GENETICS LECTURE AND LABORATORY SYLLABUS FOR A JUNIOR-LEVEL COURSE

THESIS

Presented to the Graduate Council of the
University of North Texas in Partial
Fulfillment of the Requirements

For the Degree of

MASTER OF SCIENCE

By

Kasey Harper, B.S.
Denton, Texas

August 1999

Abstract:

The following is a complete syllabus for a college level genetics course. The syllabus contains lecture outlines and notes for each chapter, along with a list of transparencies needed. The quizzes and exams are prepared and placed at the beginning of the syllabus. The beginning of the course will consist of a lecture to introduce the students to the basics of genetics, followed by many applications of genetics. The process of cell division will be mastered by the students, as well as Mendelian genetics, quantitative genetics, chromosome mapping, and inheritance. The replication, synthesis, and organization of DNA are also discussed within the lectures. The final topics that will be covered using this syllabus are genetics of cancer and immunology and population genetics. These topics are essential for a detailed genetics course. The syllabus is written in great detail, and will require a full semester to be completed. The book used in association with this syllabus is Essentials of Genetics by William S. Klug and Michael R. Cummings.

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Ever since junior high school, I have been interested in biology. I credit this interest to one teacher, Ms. Pullman. She taught Earth science at Blalack Junior High School in Carrollton, Texas. The same year that I advanced into high school, Ms. Pullman began teaching there. She taught the life sciences at Newman Smith High School. She was a wonderful influence on me, and she taught me that science requires a certain amount of determination and dedication. Ms. Pullman's class was anything but easy, her response to this was that biology was only going to get more difficult as the years passed.

As I advanced to college, I knew I wanted to be involved in the discipline of science. I attended Southwestern University in Georgetown, Texas. Although I knew I possessed the will and determination to complete a degree in biology, I was unsure how I was going to be able to emotionally proceed through college. The first day I was on campus, I met with a guidance counselor. The title she bore was deceiving. I assumed that she was going to help me with my schedule, as well as encourage me to do the best I could at Southwestern, but instead she belittled me in her office. As she was looking over my files, for the first time, she asked me if I had a learning disability. Of course I responded by saying no, and I asked her why she would think such a thing. She informed me that my GPA was excellent in high school, but my SAT scores were only mediocre. I honestly feel that she did everything she could to convince me that I would not be able to handle the pressure of science. I was distraught by her accusation of a learning disability. After I cried about what she had said, I realized that she did not know me personally, and that she had only seen my file for a matter of minutes, before she decided to destroy my self-confidence. It was her demeaning remarks about my intelligence that made me take science even more seriously, and earn a position on the Dean's List, of which I informed her by sending her a copy of the announcement.

I found myself in a new territory at Southwestern. It did not take long for me to become attached to some of the teachers, and stay away from some of the others. My first semester there was tough, but I made it through. By the end of my first semester, I was convinced that medical school was for me. I continued with my education, enrolling in a genetics course along the way. The only reason that I enrolled in this course was because one of my favorite professors was the instructor. I would soon learn though, that

I possessed a love and passion for genetics more than any other sub-discipline of science. Genetics was a difficult course; in fact, I failed the first exam. As I discussed my grade with my professor, he recommended that I drop the course, but his lack of confidence in me only strengthened my determination to excel in genetics. I began studying the material everyday, and then I received a perfect score on the second exam, which had never been accomplished on any of his tests. I proved to myself, as well as to my professor, that I possessed the intelligence needed for such a course.

By the end of my junior year, I realized that medical school was not in my future. It did not take long for me to arrive at the conclusion that I wanted to become a professor. I also knew that genetics would be the main course that I would like to instruct. There are many different qualities required for a "good" professor, and I believe that I posses all of them. Following graduation, I entered the masters program at the University of North Texas. I opted to complete an entire syllabus, including exams, lecture notes and outlines, as well as a laboratory guide to constitute my thesis.

I enjoy offering my education and knowledge to anyone who will listen. I began to notice myself applying the information that I learned in genetics to everyday events. My goal in life is to spread knowledge, not only about genetics, but anything and everything I can. I honestly feel that knowledge is the key to a happy and successful life. As a professor, I know that it will not only be the students who are learning, but I will learn new facts as well. These new facts will not only come out of the text, which I have already read front to back, but also from the research that is needed to keep up with the progression of genetics. During the course of my studies at the University of North Texas, I enrolled in a medical genetics course. A requirement for this course was to complete a review of three journal articles. I will never forget the reasoning my professor gave for these assignments. She informed the students that these journals would not only increase our awareness of what is happening in the field, but that it would increase hers as well. I believe that she was able to read these articles, as well as our reviews, and gain knowledge about the progress within the field of genetics. Since there are no two students exactly the same, I suspect that during my lectures I will be asked questions about which I would not have previously thought. This is a way that I can learn more about the course material myself.

During the course of my thesis work, I was required to read a genetics textbook, with which I had not previously read. As I read this book, I realized that there was still much information to which I had not previously been exposed. My thesis work offered a new way for me obtain knowledge that I did not already possess. In fact, I was able to process much of the information that I already knew, in a new way. Preparing lectures of material in a form that is comprehensible requires a lot of patience and understanding. Most of all, I have come to the realization that there is still a lot of information that I need to learn. I personally learn a lot of information by reading, but I realize that not everyone learns in that fashion. Many students today are visual learners. This is a concept that I took in consideration when forming my lecture notes; I was able to list over one hundred transparencies that would be needed for this course. I believe that I will be able to apply my lectures to any student, no matter what their way of learning is.

Through the course of my masters program, I became a substitute teacher at Blalack Junior High School. I believe that this experience has been extremely beneficial to me, especially since I want to become a professor. Although these were not college students and the information was not college level, I know that I learned a lot from being a teacher. Patience is obviously a requirement for becoming a professor, and I know learned a lot about patience at the junior high school. Aside from the discipline problems I faced at the junior high level, I loved teaching. I know that I would rather teach serious college students, but this gave me an opportunity for first-hand experience with teaching. This job also helped me overcome my fear of speaking in front of large audiences. I know that many teachers still get nervous speaking in front of a large classroom, but the more it is performed, the easier it becomes. Substitute teaching also allowed me to become exposed to other disciplines. I was able to teach a variety of courses, which I believe also helped me in making my decision to teach a genetics course. Although genetics is not offered at the junior high level, basic science is, and there is no other course that I would rather teach than science.

I chose Essentials of Genetics, the third edition by William S. Klug and Michael R. Cummings, as the textbook for this course. I believe this text covers all the material needed to educate an individual in the discipline of genetics. It includes twenty-one chapters concerning topics about DNA, mitosis and meiosis, recombinant DNA

technology, population genetics, and much more. The text is very well written and easy to understand. It offers a detailed look at all the components of genetics. It contains study questions as well as summaries for each chapter to provide an even better way to retain the knowledge from my lectures. The course that I have outlined is intended for a junior or senior college student. Some prior biological information will be required in order to excel in this course. The first three lectures are devoted to DNA, mitosis, and meiosis. These seem to be the most difficult concepts for students to grasp. I intend on making certain that all the students are familiar with these topics, otherwise they will be confused during the remaining lectures of the semester. The course grade is based on three exams, a final exam, and four quizzes. There will be a laboratory course that will be taught in association to this genetics course, but it will be graded separately.

I believe that genetics will be very important in the future. Genetics is still a fairly new subset of biology, and I think that its progression will offer many more insights to DNA and disorders both common and rare. Recombinant DNA technology is changing everyday, offering new ways to cope with many of the disorders that affect humans. There are ways to incorporate gene therapy today that were not available even five years ago. Along with this new knowledge, comes the responsibility of utilizing it without compromising human ethics. This text covers some of the ethical issues that are associated with these new techniques. It is my intention to teach the information about these new techniques, as well as the ethical issues involved. Ethics is a very sensitive subject though, especially with all the different religious and cultural beliefs present today. On the other hand, there is no way to get around this issue when discussing genetics. I will obviously have to be careful when mentioning such a topic, and I am sure that I will make mistakes, but that is what learning is all about. I do believe that the new DNA technology could possibly do harm to others if strict guidelines are not set. For example, genetic information is presently obtained in prenatal diagnosis. Many people find this a blessing, while others feel that is an excuse to abort fetuses with a disorder. These are the kinds of issues that I believe will arise in my lectures, but I think that is good because it will offer the students a chance to hear the opposing view of their beliefs and possibly open their minds to consider the other side. I know that I will have to be

unbiased and fair when discussing these topics with my students, but I feel confident that I have the willpower to perform such a task.

My ultimate goal is to offer students the knowledge that I have received and allow them to use it in their own way. I am sure that I will come across many students who want to become physicians, and I believe that a genetics course will certainly help prepare them for the medical field. Other students, who will not be proceeding to medical school, will still gain knowledge that can be used everyday from this course. Whether or not the information obtained in this course is used in the medical field, in a research situation, or just in everyday occurrences, I believe that it will only enhance the knowledge of each student.

I aspire to be the best professor I can; therefore with each course I take I pay close attention to the professor. I can take many different qualities from many of my past professors and incorporate them in my teaching, in order to teach to the best of my ability. My best professors were the ones that I felt I could talk to, and that is exactly what I want to convey to my students. I will definitely be willing to listen to arguments about information that I provide, about assignments, and about exams. I have also had a few bad experiences with my professors. I find that professors who look down on students as being inferior are often disliked. I expect to listen to my students with an open ear at any time. I do not feel that any idea is stupid, and the fact that students are able to create questions about information only informs me that they are thinking about what has been presented. This is the best way for knowledge to be transferred, getting a student to think about a lecture, means that the information will most likely be retained. I also plan to be organized in my lectures. I have encountered a few professors that would just speak about a topic without a guideline. I found this very distracting and uncomfortable. I have prepared detailed lecture notes and outlines just for this case. The notes that I have created will allow me to be prepared for every lecture, so that I can avoid being disorganized. I believe that this organization will allow the students more comfort in the course.

I took my thesis very seriously. I have completed very detailed lecture notes, as well as a basic outline that I plan to distribute to the students before class. Attendance will be very important to me; therefore, the outlines will be available on a trial basis.

Since I know there will be some students who will not feel the need to attend class when an outline is offered, I will pay close attention to the attendance rate, in order to be sure that the notes are not being abused. I also have incorporated questions into each test that are not found in the outlines, in hopes that every student will realize that attending my class is necessary. I am content with my choice to create a syllabus as my thesis project. Reading the genetics textbook has offered me another opportunity to gain more knowledge, which I enjoy. I know that I will be able to provide the knowledge of genetics to students who are willing to learn. Most importantly, I know that the students will not be the only ones learning in this course. After all, everyone will always be a student.

Genetics Syllabus

Lecture	Topic	Chapter
1.	An Introduction to Genetics	1
2.	Cell Division and Chromosomes (Mitosis)	2
3.	Cell Division and Chromosomes (Meiosis)	2
4.	DNA: The Physical Basis of Life	9
5.	DNA: The Physical Basis of Life	9
6.	DNA: Replication and Synthesis	10
7.	DNA: Replication and Synthesis	10
8.	**Quiz 1**, Mendelian Genetics	3
9.	Mendelian Genetics	3
10.	Modification of Mendelian Ratios	4
11.	Modification of Mendelian Ratios; Review for Test	4
12.	EXAM 1	
13.	Quantitative Genetics	5
14.	Quantitative Genetics	5
15.	Linkage and Chromosome Mapping	6
16.	Extranuclear Inheritance	7
17.	Chromosome Variation and Sex Determination	8
18.	Chromosome Variation and Sex Determination	8
19.	**Quiz 2**, Organization of DNA in Chromosomes and Genes	11
20.	Organization of DNA in Chromosomes and Genes	11
21.	Storage and Expression of Genetic Information	12
22.	Storage and Expression of Genetic Information	12
23.	EXAM 2	
24.	Regulation of Gene Expression	13
25.	Regulation of Gene Expression	13
26.	Proteins: The End Product of Genes	14
27.	DNA- Mutation, Repair, and Transposable Elements	15
28.	DNA- Mutation, Repair, and Transposable Elements	15

29.	Genetics of Bacteria and Bacteriophages	16
30.	Genetics of Bacteria and Bacteriophages	16
31.	**Quiz 3**, DNA Biotechnology: Techniques and Analysis	17
32.	DNA Biotechnology: Techniques and Analysis	17
33.	DNA Biotechnology: Applications and Ethics	18
34.	DNA Biotechnology: Applications and Ethics, Review for exam	18
35.	EXAM 3	
36.	Genetics of Cancer and Immunology	19
37.	Population Genetics	21
38.	Population Genetics	21
39.	**Quiz 4**, review for FINAL	
40.	**FINAL EXAM**	

Grading:

3 Exams –	20% each
Average of 4 Quizzes –	15%
Final Exam –	25%

Text: Essentials of Genetics Third Edition. William S. Klug and Michael R. Cummings. Prentice-Hall Inc. © 1999.

Transparencies for Genetics

Transparency number	Page in textbook	Figure/table
1	5	f. 1-2
2	7	f. 1-4
3	8	f. 1-5
4	19	f. 2-1
5	20	f. 2-3
6	22	t. 2.1
7	23	f. 2-5
8	25	f. 2-7
9	27	f. 2-9
10	33	f. 2-12
11	34	f. 2-13
12	45	f. 3-1
13	47	f. 3-2
14	48	f. 3-3
15	49	f. 3-5
16	49	f. 3-6
17	52	f. 3-8
18	56	t. 3.1
19	58	f. 3-12
20	72	f. 4-3
21	74	f. 4-5
22	75	f. 4-6
23	81	f. 4-11
24	82	t. 4.2
25	186	f. 9-1
26	188	f. 9-2
27	189	f. 9-3
28	190	f. 9-4
29	191	f. 9-5
30	194	f. 9-7
31	199	f. 9-12
32	202	f. 9-14
33	204	f. 9-16
34	212	f. 10-1
35	213	f. 10-2
36	219	f. 10-9
37	220	f. 10-10
38	221	f. 10-11
39	222	f. 10-12
40	225	f. 10-13
41	225	f. 10-14
42	227	f. 10-15
43	97	f. 5-5 and -6

90	323	f. 15-1
91	324	f. 15-2
92	325	f. 15-3
93	326	f. 15-4
94	334	f. 15-11
95	336	f. 15-12
96	351	f. 16-3
97	353	f. 16-6
98	355	f. 16-9
99	357	f. 16-11
100	359	f. 16-14
101	361	f. 16-15
102	379	f. 17-9
103	380	f. 17-11
104	382	f. 17-12
105	383	f. 17-13
106	386	f. 17-15
107	388	f. 17-17
108	389	f. 17-19
109	402	f. 18-6
110	403	f. 18-7
111	409	f. 18-15
112	413	f. 18-18
113	427	f. 19-2
114	429	f. 19-4
115	430	f. 19-5
116	433	f. 19-8
117	435	f. 19-10
118	475	t. 21.2
119	475	f. 21-1
120	476	f. 21-2
121	477	f. 21-3
122	480	f. 21-5
123	484	f. 21-12
124	485	f. 21-13

Name: Hour:

Genetics Quiz 1

1. Define genetics-

2. Describe the different centromere placements.

3. Briefly describe mitosis.

4. Describe the differences between RNA and DNA, at least 3.

5. Give a brief description of two unique kinds of chromosomes.

6. What is the tetranucleotide hypothesis?

7. What mode does our DNA replicate in?

8. Define biologically active-

9. What is the function of polymerase I?

10. What is an Okazaki fragment?

Name: Hour:

Genetics Quiz 2

1. What is meant by the term maternal effect?

2. What is the multi-factor or multiple-gene hypothesis?

3. Define aneuploidy and be sure to include at least two different "types."

4. Describe genomic imprinting.

5. What is a harlequin chromosome?

6. What does broad-sense heritability measure?

7. Show what a single crossover, between the A and B alleles, would look like involving the two chromosomes below, and show what the gametes would be.

8. Why are calico cats usually female? What can you conclude about a male calico cat?

9. Name the four structural changes that occur in chromosomes.

10. What are the mitochondrial gene products?

Name: Hour:

Genetics Quiz 3

1. List and briefly describe three components of promoters.

2. Contrast positive and negative control systems.

3. What are the three different modes of recombination in bacteria?

4. List at least four levels of protein organization and give an example for each.

5. What is an enzyme, and why are they essential for living organisms?

6. Contrast a somatic and a gene mutation.

7. Why do F^- recombinants never result from a cross between Hfr and F^-?

8. What is the role of reverse transcriptase during the infection of an animal cell by a retrovirus?

9. Using sickle-cell anemia as a basis or an example, describe what is meant by a molecular or genetic disease.

10. Describe tautomerism and the way in which this chemical event may lead to mutation.

Name: Hour:

Genetics Quiz 4

1. Define protoncogenes-

2. Define oncogenes-

3. What are the three mechanisms often used to describe the conversion of proto-oncogenes to oncogenes?

4. List the 5 classes of antibodies, be sure to include where they are found.

5. List three common disorders of the immune system.

6. Define natural selection-

7. What are the three types of selection?

8. Calculate the frequencies of the *AA, Aa, and aa* genotypes after one generation if the initial population consists of 0.2*AA*, 0.6*Aa*, and 0.2*aa* genotypes.

9. What is the basis for inbreeding depression?

10. If 4% of the population in equilibrium expresses a recessive trait, what is the probability that the offspring of two individuals who do not express the trait will express it?

Name: Date:

Genetics Exam 1

I. **Matching**: Match the term on the left with the appropriate definition on the right. Each definition will only be used one time. (10 points)

1. Homunculus
2. Polyploid
3. Karyokinesis
4. p53
5. Alleles
6. Mutation
7. Epistasis
8. Hemizygous
9. Nucleotide
10. Holoenzyme

a. forms of single genes that determine phenotype.
b. a nucleoside with a phosphate group attached.
c. a sex-cell containing a complete miniature adult.
d. one gene or gene pair masks the expression of another gene or gene5. pair.
e. active form of a DNA polymerase 6. molecule.
f. an organism with more than 2 sets of chromosomes.
g. Male sex genes.
h. nuclear division.
i. Tumor suppressor gene.
j. Source of new alleles.

II. **Definitions**: Give a complete, but brief definition for the following five vocabulary words. (10 points)

11. **Gene conversion-**

12. **Okazaki fragments-**

13. **Sex-influenced inheritance-**

14. **Endosymbiont hypothesis-**

15. **Monohybrid cross-**

III. **Short Answer**: Please write answers in paragraph form, be sure to include all information asked for in the question. Partial Credit will be given. SHOW WORK!

16. Describe the three possible replication methods for DNA; be sure to indicate which one is correct. (5 points)

17. Show a punnet square for a cross between **Aa x Aa** and report ratios of expected progeny. (5 points)

18. An example of a simple recessive trait in humans is albinism. Determine the genotypes of both the parents and the offspring for the following families:
    a. Two nonalbino (normal) parents have five children, four are normal and one is albino. (2 points).

    b. A normal male and an albino female have six children, all of which are normal. (2 points)

19. The F_2 results of two of Mendel's monohybrid crosses are shown below. State a null hypothesis to be tested using χ^2 analysis. Calculate the chi-square value and determine the *p* value for both. Interpret the *p* values as well. Which of the two crosses shows a greater amount of deviation? (5 points)

    a. Full pods 882 Constricted pods 299

    b. Violet flowers 705 White flowers 224

20. Define the function of the following cellular components: (6 points)

    a. **chromatids-**

    b. **nucleolus-**

    c. **ribosomes-**

    d. **mitochondria-**

    e. **centriole-**

    f. **centromere-**

21. How are chromosomes generally named on the basis of centromere placement? (4 points)

22. Give a few examples of how genetic research has been applied to agriculture and to medicine. (3 points)

23. Suppose a husband and wife both had normal vision and both of their fathers were red-green color-blind, an inherited X-linked recessive condition. What is the probability that their first child will be:

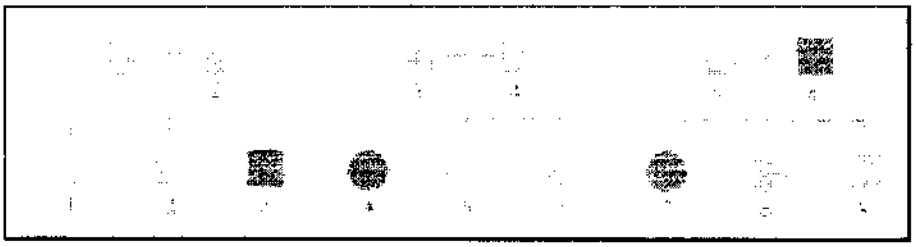
    a. a normal son? (1 point)

    b. a normal daughter? (1 point)

    c. a color-blind son? (1 point)

    d. a color-blind daughter? (1 point)

24. The three pedigrees below all involve a single human trait. (8 points)



a. Which sets of the following conditions, if any, can be excluded?

Conditions: dominant *and* X-linked
dominant *and* autosomal
recessive *and* X-linked
recessive *and* autosomal

b. Which individual in generation II was most instrumental for the set(s) of conditions that you excluded? [Answer none apply if no conditions were excluded].

c. List all the possible genotypes for the individuals listed below. Use A and a as the genotype symbols.

II-1, II-6, and II-9

25. Briefly discuss the Hershey-Chase experiment. (5 points)

26. Describe the various characteristics, at least five, of the Watson-Crick double helix model for DNA. (5 points)

27. In the Meselson-Stahl experiment, which of the three modes of replication could be ruled out after one round of replication and why? (3 points)

28. What is meant by the term "biologically active" DNA? (3 points)

29. Distinguish disjunction from nondisjunction, basic definitions. (2 points)

30. Summarize the functions of polymerase I, II, and III. (3 points).

31. Describe meiosis in **detail**. (10 points)

32. Draw the complementary strand of RNA to this strand of DNA: (5 points)

5'-TACTCCGATCCGGTTAAGC-3'

Name: Hour:

Genetics Exam 2

I. **Matching**: Match the term on the left with its definition on the right, each definition will only be used once. (10 points)

1. Complete linkage	a. Restores the function lost by a previous mutation at another site.
2. Chiasmata	b. RNAs that are capable of splicing themselves.
3.Crossing over	c. Only part of the chromosome is lost.
4. Suppressive mutation	d. When two randomly selected genes are so close together that crossover events are too infrequent to detect.
5. Autogamy	e. The amount of DNA contained in the haploid genome.
6. Partial monosomy	f. The process of self-fertilization resulting in homozyosis.
7. Telomeres	g. Points of overlap, which are created when synapsed homologous chromosomes are in meiosis wrap each other.
8. Nucleoid	h. The structure that holds DNA in bacteria.
9. C value	i. Physical exchange leading to recombination.
10. Ribosomes	j. Regions at the end of chromosomes.

II. **Definitions**: Give a brief, but complete definition for the following five vocabulary words. (10 points)

11. **Quantitative inheritance-**

12. **Somatic cell hybridization-**

13. **Heterokaryon-**

14. **Gene amplification-**

15. **Wobble Hypothesis-**

III. **Short Answer:** Please write answers in paragraph form, be sure to include all information asked for in the question. Partial credit will be given. SHOW WORK!

16. List the nine general features of the genetic code. (5 points)

17. Define and discuss the following terms: (5 points)
    a. **polygenic-**

    b. **additive alleles-**

    c. **monozygotic** as compared to **dizygotic twins-**

    d. **concordance** as compared to **disconcordance-**

    e. **heritability-**

18. What is a Barr body?

    -Indicate the number of Barr bodies in the following individuals: (6 points)
        a. Klinefelters syndrome
        b. Turner syndrome

c. 47, XYY

d. 47, XXX

e. 48, XXXX

19. What is "exon shuffling?" (3 points)

20. List the start and stop codons. (2 points)

Start: Stop:

21. If the make believe genes, *out-of-shape (os)*, *extra long wings (lw)*, and *ugly toes (ut)* are linked on chromosomes 2 of *Drosophila*, and a series of two point mapping crosses determined the following genetic distances: (3 points)

Os – ut 43 units *os – lw* 7 units *ut – lw* 36 units

What is the sequence of the genes? Draw a map of the chromosome.

22. Distinguish between the following:

a. paracentric/pericentric- (2 points)

b. autopolyploidy/allopolyploidy- (2 points)

23. In a mixed copolymer experiment using polynucleotide phosphorylase, ¾ G: ¼ C was added to form the synthetic message. The amino acid composition of the ensuing protein was determined to be :

Glycine	36/34	56%
Alanine	12/64	19%
Arginine	12/64	19%
Proline	4/64	6%

a. Indicate the percentage, or fraction, of the time each possible triplet will occur in the message. (3 points)

b. Determine a complete set of base composition assignments for all amino acids present. (3 points)

c. Considering the wobble hypothesis, predict as many specific triplet assignments as possible. (3 points)

24. Results of three crosses between strains of *Paramecium* are shown below. Determine the genotypes of each parental strain. (3 points)

a. Killer X sensitive $\Longrightarrow$ ½ Killer; ½ sensitive

b. Killer X sensitive $\Longrightarrow$ all Killer

c. Killer X sensitive $\Longrightarrow$ ¾ Killer; ¼ sensitive

25. The β-globin gene family consists of 60 kb of DNA, yet only 5% of the DNA encodes β-globin gene products. Account for the remaining 95% of DNA. (3 points)

26. A newlywed couple is very excited about having children, but the couple is aware of the high incidence of still births on the male's side of the family. The wife had studied genetics in college and asked her husband to visit a genetic counselor, where a complete karyotype analysis could be produced. It was determined that the

husband had the normal complement of 46 chromosomes, but one member of the chromosome #1 pair had an inversion that covered 70% of the length. The homolog of chromosome 1 and all others were normal.

a. How would you explain the high incidence of past stillbirths? (2 points)

b. What would you predict the probability would be for the couple to have children with this inversion? (2 points)

c. What advice would you give the couple if they were insistent on having their own children? (2 points)

27. Corn plants are measured from a test plot, and the distribution of the heights at 10-cm intervals is recorded in the following table:

Height (cm)	*Plants (no.)*
100	20
110	60
120	90
130	130
140	180
150	120
160	70
170	50
180	40

Calculate the (a) the mean, (b) the variance, (c) the standard deviation, (d) the standard error of the mean. Based on your calculations, how would you assess the variation in this population? (8 points)

28. Name and describe at least two types of banding patterns in chromosomes. (4 points)

29. Draw and label a tRNA structure including the 3'-end, 5'-end, anticodon loop, anticodon stem, D-loop, a.a. binding site, acceptor stem, TψC loop, and the variable loop. (10 points)

30. Summarize the steps in translation, you may draw pictures if it helps you. (9 points)

Name: Date:

Genetics Exam 3

I. **Matching**: Match the term on the left with the appropriate definition on the right. Each definition will only be used one time. (10 points)

1. Constitutive	a. carrier DNA molecules such as plasmids and bacteriophages.
2. Sickle-cell trait	b. Hb^S / Hb^A
3. Chaperones	c. an organism that can synthesize all essential organic compounds.
4. Hemophilia	d. produced continuously regardless of chemical make-up of environment.
5. Short interspersed elements (SINEs)	e. acts as an antigen to stimulate immune system.
6. Phototroph	f. proteins that facilitate the folding of other proteins.
7. Auxotroph	g. X-linked mutation in humans.
8. Vectors	h. bacteria which have lost the ability to synthesize one or more organic compounds.
9. Chromosome walking	i. sequences that constitute 3% of the total genome.
10. Subunit vaccine	j. isolation of adjacent clones from a library.

II. **Definitions**: Give a complete, but brief definition for the following five vocabulary words. (10 points)

11. **Transgenic-**

12. **Recombinant DNA-**

13. **Complete versus generalized transduction-**

14. **Transposons-**

15. **Attenuation-**

III. **Short Answer**: Please write answers in paragraph form, be sure to include all information asked for in the question. Partial Credit will be given. SHOW WORK!

16. List and briefly describe the three protein factors that must be present to initiate transcription. (3 points)

17. Differentiate between polypeptide and protein. (4 points)

18. Give three ways to analyze cloned sequences. (3 points)

19. Define and compare the four levels of protein organization. (4 points)

20. List and explain at least 5 different categories of mutations. (5 points)

21. Describe, briefly, the lytic and lysogenic pathways of phage lambda in *E. coli*. (4 points)

22. Explain the one gene/one enzyme hypothesis. (4 points)

23. Give 5 examples of post-translational modification. (5 points)

24. List and explain the 7 basic steps in recombinant DNA technology. (4 points)

25. Explain the process for excision repair of DNA. (3 points)

26. Give at least three examples of diagnosing and screening for genetic disorders. (3 points)

27. When the interrupted mating technique was used with five different strains of Hfr bacteria, the following order of gene entry and recombination was observed. On the basis of these data, draw a map of the bacterial chromosome, and do the data support the concept of circularity?

Hfr Strain	*Order*
1	T C H R O
2	H R O M B
3	M O R H C
4	M B A K T
5	C T K A B

28. Pretend that you have recovered a cloned DNA segment of interest, and determined that the insert is 1300 bp in length. To characterize the cloned segment, you isolated the insert and decided to construct a restriction map. Using enzyme I and enzyme II and gel electrophoresis, you determine the number and size of the fragments produced by the enzymes, as follows:

Enzymes	*Restriction Fragment Sizes (bp)*
I	350 bp, 950 bp
II	200 bp, 1100 bp
I and II	150 bp, 200 bp, 950 bp

From these data, construct a restriction map, showing the positions of the restriction sites relative to one another, as well as the distance between them in base pairs. (3 points)

29. Explain the steps in PCR. (4 points)

30. Give an explanation of the technique known as DNA fingerprinting, be sure to include possible applications. (3 points)

31. Donor DNA was obtained from a prototrophic bacterial strain ($a^+ b^+ c^+$), and the recipient was auxotrophic for all three genes ($a^- b^- c^-$) in a transformation experiment. The following data were obtained:

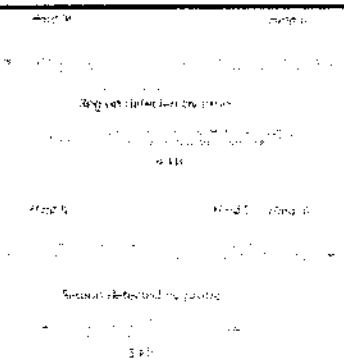
$a^+ b^- c^-$	180	$a^+ b^- c^+$	2
$a^- b^+ c^-$	150	$a^+ b^+ c^-$	210
$a^- b^- c^+$	179	$a^- b^+ c^+$	1
$a^+ a^+ a^+$	3		

What general conclusions can be drawn about the linkage relationship between the three genes? (4 points)

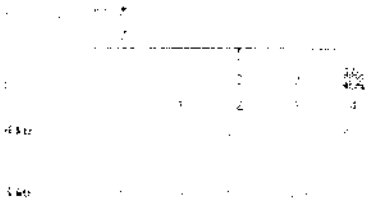
32. If the human genome contains 100,000 genes, and the mutation rate at each of the loci is 5×10^{-5} per gamete formed, what is the average number of new mutations that exist in each individual? (2 points)

33. Explain the different steps in bacterial transformation. (4 points)

34. A mutation in the clotting factor VIII, causes one form of hemophilia. There is an RFLP for the enzyme *Hin*dIII contained in an intron of the factor VIII gene that can often be used in screening, as shown below:



A female whose brother has hemophilia is at a 50 % risk of being a carrier of this disorder. To test her status, DNA is obtained from white blood cells of family members, cut with *Hin*dIII, and the fragments probed and visualized by Southern blotting. The results are shown below. Determine whether any of the females in generation II are carriers for hemophilia. (3 points)



35. The human insulin gene contains introns, despite the fact that bacterial cells will not excise introns from mRNA, how can a gene like this be cloned into a bacterial cell and produce insulin? (2 points)

36. A series of mutations in the bacterium *Salmonella typhimurium* results in the requirement of either tryptophan or some related molecule in order for growth to occur. From the data shown below, suggest a biosynthetic pathway for tryptophan: (4 points)

Mutation	Minimal Medium	Anthranilic Acid	Indole Glycerol Phosphate	Indole	Tryptophan
trp-8	-	+	+	+	+
trp-2	-	-	+	+	+
trp-3	-	-	-	+	+
trp-1	-	-	-	-	+

37. What is SCID? Be sure to include what causes it, possible therapy actions, and a person who has been affected by it. (5 points)

38. Briefly describe the operon model. (5 points)

Name: Date:

Genetics Final Exam

I. **Matching**: Match the term on the left with the appropriate definition on the right. Each definition will only be used one time. (10 points)

1. Translation	a. Differential reproduction of some members of a species, resulting from variable fitness conferred by genotype differences.
2. Chromatin	b. Related by a common ancestor within a few previous generations.
3. Fixation	c. Chromosome or chromosome fragment with no centromere.
4. Bivalents	d. An enzyme that participates in DNA replication by unwinding the double helix near the replication fork.
5. Lysis	e. Used to detect mutagens and carcinogenic compounds.
6. Disjunction	f. A derivation of the amino acid sequence of a polypeptide, from the base sequence of an mRNA molecule in association with a ribosome.
7. Allele	g. A class of serum proteins having the properties of antibodies.
8. Spacer DNA	h. Disintegration of a cell brought on by the rupture of its membrane.
9. Telocentric chromosome	i. DNA segments of a gene that are transcribed and translated into a protein.

10. Helicase	j. Term used to describe the complex of DNA, RNA, histones and nonhistone proteins that make up a chromosome.
11. Acentric chromosome	k. The unit of distance between genes on a chromosome.
12. Consanguine	l. A condition in which all members of a population are homozygous for a given allele.
13. Pilus	m. Observed properties of an organism that are genetically controlled.
14. Natural Selection	n. Enzymes that convert DNA from one form to another.
15. Telocentric chromosome	o. Sequences found between genes that are usually repetitive.
16. Exon	p. Separation of chromosomes at the anaphase stage of the cell cycle.
17. Centimorgan	q. A filament like projection from the surface of a bacterial cell.
18. Immunoglobulin	r. Synapsed homologous chromosomes in the first prophase of meiosis.
19. Ames Test	s. A chromosome with the centromere on the end.
20. Phenotype	t. One of the possible mutation states of a gene.

II. **Definitions**: Give a complete, but brief definition for the following ten vocabulary words. (10 points)

21. **Genetics-**

22. **Prototroph-**

23. **Mutation-**

24. **Homunculus-**

25. **Intercalating agent-**

26. **Lytic phase-**

27. **Lagging strand-**

28. **F^+ cell-**

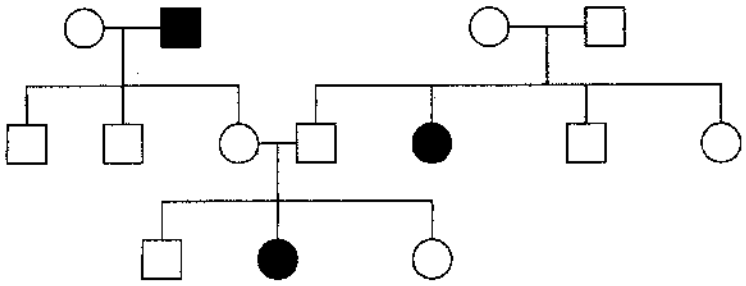
29. **Hardy-Weinberg law-**

30. **Frameshift mutation-**

III. **Short Answer**: Please write answers in paragraph form, be sure to include all information asked for in the question. Partial Credit will be given. SHOW WORK!

31. Contrast spermatogenesis and oogenesis. What is the significance of the formation of polar bodies?

32. The following pedigree follows the inheritance of myopia (near-sightedness) in humans. Predict whether the disorder is inherited as a dominant or a recessive trait. Based on your prediction, indicate the most probable genotype for each individual.



33. In diploid yeast strains, sporulation and subsequent meiosis can produce haploid ascospores. These may fuse to reform diploid cells. When ascospores from a segregational *petite* strain fuse with those of a normal wild type strain, the diploid zygotes are all normal. However, following, meiosis, ascospores are ½ *petite* and ½ normal. Is the segregational *petite* phenotype inherited as a dominant or a recessive gene? Explain.

34. In the guinea pig, there are four alleles that can contribute to coat color: *C (black)*, c^k *(sepia)*, c^d *(cream)*, or c^a *(albino)*. There is a progressive order of dominance between these alleles when they are present heterozygously, $C > c^k > c^d > c^a$. For the following

crosses, determine the genotype of each individual and predict the phenotypic ratios of the offspring.

a. sepia X cream, where both had an albino parent.

b. sepia X cream, where the sepia individual had an albino parent, and the cream individual had two sepia parents.

c. sepia X cream, where the sepia individual had two full-color parents, and the cream individual had two sepia parents.

d. sepia X cream, where the sepia individual had two full-color parents, and the cream individual had two full-color parents.

35. Define plaque, lysogeny, and prophage.

36. Outline the current model for DNA synthesis, briefly.

37. In a cross in *Drosophila*, a female that was heterozygous for the autosomally linked genes *a, b, c, d,* and *e* (*abcde*/+++++) was test-crossed to a male that was

homozygous for all recessive alleles. Even though the distance between each of the above loci was at least 3 map units, only four phenotypes were recovered:

Phenotype	**No. of Flies**
+ + + + +	440
a b c d e	460
+ + + + *e*	48
a b c d +	52
Total =	1000

Why are there many expected phenotypes missing? Can any of these loci be mapped from the data given here? If so, determine the map distances.

38. The synthesis of flower pigments is known to be dependent upon enzymatically controlled biosynthetic pathways. In the crosses below, postulate the role of mutant genes and their products in producing the observed phenotypes.

    (a) P_1: white strain A X white strain B

    F_1: all purple

    F_2: 9/16 purple: 7/16 white

    (b) P_1: white X pink

    F_1: all purple

    F_2: 9/16 purple: 3/16 pink: 4/16 white

39. Why do you suppose that a random mutation is more likely to be deleterious than beneficial?

40. Consider the allele frequencies $p = 0.70$ and $q=0.30$, where selection occurs against the homozygous recessive genotype. What will the allele frequencies be following one generation if:

a. $s = 1.0$ b. $s = 0.5$ c. $s = 0.1$ d. $s = 0.01$

41. Define tumor suppressor genes, why are most tumor suppressor genes expected to be recessive?

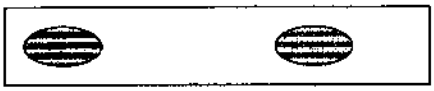
42. The DNA sequence surrounding the site of sickle-cell mutation in β-globin is shown for normal and mutant genes:

5' G A C T C C T G A G G A G A A G T 3'
3' C T G A G G A C T C C T C T T C A 5'
Normal DNA

5' G A C T C C T G T G G A G A A G T 3'
3' C T G A G G A C A C C T C T T C A 5'
Sickle-cell DNA

Each type of DNA is denatured into single strands and applied to a filter. This paper is then hybridized to an ASO of the following sequence:

5' G A C T C C T G A G G A G A A G T – 3'. Which, if either spot, will hybridize to this probe and why?



Normal DNA Sickle-cell DNA

43. What is the role for each of the following in recombinant DNA studies?
    a. Restriction endonucleases

    b. Vectors

    c. Host cells

44. What do the following symbols represent in immunoglobulin structure:
    a. VL
    b. CH
    c. IgG
    d. J
    e. D

45. How do translocations such as the Philadelphia chromosome lead to oncogenesis?

46. Describe the role of transcription factors in the regulation of gene expression.

47. Briefly describe the structure of RNA.

48. Describe the differences between Klinefelter and Turner syndromes.

49. List a few examples when inbreeding can be good.

50. What does genomic imprinting mean?

A geneticist is assessing data that fell into two phenotypic classes, observed values of 250:150. She decided to perform a chi-square analysis using two different null hypotheses: a. the data fit a 3:1 ratio, and b. the data fit a 1:1 ratio. Calculate the χ^2 values for each of these hypotheses. What can be concluded about each hypothesis?

51. χ^2 value for null hypothesis a.

52. χ^2 value for null hypothesis b.

53. Mendel crossed peas having round seeds and yellow cotyledons. All the F_1 plants had round seeds with yellow cotyledons. Diagram this cross through the F_2 generation using a Punnett square.

54. Does inbreeding cause an increase in frequency of recessive alleles within a population?

55. Calculate the frequencies for *AA*, *Aa*, and *aa* genotypes after one generation if the initial population consists of 0.2 *AA*, 0.6 *Aa*, and 0.2 *aa* genotypes.

56. What will the genotype frequencies be after the second generation? (From 54).

57. List at least 3 disorders of the immune system.

58. What is the HLA system?

59. What are the functions of gatekeeper and caretaker genes?

60. What individual would want to be tested to see if she carries the *BRCA 1 and BRCA 2* genes?

Lecture Outline

Chapter One: An Introduction to Genetics

I. Prehistoric Times: Domesticated Animals and Cultivated Plants.

A. Between 8000 and 1000 B.C.

1. Various Breeds of Animals.

B. 5000 B.C.

1. Cultivation of Plants.

a. Maize

b. Wheat

c. Rice

d. Date Palm

II. The Greek Influence: Hippocrates and Aristotle.

A. Explanation of Heredity Basis of Reproduction.

1. The source of the physical substance of the offspring.

2. The nature of the generative force that directs the physical substance as it develops into an adult organism.

III. 1600-1850: The Dawn of Modern Biology.

A. Roman Times

1. Plant grafting and animal breeding

B. Middle Ages

1. Naturalists

C. Between 1600 and 1850

1. 1600

a. William Harvey

1. **Epigenesis**

2. Transparency 1 (Page 5, Figure 1-2)

3. **Theory of Performation**

4. **Homunculus**

2. 1808

a. John Dalton

1. **Atomic Theory**

3. 1830
    a. Matthias Schleiden and Theodor Schwann
        1. **Cell Theory**
    b. Francesco Redi, Lazzaro Spallanzani, and Louis Pasteur
        1. Disproved the idea of **spontaneous generation**
    c. Carolus Linnaeus
        1. Helped popularize the idea of **fixity of species**

IV. Charles Darwin and Evolution

A. *The Origin of Species*
    1. 1859
    2. Existing species arose by descent with modification from other ancestral species
    3. H.M.S. *Beagle*
    4. **Theory of natural selection**
B. Alfred Russel Wallace
C. *Variations in Animals and Plants under Domestication*
    1. 1868
    2. Attempt to provide a more definitive explanation of how heritable variation arises gradually over time
D. **Pangenesis**
E. Lamarck
    1. previously formalized this idea in 1809, acquired characteristics
F. Gregor Mendel
    1. Demonstrated a number of statistical patterns underlying inheritance

V. Basic Concepts of Genetics

A. **Genetics-** The branch of biology concerned with heredity and variation
B. Genetic Material
    1. Transparency 2 (Page 7, Figure 1-4)
    2. **Eukaryotic** versus **Prokayotic**
    3. **Nucleus**
    4. **Nucleoid region**
    5. Genetic material is located within the protein coat of a virus

6. Eukaryotes and prokaryotes, **DNA** stores genetic information; viruses, either DNA or **RNA** serves this function, but never both
7. DNA
   a. Deoxyribonucleic acid
   b. Double stranded (usually)
   c. Double helix
8. RNA
   a. Ribonucleic acid
   b. Single stranded
   c. mRNA
   d. rRNA
   e. tRNA
9. **Genes**
   a. Informational storage unit
   b. Functions: replication, mutation, and expression
10. Chromosome
   a. Transparency 3 (Page 8, Figure 1-5)
   b. Tightly coiled and condensed
   c. **Chromatin**
   d. **Diploid Number (2*n*)**
   e. **Homologous**
   f. **Centromere**
   g. **Loci**
   h. **Haploid (*n*)**
   i. **Polyploid**
11. Mitosis
   a. Somatic cells
   b. Genetic material duplicated
   c. Two progeny
   d. Same amount of genetic material
12. Meiosis

a. Germ cells
b. Genetic material duplicated
c. Four progeny
d. Half of genetic material

13. Genetic Variation
    a. **Chromosomal Mutations**
    b. **Gene Mutations (aberrations)**
    c. **Genotype**
    d. **Alleles**
    e. **Phenotype**

14. **Nucleotides**
    a. Four
    b. **Nitrogenous bases**
    c. **Triplet**
    d. **Amino acids**

15. Expression of Genetic Code
    a. **Transcription**
        1. **mRNA**
        2. **ribosome**
    b. **Translation**

16. Proteins
    a. Biological catalysts (enzymes)
        1. Lower **activation energy**
    b. Nonenzymatic roles

VI. Investigative Approaches in Genetics

1. **Transmission genetics**
    a. Gregor Mendel
    b. **Pedigree analysis**
2. **Cytogenetics**
    a. **Chromosomal theory of inheritance**
    b. **Karyotypes**

3. **Molecular Genetics**
    a. Cloning
    b. **Recombinant DNA**
    c. **DNA biotechnology**
4. **Population genetics**

VI. Genetic Advances in Agriculture and Medicine

1. Plants
    a. More vigorous growth
    b. Increased resistance
    c. Combinations of superior traits
    d. Desirable qualities
2. Animals
3. Medicine
    a. Disorders and their gene
    b. Cancer
    c. **Genetic Counseling**
    d. **Immunogenetics**
    e. **Gene Therapy**
    f. **Human Genome Project**

Lecture Content

Chapter One: An Introduction to Genetics

I. Prehistoric Times: Domesticated Animals and Cultivated Plants

A. Between 8000 and 1000 B.C, various breeds of horses, dogs, camels, and oxen were domesticated for different roles.

B. 5000 B.C marked the beginning of the cultivation of Plants.

1. It is thought that people cultivated plants for eating.
2. These plants include maize (corn), wheat, rice, and the date palm.

II. The Greek Influence: Hippocrates and Aristotle

A. There were two hypothesis to explain the heredity basis of reproduction.

1. One of these theories was associated with the source of the physical substance of the offspring.
2. The second considered the nature of the generative force that directs the physical substance as it develops into an adult organism.

III. 1600-1850: The Dawn of Modern Biology

A. In Roman times, plant grafting and animal breeding were emphasized.

B. In the middle ages, naturalists were aware of the impact of heredity on the organisms that they studied, and they were faced with reconciling their findings with their religious beliefs.

C. William Harvey is credited with the theory of **Epigenesis**.

1. This states that an organism is derived from substances present in the egg, which differentiate into adult structures during embryonic development.
2. In other words, the structures are not present initially, but form in the embryo.

D. The **Theory of Preformation** was put forth in the seventeenth century.

1. This stated that sex cells contain a complete miniature adult, which is called a **Homunculus**.
2. This theory was popular well into the eighteenth century.
3. Transparency 1, page 5, figure 1-2.

E. In 1808, John Dalton derived the **atomic theory**, which stated that all matter is composed of small invisible units called atoms.

F. In 1830, improved microscopes became available allowing for better investigations.

1. Matthias Schleiden and Theodor Schwann proposed the **cell theory** at this time.
   a. This stated that all organisms are composed of basic visible units called cells.
   b. These cells are derived from similar preexisting structures.
2. Francesco Redi, Lazzaro Spallanzani, and Louis Pasteur disproved the idea of **spontaneous generation**.
   a. This stated that, the creation of living organisms was derived from nonliving components.
   b. Therefore, living organisms were now thought to be derived from preexisting structures or to consist of cells.
3. Carolus Linnaeus was another influential scientist at this time.
   a. During the nineteenth century, he helped to popularize the idea of **fixity of species**.
   b. This stated that animal and plant groups remain unchanged in form from the moment of their existence on earth.
   c. He is also known for the binomial system of classification, which consist of two latin names that are underlined.

IV. Charles Darwin and Evolution

A. *The Origin of Species*, was published in 1859; it was a book length statement of his theory of evolution.
   1. It explained that existing species arose by descent with modification from other ancestral species.
   2. Darwin got many of his ideas while on the H.M.S. *Beagle* between 1831 and 1836.
      a. This was a five-year voyage that had the intention of returning slaves.
      b. The ship traveled to the Galapagos Islands, which are very well known in association with Darwin.
   3. Darwin's **theory of natural selection** attempts to explain the causes of evolutionary change.

B. Alfred Russel Wallace, had the same ideas that Darwin had about evolution.
   1. He noticed that more offspring were created than the environment could support, which lead to a struggle for survival between them.

2. The offspring with heritable traits were able to adapt better to the environment, yielding a better survival rate.
3. Consequently, these are the organisms that would live to reproduce again.

C. *Variations in Animals and Plants under Domestication* was published in 1868, and attempted to provide a more definitive explanation of how heritable variation arises gradually over time.

D. **Pangenesis** is a theory of development that postulated the existence of pangenes, small particles from all parts of the body that concentrate in the gametes, passing traits from one generation to the next, and blending the traits of the parents in the offspring.
   1. Darwin coined the term **gemmules** to describe the physical units representing each body part that were gathered by the blood in the semen.
   2. Darwin felt that the gemmules could respond to the external environment in an adaptive way.
   3. He also believed that these adaptations would be passed on to the offspring by acquired characteristics.

E. Lamarck had previously formalized this idea in 1809 of **acquired characteristics**.
   1. This stated that organisms acquire or lose characteristics that then become heritable.
   2. An example would be the giraffe; the giraffe stretches his neck to reach food, leading to a longer neck, which he would then pass on to his offspring.

F. Gregor Mendel demonstrated a number of statistical patterns underlying inheritance and developed a theory involving hereditary factors in the germ cells to explain these differences (Ch. 3).
   1. Mendel actually wrote Darwin a letter describing his experiments because he wanted Darwin's view on them.
   2. The letter was found on Darwin's desk following his death; it had not even been opened.

G. It was not until the twentieth century, that chromosomes were discovered.
   1. Although little was known about chromosomes, many scientists had ideas on how they contributed to the process of life.

2. It soon became clear that heredity and development were dependent on the "information" contained within these chromosomes.

V. Basic Concepts of Genetics

A. **Genetics** is the branch of biology concerned with heredity and variation.

B. There are two kinds of cells, **eukaryotic**, which are found in animal cells, and **prokaryotic**, which are found in bacteria.

1. A **Nucleus** is present in a eukaryotic cell, and is where the chromosomes are kept.
2. The **Nucleoid region** is found in a prokaryotic cell, and is where the genetic material is found, but it is not enclosed like the eukaryotic genetic information.
3. The genetic material of a virus, is located in a protein coat.
4. In eukaryotes and prokaryotes, **DNA** stores genetic information.
5. Viruses have either DNA or **RNA** to serve this function.
6. DNA is deoxyribonucleic acid.
   a. It is double stranded (usually).
   b. And it is in the form of a double helix.
7. RNA is ribonucleic acid
   a. It is single stranded.
   b. There are three main types of RNA, mRNA, rRNA, and tRNA.
8. **Genes** are defined as the functional unit of heredity.
   a. They are the informational storage unit.
   b. The functions of genes include replication, mutation, and expression.
9. Viruses and bacteria usually only have a single chromosome, whereas eukaryotes have many.
   a. Chromosomes are tightly coiled and condensed, but very long.
   b. Chromosomes replicate in the processes known as mitosis and meiosis (Ch. 2).
   c. **Chromatin** uncoils during interphase where it can be studied under an electron microscope.
10. There are some exceptions, but most eukaryotes have the **Diploid Number (2*n*)** of chromosomes in each somatic cell.

a. These chromosomes occur in pairs.

b. There is a nearly identical appearance between the two chromosomes, which are called **homologous chromosomes**, when they are in their pairs.

11. The **Centromere** is the point of spindle fiber attachment during division

a. All chromosomes have a centromere.

b. In homologous chromosomes, the centromere is located at the same place on each chromatid.

12. **Loci** are also known as gene sites, this is where the homologous chromosomes pair up with one another during gamete formation.

13. Some organisms are almost always **haploid (*n*)**, which is the half the number of diploid.

a. Haploid organisms contain only one set of chromosomes.

b. Yeasts are usually haploid all their life.

14. Other organisms can be **polyploid**, which means having more than two sets of chromosomes; in fact, many plant species are polyploid.

15. Mitosis is the process in which the genetic material of eukaryotic cells is duplicated and distributed during cell division.

a. This occurs in the somatic cells of an organism.

b. The final result is two progeny cells, which contain the same amount of genetic material (*n*).

16. Meiosis is the process in which cell division produces gametes in animal and spores in most plants.

a. This only occurs in germ cells of organisms.

b. The final result is four progeny, which contain only half the amount of genetic material that was started with (*n*).

C. There are two sources for genetic variation:

1. One source is through **chromosomal mutations**.

2. The second source is through **gene mutations (aberrations)**, which include duplication, deletion, or rearrangement of chromosome segments.

a. **Genotype** refers to the stored information in the DNA.

b. **Alleles** are alternative forms of genes, which result from mutations.

c. **Phenotype** refers to the characteristics of an organism.

D. **Nucleotides** are identified as four different blocks in a segment of DNA constituting a gene.

a. The sequence of these nucleotides makes up a gene.

b. The gene encodes the chemical nature of a protein, which is the end result in genetic expression.

c. **Mutations** are produced when the sequence of nucleotides is altered.

E. The genetic code is a **triplet**, which takes three nucleotides to constitute a "code word".

a. Almost all triplets code for one of the twenty **amino acids**.

b. Amino acids are the building blocks of proteins.

F. Transcription is the transfer of genetic information from DNA by the synthesis of an RNA molecule copied from a DNA template.

1. **mRNA** is an RNA molecule transcribed from DNA and translated into the amino acid sequence of a polypeptide.

2. A **Ribosome** is a ribonucleoprotein organelle consisting of two subunits, each containing RNA and protein; they are the site of translation of mRNA codons into the amino acid sequence of a polypeptide chain.

G. Translation is the derivation of the amino acid sequence of a polypeptide from the base sequence of a mRNA molecule in association with a ribosome.

H. Proteins are biological catalysts (enzymes).

1. They control cellular metabolism and determine which carbohydrates, lipids, nucleic acids, and other proteins are present in the cell.

a. They perform their function by lowering the **activation energy**.

b. When the activation energy is lowered, the rate process increases.

2. Proteins also have nonenzymatic roles.

a. Some other examples of proteins are hemoglobin, collagen, immunoglobins, and some hormones.

b. These proteins function in a specific way, but do not necessarily increase the rate of a reaction.

VI. Investigative Approaches in Genetics

A. **Transmission genetics** is when the patterns of inheritance of specific traits are examined.

1. Gregor Mendel performed the first significant experiment dealing with transmission genetics.
   a. He described the benefit of **pedigree analysis**.
   b. Mendel worked with pea plants.
      - He chose these organisms because selective matings between humans is almost impossible.
      - Patterns of inheritance are traced through as many generations as possible, when working with pedigrees.
      - This type of analysis leads to inferences concerning modes of inheritance of the trait under investigation.
2. **Cytogenetics** is the study of chromosomes.
   a. The **chromosomal theory of inheritance**, viewed the chromosome as the carrier of genes and the functional unit of transmission of genetic information.
   b. This was the cornerstone for further studies in genetics during the first half of this century.
   c. **Karyotypes**, illustrate the chromosomes characteristic of any species arranged in a standard sequence.
      - These are useful in the investigation of chromosome structure and abnormalities.
      - Transparency 6, page 21, figure 2-4.
3. **Molecular genetics** was initiated in the early 1940s.
   a. **Recombinant DNA** is when genes from another organism are literally spliced into bacterial or viral DNA and cloned.
   b. **DNA biotechnology** is when genes are identified, sequenced, cloned and manipulated.
4. **Population genetics** is used when scientists want to determine how and why certain genetic variation is maintained in populations, while other variation is diminishes or is lost over time.
   a. This information is critical for the understanding of the evolutionary process.

b. It also allows us to predict the gene frequencies in future generations.

VI. Genetic Advances in Agriculture and Medicine

1. Plants have been improved in four ways:
   a. More vigorous growth which increases the yields of crops.
   b. Increased resistance, which includes resistance to predators and pests.
   c. A combination of superior traits by creating hybrids of two different strains; even combinations of two different species.
   d. Desirable qualities such as increased protein value, increased amount of limiting amino acids, and reducing the vulnerability to certain weather conditions by making them a smaller size.
2. Animals have also been improved in many ways.
   a. Chickens that grow faster and lay a larger number of eggs.
   b. Artificial insemination is often used with the larger animals to produce prize-winning offspring.
3. Medicine has benefited from the knowledge obtained from studying genetics.
   a. Numerous disorders have been discovered to result from a single mutation or a specific chromosomal abnormality, such as sickle-cell anemia and hemophilia.
   b. Most if not all forms of cancer have a genetic basis.
   c. **Genetic Counseling** provides couples with objective information on which they can base their own decisions concerning childbearing.
   d. **Immunogenetics** has made compatible blood transfusions, and organ transplants possible, as well as aiding in the formation of different immunosuppressive drugs.
   e. **Gene Therapy** is when genetic disorders can be treated by inserting normal copies of genes into the cells of afflicted individuals.
   f. The **Human Genome Project** is a very interesting project occurring at this time.
      - The entire human genome will be sequenced.
      - Scheduled for completion in the year 2003.

Lecture Outline

Chapter Two: Cell Division and Chromosomes

I. Cell Structure

A. **Plasma Membrane**

1. This membrane is not passive
2. **Cell wall** made up mainly of **cellulose**

B. **Cell coat**

1. Glycoproteins and polysaccharides
2. Biochemical identity under genetic control
   a. **AB** and **MN** antigens
   b. **Histocompatibility antigens**
   c. **Receptor molecules**

C. Eukaryotic Cells

1. Membrane bound organelles
   a. Transparency 3 (Page 19, Figure 2-1)
   b. Nucleus
      1. **Chromatin**
      2. **Chromosomes**
   c. **Nucleolus**
   d. **Endoplasmic reticulum (ER)**
      1. Smooth
      2. Rough
   e. **Mitochondria**
      1. Both plant and animal cells
      2. Cell respiration
   f. **Chloroplasts**
      1. Plant cells
      2. **Photosynthesis**
   g. Chloroplasts and mitochondria distinct DNA
      1. Endosymbiont Hypothesis
   h. **Centrioles**

1. **Centrosome**
2. **Basal body**

i. **Cytoplasm**

1. Composition
2. Cytoskeleton

D. Prokaryotic cells

a. Genetic material

b. **Nucleoid region**

II. Homologous Chromosomes, Haploidy, and Diploidy

A. Chromosome Structure

1. **Centromere**
2. Arms of the chromosome

a. Transparency 4 (Page 20, Figure 2-3)

b. Arm ratios

1. **Metacentric**
2. **Submetacentric**
3. **Acrocentric**
4. **Telocentric**

c. **p arm** (petite)

d. **q arm** (next letter in the alphabet)

B. Diploid number

1. Pairs
2. **Homologous chromosomes**

a. Genetic similarities

1. **Loci**
2. **Biparental inheritance**

3. **Sex-determining chromosomes**

C. Human Chromosomes

1. The diploid number for humans is 46
2. **Sister chromatids**

D. **Haploid genome**

1. Transparency 5 (Page 22, Table 2.1)

III. Mitosis and Cell Division

A. **Asexual reproduction**

B. **Zygotes**

C. Cell replacement in certain tissues

1. Epidermal skin cells
2. Reticulocytes
3. Cancer

D. **Cytokinesis**

E. **Karyokinesis**

F. Interphase and the Cell Cycle

1. **Interphase**
   a. **S phase**
   b. Two periods within interphase where DNA is not replicated
      1. Transparency 6 (Page 23, Figure 2-5)
      2. **G1 (gap I)** [Before S] and **G2 (gap II)** [After S]
         - Metabolic activity, cell growth, and cell differentiation
         - Volume doubled
         - **G0 stage** and **G1 checkpoint**
   c. Time constraints
2. **Prophase**
   a. Transparency 7 (Page 25, Figure 2-7)
   b. Centrioles
      1. **Centrosome**
      2. Composition
   c. **Spindle fibers**
   d. Nuclear envelope
3. **Prometaphase** and **Metaphase**
   a. **Metaphase plate**
   b. **Kinetechore**
   c. Arms of chromosomes

4. **Anaphase**
    a. Disjunction
    b. **Daughter chromosome**
    c. At the completion of anaphase, the chromosomes have migrated to the opposite poles of the cell
5. **Telophase**
    a. **Cytokinesis**
    b. Plant and animal cells
        1. **Cell plate** is synthesized and laid down across the region of the metaphase plate
            - **Middle lamella**
            - Primary and Secondary cell wall
        2. **Cell furrow**
        3. End result

III. Cell Cycle Control

A. Mechanisms are under genetic control
    1. Mutations
        a. **cdc mutations** (cell division cycle mutations)
        b. **cdc kinases**, work in conjunction with **cyclins**
            1. **Cdk protein**, for **Cyclin-dependent kinase protein**
            2. There are three major checkpoints
            3. Transparency 8 (Page 27, Figure 2-9)
        c. **G1/S checkpoint**
        d. **G2/M checkpoint**
        e. **M checkpoint**
B. Impaired cell cycle control
C. ***p53* gene's**
    1. **Apoptosis**
    2. **Tumor-suppressor gene**

IV. Meiosis and Sexual Reproduction

A. **Meiosis**

1. **Prophase I**
    a. **Synapse**
    b. **Crossing over**
    c. Five substages
        1. **Leptotene stage**
            - **Chromomeres**
            - **Homology search**
        2. **Zygotene stage**
            - **Rough pairing**
            - **Synaptonemal complex**
            - **Bivalents**
        3. **Pachytene stage**
            - **Synapsis**
            - **Chromatids**
            - **Sister chromatids** and **non-sister chromatids**
            - **Tetrad**
        4. **Diplotene stage**
            - Two pairs of sister chromatids
            - **Chiasma**
        5. **Diakinesis stage**
2. Metaphase I, Anaphase I, and Telophase I
    a. Similar to the stages of mitosis
    b. Metaphase I
    c. Anaphase I
        1. **Disjunction**
        2. **Nondisjunction**
        3. **Segregation of dyads**
    d. **Telophase I**
        1. **Interkinesis**
3. The second meiotic division
    a. **Prophase II**

b. **Metaphase II**

c. **Anaphase II**

d. **Telophase II**

e. **Monad**

B. Spermatogenesis and Oogenesis

1. **Spermatogenesis**

a. Transparency 9 (Page 33, Figure 2-12)

b. **Primary spermatocyte**

c. **Secondary spermatocytes**

d. **Spermatids**

1. **Spermatogenesis**

2. **Spermatozoa (sperm)**

2. **Oogenesis**

a. Cytoplasm

b. **Primary oocyte**

c. **The first polar body**

1. **Secondary oocyte**

2. The first polar body

d. Second meiotic division

1. **Ootid** and **second polar body**

2. **Mature ovum**

e. Non-continuous

V. The significance of meiosis

A. Successful sexual reproduction

1. Plants

2. Fungi

a. Haploid vegetative cells

b. **Sporophyte stage** and **gametophyte stage**

1. Transparency 10 (Page 34, Figure 2-13)

2. "Alternation of generations"

VI. Specialized chromosomes

A. **Polytene chromosomes**

1. E. G. Balbiani
2. Various tissues
3. Nuclei of interphase cells
4. Bands and interbands

B. **Lampbrush chromosomes**

1. 1892
2. **Lateral loops**

Lecture Content

Chapter Two: Cell Division and Chromosomes

I. Cell Structure

A. All cells are surrounded by a **plasma membrane**, which is an outer covering that defines the cell boundary and delimits the cell from its immediate external environment.
   1. This membrane is not passive; instead, it controls materials going into and out of the cell.
   2. Plant cells have, in addition to this membrane, an outer **cell wall**, made up mainly of **cellulose**.

B. Many if not all, animal cells have a covering over the plasma membrane called the **cell coat**.
   1. The cell coat consists of glycoproteins and polysaccharides.
   2. The cell coat provides biochemical identity at the surface of the cells, which are under genetic control.
      a. For example, there are various antigenic determinants such as the **AB** and **MN** antigens found on the surface of red blood cells.
      b. Other cells have **histocompatibility antigens**, which elicit an immune response during tissue and organ transplants.
      c. There are also a variety of **receptor molecules**, which are important components at the surface of these cells.

C. Eukaryotic Cells are different from prokaryotic cells.
   1. Eukaryotic cells have membrane bound organelles.
      a. The nucleus is the organelle that houses the DNA.
         - During nondivisional phases of the cell cycle, the DNA/protein complex is in an uncoiled format and is called **chromatin**.
         - This material, during replication, condenses into structures called **chromosomes**.
         - Transparency 3, page 8, figure 1-5.
      b. Within the nucleus, there is another structure called the **nucleolus**, which is where ribosomal RNA is synthesized.

c. The **endoplasmic reticulum (ER)** compartmentalizes the cytoplasm while greatly increasing the surface area available for biochemical synthesis.
   - The ER may appear smooth, in which case it is the site for synthesis of fatty acids and phospholipids.
   - The ER may also appear rough, in which case it is associated with ribosomes, which serve as a site for the translation of genetic information contained in mRNA into proteins.

d. Another organelle present in the eukaryotic cell is the **mitochondria**.
   - Mitochondria are found in both plant and animal cells.
   - This is the site for oxidative phases of cell respiration, which generates large amounts of adenosine triphosphate (ATP).

e. **Chloroplasts** are also found within the eukaryotic cell.
   - Chloroplasts are found in plant cells.
   - They are associated with **photosynthesis**, which is the major energy-trapping process on earth.

f. Both chloroplasts and mitochondria contain a type of DNA that is distinct from that found in the nucleus.
   - Because of this unique DNA, these organelles can duplicate themselves, transcribe and translate their genetic information whenever they need to.
   - The machinery of these two organelles closely resemble that of prokaryotic cells, therefore it is thought that they were once primitive free-living organisms, which established a symbiotic relationship with primitive eukaryotic cells (Endosymbiont Hypothesis).

g. Animal cells and some plant cells contain structures called **centrioles**.
   - These bodies are contained within a specialized region called the **centrosome**.
   - They are associated with the organization of spindle fibers that function in both mitosis and meiosis.
   - In some organisms, the centriole is derived from the **basal body**, which is associated with the formation of cilia and flagella.

h. The remainder of the cell, excluding the nucleus, is composed of **cytoplasm.**

- Cytoplasm is composed of nonparticulate, colloidal material, which is referred to as cytosol, and surrounds all the cellular organelles.
- There is also an extensive system of tubules and filaments, which comprise the cytoskeleton that provides a lattice of support structures within the cytoplasm.
  - The cytoskeleton consists mainly of tubulin-derived microtubules and actin-derived microfilaments.
  - Together, it maintains the cell shape, facilitates cell mobility, and anchors the various organelles.

2. Prokaryotic cells lack membrane bound organelles.
   a. In bacteria, such as *Escherichia coli*, the genetic material is present as a long looped DNA molecule.
   b. The genetic information is located in an area known as the **nucleoid region**, which constitutes a large are throughout the cell.
   c. The DNA, although compacted, does not go through the extensive coiling process as in the eukaryotic cell.
   d. Transparency 4, page 19, figure 2-1.

II. Homologous Chromosomes, Haploidy, and Diploidy

A. Chromosomes have a very specific structure.
   1. Each chromosome contains a **centromere**, which establishes a general appearance of each chromosome.
   2. The arms of the chromosome extend from each side of the centromere.
      a. Different arm ratios are produced on the chromosome, depending on the placement of the centromere.
         - **Metacentric** is when the centromere is located at the middle of the chromosome.
         - **Submetacentric** is when the centromere is located between the middle and the end, but very close to the center of the chromosome.
         - **Acrocentric** is when the centromere is located close to the end of the chromosome.

- **Telocentric** is when the centromere is located at the end of the chromosome.
- Transparency 5, page 20, figure 2-3.

b. The shorter arm of the chromosome is called the **p arm** (petite).

c. The longer arm of the chromosome is called the **q arm** (next letter in the alphabet).

B. All somatic cells are derived from members of the same species, and contain an identical number of chromosomes, usually the diploid number.

1. Nearly all of the chromosomes exist in pairs.
2. The pairs of chromosomes are called **homologous chromosomes**.
   a. Homologous chromosomes have important genetic similarities.
      - They contain identical gene sites along their lengths, which are called **loci**.
      - One member of each pair of the homologous chromosomes is derived from the maternal parent and one from the paternal parent; therefore, each diploid organism contains two copies of each gene as a consequence of **biparental inheritance**.

   b. There is an important exception to the concept of homologous chromosomes, in many species one pair, the **sex-determining chromosomes**, are often not homologous in size, centromere placement, arm ratio, or genetic content.
      - An example would be in humans, where the male contains one Y chromosome in addition to the one X chromosome; whereas females carry two copies of the X chromosome.
      - Even though they are not exactly homologous, they do act like homologous chromosomes during meiosis.

C. Human chromosomes are very interesting to study.

1. The diploid number for humans is 46.
2. They exhibit a diversity of sizes and centromere placements.
3. In the karyotype, in figure 2-4 in the text, one can see that the chromosomes exhibit a double structure consisting of two parallel **sister chromatids**, genetically identical chromatids that are connected by a centromere.

D. The total set of genes contained on one member of each homologous pair of chromosomes constitutes the **haploid genome**.
   1. Transparency 7, page 23, table 2.1 shows the haploid number of chromosomes for a variety of organisms.
   2. Some organisms spend part of their life in the haploid state and part of their life in the diploid state.

III. Mitosis and Cell Division

A. In some single-celled organisms, such as protozoans, and some fungi and algae, mitosis provides the basis for **asexual reproduction**.

B. Mutlicellular diploid organisms begin life as single-celled fertilized eggs called **zygotes**.

C. In adult organisms, mitotic activity is prominent in wound healing and other forms of cell replacement in certain tissues.
   1. An example would be the epidermal skin cells. In humans they slough off continuously, the mitotic activity is responsible for replacing those cells.
   2. Another example is the continuous production of reticulocytes, which shed their nuclei and replenish the supply of red blood cells in vertebrates.
   3. Cancer results when the somatic cell's mitotic activity becomes uncontrolled.

D. **Cytokinesis** is the process of cytoplasmic division.
   1. When the cytoplasm begins division, it partitions its volume into two parts, and then is followed by the enclosure of both new cells within a distinct plasma membrane.
   2. The cytoplasmic organelles either replicate themselves, with existing membrane structures, or are synthesized *de novo* in each cell.

E. **Karyokinesis**, nuclear division, is more complex than cytokinesis.
   1. The chromosomes must first be exactly replicated and then accurately partitioned into daughter cells.
   2. The end result is the production of two daughter cells each with a chromosome composition identical to the parent cell.

F. Interphase is one of the phases in the cell cycle.
   1. **Interphase** is the interval between each mitotic division.

a. It was once thought that the cell devoted all of its energy to the cell's growth and normal function.
b. Today however, we know that the replication of DNA of each chromosome occurs during interphase.
c. The **S phase** is the period long before the cell enters mitosis when the DNA is synthesized
d. Investigators have determined that there are two periods within interphase where DNA is not replicated.
    - The two periods are called **G1 (gap I)** [Before S] and **G2 (gap II)** [After S].
        - Intensive metabolic activity, cell growth, and cell differentiation occur during these two periods.
        - By the end of G2, the volume of the cell has doubled, DNA has been replicated, and mitosis **(M)** is initiated.
        - Transparency 7, page 23, figure 2-5
        - At a late point in G1, the cell follows one of two paths, it will either withdraw from the cycle, which is called the **G0 stage**, or the cell will commit to initiate DNA synthesis and complete the cycle; this is called the **G1 checkpoint**.
        - Cells in the G0 stage, remain viable, but do not proliferate.
        - Cancer cells apparently avoid entering the G0 stage.
e. There are time constraints, which are known to occur within cells.
    - The actual process of mitosis occupies a small part of the overall cycle, only about an hour.
    - Most of the variation is seen in the amount of time spent in the G1 phase.

2. Prophase is the next phase within the cell cycle.
    a. Transparency 8, page 25, figure 2-7.
    b. Over half of mitosis is spent in **prophase**, a stage that is characterized by many events.
    c. At the beginning of prophase, in all animal cells, the two pairs of centrioles migrate to opposite ends of the cell.

- These structures are located just outside the nuclear envelope in an area of differentiated cytoplasm called the **centrosome**.
- It is thought that each pair of centrioles consists of one mature unit and another small, newly formed centriole.

d. Following their migration, the centrioles are responsible for the organization of cytoplasmic microtubules into a series of **spindle fibers** that run between the poles of the cell.
   - Cells of most plants, fungi, and certain algae seem to lack centrioles.
   - However, spindle fibers are present during replication.

e. As the centrioles migrate, the nuclear envelope begins to break down and gradually disappears; the nucleolus also disintegrates.

f. During this time, the chromatin begins to condense, continuing until distinct threadlike structures (chromosomes) are apparent.

g. Spindle fibers also form between the centrioles at this time as well.

3. Prometaphase and Metaphase are the following steps in the cell cycle.
   a. Metaphase, in some descriptions, is applied strictly to the chromosome configuration following migration.
   b. The equatorial plane, also known as the **metaphase plate**, is the midline region of the cell, it lies perpendicular to the axis established by the spindle fibers.
   c. The migration of the chromosomes is made possible by the binding f the spindle fibers to a structure associated with the centromere called the **kinetechore**.
      - The kinetechore consists of mutilayered plates, which form on opposite sides of each centromere.
      - Once the fibers are attached to the kinetechore, the sister chromatids are ready to be pulled to the opposite poles during the next stage.
   d. When metaphase is complete, each centromere is aligned at the plate with the arms extending outward in a random array.
4. Anaphase is the next step in mitosis.

a. During **anaphase**, sister chromatids of each chromosome separate from each other and migrate to opposite ends of the cell.
b. For complete disjunction to occur, each centromeric region must be divided into two.
c. Then, each chromatid is referred to as a **daughter chromosome**.
d. At the completion of anaphase, the chromosomes have migrated to the opposite poles of the cell.

5. **Telophase** is the final stage of mitosis.
   a. **Cytokinesis** is the most significant event in telophase, it is the division of cytoplasm.
   b. The mechanisms employed during telophase are very different in plant and animal cells.
      - In plant cells, a **cell plate** is synthesized and laid down across the region of the metaphase plate.
        - This cell plate becomes the **middle lamella**.
        - The primary and secondary layers of the cell wall are then deposited between the cell membrane and the middle lamella on both sides of the boundary between the two resulting daughter cells.
      - Animal cells have to undergo a constriction of the cytoplasm.
        - The complete constriction of the cell membrane produces the **cell furrow**.
        - The cell furrow is characteristic of newly divided cells.
      - The end result however is the same; two distinct cells are formed.
   c. In each new cell, the chromosomes begin to uncoil and become chromatin again, the nuclear envelope re-forms, and the nucleolus gradually re-forms.

III. Cell Cycle Control

A. The mechanisms, which exist to regulate cell cycle control, are under genetic control.
   1. This has been demonstrated by the discovery of mutations that disrupt this control.

a. This was first discovered in yeast, but now are evident in all organisms; these mutations were originally designated as **cdc mutations** (cell division cycle mutations).

b. The products of these genes are enzymes, which are called **cdc kinases**, which serve as "master control" molecules that work in conjunction with **cyclins**.

- When a *cdc* kinase works in conjunction with a cyclin, it is called a **Cdk protein**, for **Cyclin-dependent kinase protein**.
- Transparency 9, page 27, figure 2-9.

c. The studies of these mutations have established that at least three major checkpoints exist.

- The first of these checkpoints is the **G1/S checkpoint**; it monitors the size that the cell has achieved since the previous mitosis, as well as whether or not the DNA has been damaged.
  - If the cell is too small, or if the DNA is damaged, the cycle is arrested until these conditions are corrected.
  - If the conditions are both "normal," then the checkpoint is traversed and the cell proceeds to the S phase of the cycle.
- The second checkpoint is the **G2/M checkpoint**; it monitors the physiological conditions in the cell, and if DNA replication, or DNA repair is not complete, it will not allow the cell to enter mitosis.
- The final checkpoint is the **M checkpoint**; it monitors the formation of the spindle fiber system and the attachment of the spindle fibers to the kinetechore; if the fibers are not formed, or the attachment is inadequate, the cell is arrested in mitosis.

B. There are times, when the cell cycle control becomes impaired.

1. If a cell is allowed to proceed through the cycle and it has incurred damage to the DNA, it may begin a series of uncontrolled cell divisions.
2. This is the definition of a cancerous cell.

C. The ***p53* gene's** protein product functions during the regulation of **apoptosis**, programmed cell death.

1. When a normal *p53 gene* product is available, a proliferative cell that has incurred severe damage to the DNA will be targeted for cell death at the G1/M checkpoint.
2. However, if the gene has a mutation and cannot force the cell into apoptosis, the cell could then begin to divide in an uncontrolled manner. (CANCER)
3. Since there have been many cancers attributed to the mutation of this gene, the *p53 gene* is also known as a **tumor-suppressor gene**.

IV. Meiosis and Sexual Reproduction

A. **Meiosis** is the process where diploid cells give rise to haploid gametes or spores.
   1. The first meiotic division is **Prophase I**.
      a. First, the homologous chromosomes find each other and **synapse**, that is they pair together.
      b. An exchange process also occurs in prophase I, called **crossing over**, this is where homologous pairs of chromosomes exchange genetic information.
      c. Prophase I is divided into five substages:
         - The **Leptotene stage** is when the interphase chromatin material begins to condense, and the chromosomes become visible.
            - Along each chromosome, there are **chromomeres**, which are localized condensations that resemble beads on a string.
            - Recent research suggests that these are used during the process of **homology search**, where the chromosome pairs try to find their homologous pair.
         - The next substage is the **Zygotene stage**, and this is where the alignment of the homologous pairs occurs.
            - The initial alignment is known as **rough pairing** and it is complete by the end of this substage.
            - As the meiosis proceeds, a much more extensive ultrastructural component, the **synaptonemal complex**, begins to form between the homologs.
            - At the end of the zygotene stage, the paired homologs represent structures referred to as **bivalents**.

- The third substage is the **Pachytene stage**, within the transition from the zygotene and pachytene stages, the chromosomes continue to coil and shorten.
  - The synaptonemal complex becomes further developed, resulting in a more intimate pairing termed **synapsis**.
  - Within the pachytene stage, it becomes evident that each homolog is a double structure.
  - Each bivalent contains four members called **chromatids**.
  - Like in mitosis, replicates are called **sister chromatids**, whereas chromatids from maternal versus paternal members of homologous pairs are called **nonsister chromatids**.
  - The four-member structure, which is now apparent, is called a **tetrad**.
- The fourth substage is called the **Diplotene stage**.
  - In this stage, it is more apparent that each tetrad consists of two pairs of sister chromatids, which now begin to separate.
  - There are still areas that remain in contact with each other however, and these are called a **chiasma**.
- The final substage is called the **Diakinesis stage**.
  - The chromosomes pull farther apart, but the nonsister chromatids remain loosely associated via the chiasmata.
  - As this stage proceeds, the chiasmata move towards the end of the tetrad in a process called **terminalization**.
  - The nucleolus and the nuclear envelope bread down, and the two centromeres of each tetrad become attached to the recently formed spindle fibers at the end of this stage.

2. Metaphase I, Anaphase I, and Telophase I are the following phases in meiosis.
   a. The first stages of meiosis, following prophase I, are very similar to the stages of mitosis.
   b. During metaphase of the first division, the chromosomes have maximally shortened and thickened, the terminal chiasmata are visible, and each tetrad

interacts with spindle fibers, facilitating the movement towards the metaphase plate.

c. During anaphase of the first division, one-half of each tetrad is pulled toward each pole, this separation process is called **disjunction**.
   - Occasionally, there are errors in this process, and separation is not achieved, this is called **nondisjunction**.
   - At the completion of the normal anaphase I, a series of dyads equal to the haploid number is present at each pole.
   - The alignment of each tetrad prior to the first anaphase is random, consequently one is pulled to one pole and the other is pulled to the other pole, in a random fashion. This random **segregation of dyads** is the basis for the Mendelian principle of **independent assortment**.

d. Many organisms have a **Telophase I** stage, where a nuclear envelope forms around the dyads, then the nucleus enters into a short interphase sometimes called **interkinesis**.
   - In other cases, the cells go directly into the second prophase.
   - If an interphase period occurs, the chromosomes do not replicate because they already consist of two chromatids.

3. The second meiotic division occurs following the completion of the first meiotic division.
   a. During **prophase II**, each of the dyads are composed of one pair of sister chromatids.
   b. During **metaphase II**, the centromeres are directed to the equatorial plane.
   c. **Anaphase II** is when the centromeres divide, and the sister chromatids are pulled to opposite poles.
   d. Finally, during **telophase II** one member of each pair of homologous chromosomes are present at each pole.
   e. It is at this time that each chromosome is referred to as a **monad**.
   f. Following cytokinesis in telophase II, four haploid gametes may result from a single meiotic event.

B. Spermatogenesis and Oogenesis are specific names for meiosis, which occur in the male and female gametes.

1. **Spermatogenesis**, the production of male gametes, takes place in the testes of the male reproductive organs.
   a. Transparency 10, page 33, figure 2-12.
   b. An undifferentiated diploid germ cell called a spermatogonium, enlarges and becomes a **primary spermatocyte**, which undergoes the first meiotic division.
   c. The products from the first meiotic division are called **secondary spermatocytes**, which contain the haploid number of dyads, and will undergo the second meiotic division.
   d. The products of the second meiotic division are known as **spermatids**, which go through a series of developmental stages.
      - The developmental stages are known as **spermatogenesis**.
      - The products are then highly specialized and are known as **spermatozoa** or **sperm**.
      - All sperm cells receive equal amounts of genetic material and cytoplasm.
2. **Oogenesis**, the formation of **ova** or eggs, occurs in the ovaries of the female reproductive system.
   a. During oogenesis, the daughter cells all receive equal amounts of genetic material, but they do not receive equal amounts of cytoplasm.
   b. During each division, the cytoplasm of the **primary oocyte**, derived from the **oogonium**, is concentrated in one of the two daughter cells. It is important for this to occur because the function of the mature ovum is to nourish the developing embryo.
   c. During the first meiotic anaphase, the tetrads of the primary oocyte separate, and the dyads move towards opposite poles.
   d. During the first telophase, the dyads present at one pole are pinched off with very little surrounding cytoplasm, which forms **the first polar body**.
      - The other daughter cell that is formed, receives almost all of the cytoplasm, and is termed the **secondary oocyte**.

- The first polar body may or may not divide again to produce two small haploid cells.

e. During the second meiotic division, the mature ovum is produced from the secondary oocyte.
   - In this division, the cytoplasm from the secondary oocyte again divides unequally and forms an **ootid** and a **second polar body**.
   - The ootid differentiates and becomes the **mature ovum**.

f. Unlike that of spermatogenesis, the divisions of oogenesis may not be continuous in some animals.
   - For example, in humans, the first meiotic division occurs in the embryonic ovary, but is arrested in prophase I.
   - It is not until many years later that the process resumes in each oocyte prior to ovulation.
   - The second division is completed after fertilization, if it occurs.

V. The significance of meiosis

A. Meiosis is critical to the successful sexual reproduction of all diploid organisms.
   1. In animals, meiosis leads to haploid gametes, while in plants, meiosis leads to haploid spores.
   2. Meiosis plays an important role in the life cycle of fungi and plants.
      a. In many fungi, the predominant stage of the life cycle is spent in the form of haploid vegetative cells.
      b. In multicellular plants, the life cycle alternates between the diploid **sporophyte stage** and the haploid **gametophyte stage**.
         - Transparency 11, page 34, figure 2-13.
         - This switching between stages is called "alternation of generations."
         - The process of meiosis and fertilization constitute the "bridge" between the sporophyte and gametophyte generations.

VI. Specialized chromosomes

A. **Polytene chromosomes** are giant chromosomes, up to 200-600μm in length.
   1. E. G. Balbiani first discovered these chromosomes in 1881.

2. They are found in various tissues including salivary glands, midgut, and malpighian excretory tubules.
3. They can be seen in the nuclei of interphase cells because they are so large.
4. The polytene chromosomes consist of many bands and interbands.
5. Thanks to the electron microscope, scientists were able to discover that these chromosomes do not separate during a mitotic division, which has led them to be so large.

B. **Lampbrush chromosomes** are specialized meiotic chromosomes.
   1. They were first discovered in the oocytes of sharks, in the year 1892.
   2. Instead of condensing, lampbrush chromosomes remain extended up to 500-800μm in a developing cell.
   3. It is not until meiosis that they revert to their normal length of 15-20μm.
   4. The chromosome has a very distinctive appearance due to its **lateral loops** from each chromomere.
      a. Each of these loops is thought to be composed of one DNA double helix, while the central axis of the chromosome is thought to be made up of two DNA helices.
      b. This is consistent with the belief that each chromosome is made up of a pair of sister chromatids.

Lecture Outline

Chapter Nine: DNA-The Physical Basis of Life

I. DNA

A. **Genetic information**

B. Prior to 1944

C. In 1944

D. James Watson and Francis Crick

II. Characteristics of the Genetic Material

A. **Replication**

B. **Storage**

C. **Expression**

1. Transparency 25, Page 186, Figure 9-1.
2. **Transcription**
3. **Translation**
4. **Central dogma of molecular genetics**

D. **Variability**

1. Mutation
2. Alteration reflected during transcription and translation
3. Gametes
4. Evolution.

III. The Genetic Material: Early Studies

A. Friedrick Meischer

1. **Nuclein**
2. Phoebus A. Levene
   a. **Nucleotides**
   b. **Tetranucleotide hypothesis**
3. Erwin Chargaff

IV. Evidence Favoring DNA in Bacteria and Bacteriophages

A. Oswald Avery, Colin MacLeod, and Maclyn McCarty

B. **Bacteriophages**

C. Transformation studies

1. Frederick Griffith
   a. **virulent strains** and **avirulent strains**
   b. Polysaccharide capsule
   c. Nonencapsulated bacteria
   d. Encapsulated bacteria
   e. Physical appearance
      - **Smooth**
      - **Rough**
      - Transparency 26, Page 188, Figure 9-2.
   f. **Serotypes**
   g. Griffith's experiment
   h. **Transformation**
   i. Henry Dawson and coworkers
   j. Lionel H. Alloway
   k. Avery, MacLeod, and McCarty
   l. **Protease** and **ribonuclease**
      - Destruction of protein and RNA
      - Transformation.
      - **Deoxyribonuclease**

D. Alfred Hershey and Martha Chase
   1. **Phage**
   2. Structure
   3. Transparency 28, Page 190, Figure 9-4
   4. Facts from existing data
   5. Radioisotopes
      a. Transparency 29, Page 191, Figure 9-5
      b. ^{32}P and ^{35}S
      c. Adsorption complex
      d. Results

E. **Lysozyme**
   1. "Naked" cells

2. John Spizizen and Dean Fraser
3. DNA purified from bacteriophages
    a. **Transfection**
    b. Strengthened idea of DNA being the storage unit

V. Observations Favoring DNA in Eukaryotes

A. Location of genetic material
B. Mitochondria and chloroplasts
C. Location of DNA and protein
D. Mutagenesis
    1. **Ultraviolet radiation (UV)**
        a. Bacteria and yeast
        b. **Action spectrum**
        c. **Absorption spectrum**
        d. Mutagenic wavelengths
        e. 260 nanometers

VI. Direct Evidence Favoring DNA in Eukaryotes

A. **Recombinant DNA technology**
    1. Isolated and spliced
    2. Insertion
    3. Present and functional
        a. Example
        b. Bacterium divides
    4. Beatrice Mintz
        a. Human β-globin gene
        b. **Transgenic organisms**

VII. RNA as the Genetic Material

A. Viruses
B. Genetic material
C. Norman Pace and Sol Spiegelman
    1. **RNA replicase**
    2. Transfection

D. **Retroviruses**

1. RNA
2. **Reverse transcriptase**

VIII. The Structure of DNA

A. 1953

B. Paper

C. Data

1. Base composition
2. X-ray diffraction

D. Nucleic acid chemistry

1. Nucleotides
2. Mononucleotides
   a. **Nitrogenous base**
   b. **Pentose sugar**
   c. **Phosphate group**
3. **Purines and pyrimidines**.
   a. **Adenine (A)** and **guanine (G)**
   b. **Cytosine (C), thymine (T), and uracil (U)**
   c. Transparency 30, Page 194, Figure 9-7
4. Pentose sugars
   a. **Ribose**
   b. **Deoxyribose**
5. **Nucleoside**
6. **Nucleotide**
7. Bonding
   a. C-1' atom
      1. Purine
      2. Pyrimidine
   b. Nucleotide
8. Nucleotides
   a. **Nucleoside monophosphate (NMP)**

b. **Nucleoside diphosphates (NDP)** and **triphosphates (NTP)**

1. Triphosphate
2. **Adenosine triphosphate (ATP)** and **guanosine triphosphate (GTP)**
3. Hydrolysis of ATP and GTP

c. **Phosphodiester bond**

1. C-3' and C-5' end
2. **Oligonucleotides** and **polynucleotides**.

E. Separating the four bases

1. Amounts
2. Conclusions
3. Refuted Levene's tetranucleotide hypothesis

F. **X-ray diffraction analysis**

1. DNA bombarded with X-rays
2. William Astbury
3. Rosalind Franklin
   a. 3.4 Angstroms
   b. Helix

G. 1953

1. Transparency 31, Page 199, Figure 9-12
2. DNA structure
   a. Two polynucleotide chains
   b. Antiparallel
   c. "Stacked"
   d. **Hydrogen bonds**.
   e. 34 angstroms
   f. **Major grooves** and **minor grooves**
   g. Diameter
3. 5'-3' and 3'-5'
4. **Complementarity**
   a. Hydrogen bonding
   b. Weak electrostatic attraction

c. Stability to helix

5. Arrangement

a. **Hydrophobic**

b. **Hydrophilic**

6. DNA replication

a. **Semiconservative**

b. Mutation

c. Nobel

VIII. Other forms of DNA

A. Transparency 32, Page 202, Figure 9-14

B. **A-DNA** and **B-DNA**

1. B-form

2. Aqueous, low-salt

C. **Single-crystal X-ray analysis**

D. A-DNA

1. More compact

2. Right-handed helix

3. Bases

E. Z-form

1. Left-handed helix

2. Diameter

3. Zigzag-conformation

4. Function

IX. The Structure of RNA

A. Ribonucleic acid

B. Structure

1. Nucleotides

2. Uracil

3. Single-stranded

C. Three forms of RNA

1. Complementary copies of DNA

2. Characterization of RNA
    a. **Svedberg coefficient (*S*)**
    b. High *S* value
3. Ribosomal RNA
    a. 80 percent
    b. Ribosomes
    c. Translation
4. Messenger RNAs
    a. Length
    b. **Primary transcripts**
5. Transfer RNA
    a. Carry amino acids
    b. Small size
6. Other forms
    a. **Small nuclear RNA (snRNA)**
    b. **Telomerase RNA**
    c. **Antisense RNA**

X. Hydrogen Bonds and the Analysis of Nucleic Acids

A. Denaturing
    1. **Annealing**
    2. Relatedness
B. Viscosity and UV absorption
C. Graph from data
    1. **Melting temperature (T_m)**
    2. High T_m
D. **Molecular hybridization**
    1. DNA and RNA
    2. Example
E. ***In situ* molecular hybridization**
    1. Fixation
    2. Single-stranded DNA or RNA

3. Radioactive or fluorescent label
4. **Fluorescent *in situ* hybridization**
   a. "Probe", transparency 33, Page 204, Figure 9-16
   b. Centromere regions

F. **Reassociation kinetics**

1. Fragmented
2. Heated
3. Reassociate
4. **C_ot**
5. C_o
6. **Half reaction time**
7. Britten and Kohne
   a. Results
   b. Faster
   c. **Repetitive DNA**

Lecture Content

Chapter Nine: DNA-The Physical Basis of Life

I. DNA

A. Genetic information influences the form and characteristics of the offspring.

B. Until 1944, it was not clear what chemical component made up genes on the chromosome and what constituted the genetic material.

C. In 1944, there emerged direct experimental evidence that the nucleic acid DNA serves as the informational basis for the process of heredity.

D. In 1953, James Watson and Francis Crick set forth their hypothesis for the double-helical nature of DNA.

II. Characteristics of the Genetic Material

A. **Replication** of the genetic material is one facet of cell division, an important function for all living organisms.

B. **Storage** can be viewed as a repository of genetic information present in cells of an organism, which may or may not be expressed.

1. At any point in time, an organism can express only part of its genetic potential.
2. For example, bacteria turn on certain genes in response to specific environmental conditions, and turn them off again when that condition is changed.

C. **Expression** of the stored genetic material is a complex process and is the basis for the concept of **information flow** within the cell.

1. Transparency 25, Page 186, Figure 9-1.
2. The initial step in this process is **transcription** of the DNA, which results in three different types of RNA, **mRNA (messenger RNA)**, **tRNA (transfer RNA)**, and **rRNA (ribosomal RNA)**.
   a. Of the three RNAs, mRNA is the one that is translated into a specific protein.
   b. Each mRNA is the product of a specific gene and leads to the synthesis of a different protein.
3. The following step is called **translation**, and it occurs in conjunction with ribosomes and involves tRNA.
   a. The tRNA adapts the chemical information into the specified amino acids.
   b. These amino acids together make up a protein.

4. The processes all together are called the **central dogma of molecular genetics**: "DNA, which makes RNA, which makes proteins."

D. The genetic material is also the source of **variability** among organisms through the process of mutation.
   1. Mutation is a change in the chemical composition of DNA.
   2. A mutation causes an alteration that will be reflected during transcription and translation, affecting the specified protein.
   3. If the mutation is present in the gametes, then it will be passed on to future generations.
   4. Genetic variation provides the raw material for the process of evolution.

III. The Genetic Material: Early Studies

A. DNA was first studied in 1868 by a Swiss chemist named Friedrick Meischer.
   1. He was able to isolate cell nucleuses and derive an acid substance containing DNA that he named **nuclein**.
   2. Phoebus A. Levene's observations in 1910, concluded that there were approximately equal amounts of four similar molecules called **nucleotides** within DNA.
      a. Levene postulated that these molecules were repeated over and over.
      b. This was the basis for his **tetranucleotide hypothesis** for the structure of DNA.
   3. In 1940, Erwin Chargaff showed that Levene's proposal was incorrect, by demonstrating that most organisms do not contain equal amounts of the four nucleotides.

IV. Evidence Favoring DNA in Bacteria and Bacteriophages

A. In 1944, Oswald Avery, Colin MacLeod, and Maclyn McCarty published a paper concerning the chemical identity of a "transforming principle" in bacteria, which lead to acceptance of DNA as the genetic material.

B. The initial studies were performed on bacteria and the viruses that infect them, called **bacteriophages**.
   1. The bacteriophages perform rapid growth because they complete life cycles in hours.

2. They are also very easily manipulated, and mutations may easily be induced and selected for.

C. Transformation studies were used by Avery, MacLeod, and McCarty.

1. Frederick Griffith, a medical officer in the British Ministry of Health, studied *Diplococcus pneumoniae*, now called *Streptococcus pneumoniae*, in 1927.

a. He used different strains of this bacterium, some were **virulent strains**, that is they caused pneumonia in certain vertebrates, and others were **avirulent strains**, that is they did not cause illness.

b. Virulent strains have a polysaccharide capsule, while avirulent strains do not.

c. The nonencapsulated bacteria are easily engulfed and destroyed by phagocytic cells within the host's circulatory system.

d. The bacteria with the polysaccharide capsule are not as easily engulfed, and they are able to multiply and cause pneumonia.

e. The presence or absence of this capsule can be differentiated by physical appearance.

- Encapsulated bacteria form a **smooth**, shiny surfaced colony (**S**).
- The nonencapsulated bacteria form **rough** colonies (**R**).
- Transparency 26, Page 188, Figure 9-2.

f. There are dozens of different **serotypes** within this group of bacteria.

- The serotypes are due to the detailed chemical structure of the polysaccharide capsule.
- They can be identified by using immunological techniques, and are usually denoted with Roman numerals.
- Griffith used the avirulent type II*R*, and the virulent type III*S*.

g. Griffith had some knowledge of these strains of bacterium from work done by other people.

- He knew that only living virulent cells would produce pneumonia in mice, and heat-killed avirulent cells would not produce pneumonia when injected into mice.
- Griffith's critical experiment was when he injected mice with living avirulent cells II*R* and heat-killed virulent cells III*S*.

- He expected that death would not occur because he gave a double dose of "nothing."
- He was amazed when after just five days all the mice that had received the double injection were dead.
- When he analyzed blood samples from the dead mice, he found living III*S* bacteria in them!

h. Griffith concluded that the heat-killed III*S* bacteria were responsible for converting live avirulent II*R* cells into virulent III*S* cells, he called this phenomenon **transformation**.

i. By 1931, Henry Dawson and his coworkers showed that transformation could occur *in vitro*; that is injection into mice was not necessary for it to occur.

j. By 1933, Lionel H. Alloway had refined the *in vitro* experiments by using extracts from *S* cells added to *R* cells.

k. Then, in 1944, Avery, MacLeod, and McCarty published their results in the field of molecular genetics.

- They reported to have obtained the transforming principle in a highly purified state, and that beyond reasonable doubt is was DNA (Transparency 27, Page 189, Figure 9-3).
- They began their isolation procedure with large quantities of type III*S* virulent cells.
- They then centrifuged, collected, and heat-killed the cells.
- The result was a soluble filtrate that retained the ability to induce transformation of type II*R* cells.

l. The soluble filtrate was later treated with **protease**, a protein-digesting enzyme, and an RNA-digesting enzyme called **ribonuclease**.

- This treatment caused the destruction of all the activity of any remaining protein or RNA, yet transformation still occurred.
- The conclusion was then drawn that neither protein nor RNA was responsible for transformation.

- The final concluding factor was when a DNA-digesting enzyme, **deoxyribonuclease** was used, and the transformation activity was destroyed.

D. In 1952, Alfred Hershey and Martha Chase published a paper describing the results of experiments, now known as the Hershey-Chase experiment, designed to clarify the events leading to phage reproduction.
   1. This bacteriophage, or just **phage**, has as its host *Escherichia coli*, and consists of a protein coat surrounding a core of DNA.
   2. The phage has a hexagonal head and a tail.
   3. Transparency 28, Page 190, Figure 9-4.
   4. Hershey and Chase knew a few facts from existing data, such as:
      a. T2 phages consist of approximately 50 percent protein and 50 percent DNA.
      b. Infection is initiated by adsorption of the phage by its tail fibers to the bacterial cell.
      c. The production of new viruses occurs within the bacterial cell.
   5. Hershey and Chase used different radioisotopes to follow the molecular components of phages during infection.
      a. Transparency 29, Page 191, Figure 9-5.
      b. They used the ^{32}P and ^{35}S, which are forms of radioactive phosphorus and sulfur.
      c. Since DNA contains phosphorus and not sulfur, while protein contains sulfur and not phosphorus, these radioactive isotopes are good.
      d. When the labeled phage and unlabeled bacteria are mixed, an adsorption complex is formed as the phages attach their tail fibers to the bacterial cell.
         - These complexes were isolated and then put into a blender to remove the attached phages.
         - Hershey and Chase traced the radioisotopes and were able to demonstrate that most of the ^{32}P-labeled DNA had been transferred into the bacterial cell following the act of adsorption.
         - Most of the ^{35}S-labeled protein remained outside the bacterial cell, but was recovered from the phage "ghosts" (empty phage coats).

- After the separation, the bacterial cells were eventually lysed as new phages were produced.

e. Hershey and Chase interpreted these results as indicating that the protein coat of the phage remains outside the bacteria and is not involved in the production of new phages, whereas the DNA enters the host cell, and directs phage multiplication.

E. In 1957, several reports demonstrated that if *E. coli* were treated with the enzyme **lysozyme**, the outer wall of the cell could be removed without destroying the bacterium.

1. These cells are "naked" so to speak, with only the cell membrane as the outer boundary.
2. John Spizizen and Dean Fraser reported (independently) that they were able to initiate phage multiplication with disrupted T2 particles using protoplasts.
3. In 1960, similar reports were made using the DNA purified from bacteriophages.
   a. This information showed that the process of infection by only the viral nucleic acid, called **transfection**, that phage DNA contains all the necessary information for production of mature viruses.
   b. These reports strengthened the idea of DNA being the storage unit for genetic information again.

V. Observations Favoring DNA in Eukaryotes

A. It was always thought that the genetic material should be found where it functions, in the nucleus as part of the chromosomes.

B. Both mitochondria and chloroplasts are known to perform genetic functions, and DNA is also present in these organelles.

C. DNA is found only where primary genetic function is known to occur, while protein is found everywhere in the cell.

D. This information again provided strength to the argument that DNA was the genetic material.

E. Mutagenesis is the process of mutation.

1. **Ultraviolet radiation (UV)** is one number of agents capable of inducing mutations in the genetic material.

a. Bacteria and yeast can be bombarded with UV radiation and the rate of mutations can be recorded.
b. When the data are plotted, an **action spectrum** of UV radiation as a mutagenic agent is obtained.
c. This action spectrum may be compared with the **absorption spectrum** of any molecule suspected to be the genetic material
d. The molecule that is serving as the genetic material is expected to absorb at the wavelengths found to be mutagenic.
e. UV light is most mutagenic at the wavelength of 260 nanometers, and both DNA and RNA absorb it most strongly at this wavelength.
    - Protein, on the other hand, absorbs most strongly at 280 nm, yet no significant mutagenic effects are observed at this wavelength.
    - This evidence supports the idea that a nucleic acid is the genetic material and tends to exclude protein.

VI. Direct Evidence Favoring DNA in Eukaryotes

A. Utilizing **recombinant DNA technology** provides the strongest evidence that DNA is definitely the genetic material.
    1. In this procedure, segments of eukaryotic DNA corresponding to specific genes are isolated and literally spliced into bacterial DNA.
    2. After insertion into the bacterial cell the genetic expression is monitored.
    3. The presence of the corresponding eukaryotic protein product demonstrates directly that this DNA is present and functional in the bacterial cell.
        a. For example, human genes that specify for the protein that produces insulin have been isolated and injected into bacteria.
        b. As the bacterium divides, the eukaryotic DNA is replicated along with the host DNA and is distributed into the daughter cells, synthesizing the corresponding proteins.
    4. Beatrice Mintz's work has also strengthened this argument.
        a. This research has demonstrated that DNA encoding the human β-globin gene, when injected into a fertilized mouse egg, is later present and expressed in the adult mouse tissue.

b. The protein is then transmitted to that mouse's progeny as well, these mice are known as **transgenic organisms**.

VII. RNA as the Genetic Material

A. There are some viruses that contain an RNA core instead of a DNA core.

B. It appears that RNA must serve as the genetic material in these viruses.

C. In 1965 and 1966, Norman Pace and Sol Spiegelman demonstrated that RNA from the phage Qb could be isolated and replicated *in vitro*.

1. The replication could only proceed in the presence of the enzyme **RNA replicase**, which was isolated from *E. coli* cells.
2. The RNA replicated *in vitro*, and was added to *E. coli* protoplasts, infection and viral multiplication (transfection) occurred.
3. Therefore, RNA synthesized in a test tube can serve as the genetic material in these phages by directing the production of all necessary components for viral replication.

D. **Retroviruses** are another RNA-containing virus, which replicates in an unusual way.

1. Their RNA serves as a template for producing a complementary strand of DNA.
2. They do this by using the enzyme **reverse transcriptase**, in a process called **reverse transcription**.
3. Once the DNA strand is present, transcription yields retroviral RNA chromosomes if the DNA is expressed.
4. Retroviruses include the Human Immunodeficiency Virus (HIV) which causes AIDS.

VIII. The Structure of DNA

A. In 1953, James Watson and Francis Crick proposed that the structure of DNA is in the form of a double helix.

B. They published a short paper in the journal *Nature*.

C. The data that were most crucial to Watson and Crick's discovery came in two forms:

1. Base composition analysis of hydrolyzed samples of DNA.
2. X-ray diffraction studies of DNA.

D. Nucleic acid chemistry was well known to Watson and Crick, and aided them in their building of the DNA molecule.

1. DNA is nucleic acid, and nucleotides are the building blocks of all nucleic acid molecules.
2. Sometimes they are called mononucleotides, and they always consist of three essential parts:
   a. A **nitrogenous base**.
   b. A **pentose sugar** (a five-carbon sugar).
   c. A **phosphate group**.
3. There are two types of nitrogenous bases: **purines and pyrimidines**.
   a. There are two types of purines: **adenine (A)** and **guanine (G)**.
   b. There are three types of pyrimidines: **cytosine (C), thymine (T), and uracil (U)**.
   c. Both DNA and RNA contain A, C, and G; only DNA contains T, while only RNA contains U.
   d. Transparency 30, Page 194, Figure 9-7.
4. The pentose sugars found in nucleic acids give them their names:
   a. **Ribonucleic acids (RNA)** contain **ribose**.
   b. **Deoxyribonucleic acids (DNA)** contain **deoxyribose**.
   c. The presence of a hydroxyl group at the C-2' position thus distinguishes RNA from DNA.
5. A **nucleoside** is a molecule composed of a purine or pyrimidine base and a ribose or deoxyribose sugar.
6. A nucleoside with a phosphate group attached to it is called a **nucleotide**.
7. The bonding between components of a nucleotide is highly specific.
   a. The C-1' atom of the sugar is involved with the chemical linkage to the nitrogenous base.
      - If the base is a purine, the N-9 atom is covalently bonded to the sugar.
      - If the base is a pyrimidine, the N-1 atom is covalently bonded to the sugar.

b. In a nucleotide, the phosphate group may be bonded to the C-2', C-3', or the C-5' atom of the sugar.

8. There are many other ways to describe nucleotides as well.
   a. One way is **nucleoside monophosphate (NMP)**.
   b. The addition of one or two phosphate groups results in **nucleoside diphosphates (NDP)** and **triphosphates (NTP)**.
      - The triphosphate serves as the precursor molecule during nucleic acid synthesis within the cell.
      - **Adenosine triphosphate (ATP)** and **guanosine triphosphate (GTP)** are important in the bioenergetics of cells because of the large amount of energy that is involved in the addition or removal of the terminal phosphate group.
      - The hydrolysis of ATP and GTP to ADP and GDP and inorganic phosphate (P_i) is accompanied by a large release of energy, which can drive other reactions in the cell.
   c. A **phosphodiester bond** is formed between two mononucleotides because phosphoric acid has been joined to two alcohols by an ester linkage on both sides.
      - Each of these bonds has a C-3' and C-5' end.
      - Short chains of fewer than 20 nucleotides are called **oligonucleotides**, while longer chains are referred to as **polynucleotides**.
      - These long polynucleotides explain the most important property of DNA, storage of vast quantities of genetic information.

E. Between the years 1949 and 1953, Erwin Chargaff and his colleagues used chromatographic methods to separate the four bases in DNA samples from various organisms.
   1. They also determined the amounts of the four nitrogenous bases from each source.
   2. Chargaff and colleagues made some important conclusions:

a. The amount of adenine residues is proportional to the amount of thymine residues in DNA, while the amount of guanine residues is proportional to the amount of cytosine residues.
b. The sum of the purines equals the sum of the pyrimidines.
c. The percentage of G + C does not always equal the percentage of A + T.

3. These conclusions were very important in Watson and Crick's discovery of the structure of DNA, and it refuted Levene's tetranucleotide hypothesis, which stated that the four bases were present in equal amounts.

F. **X-ray diffraction analysis** had been used previously on the structure of DNA.
1. This is where DNA is bombarded with X-rays, and the rays are scattered due to the molecule's atomic structure.
2. X-ray diffraction had been used as early as 1938, but by 1947 William Astbury had detected a periodicity within the structure of the molecule of 3.4 Angstroms.
3. Between 1950 and 1953, Rosalind Franklin, obtained better quality X-ray data from more purified DNA samples.
a. This data confirmed the 3.4 angstroms, that Astbury had previously suggested.
b. Her data also suggested the structure was some sort of helix.
c. She is often included with Watson and Crick for the discovery of the DNA structure, but she did not win the Nobel Prize.
d. Although she did much of the X-ray work that led to the discovery of the structure, she never proposed an actual model from her work.

G. In 1953, Watson and Crick published their analysis of DNA structure.
1. Transparency 31, Page 199, Figure 9-12.
2. The DNA structure has many features:
a. There are two long polynucleotide chains, which are coiled around a central axis forming a right-handed double helix.
b. The two chains run in opposite directions of each other; that is they are antiparallel.
c. The bases of both chains are flat, that is they are "stacked" on top of one another, and are located 3.4 angstroms apart on the inside of the structure.
d. The nitrogenous bases are paired to one another by **hydrogen bonds**.

e. Each complete turn of the helix is 34 Angstroms; therefore, there are ten bases in each chain per turn.

f. There are alternating larger **major grooves** and smaller **minor grooves** along the axis.

g. The double helix measures 20 Angstroms in diameter.

3. The antiparallel nature of the two chains is an important part of the double-helix model; one chain runs 5'-3', while the other runs 3'-5'.

4. The specific A=T and G=C base pairing is the basis for the concept of **complementarity**, which is very important in the process of DNA replication and gene expression.

   a. The hydrogen bonding which holds these bases together is very important.

   b. Hydrogen bonding is a very weak electrostatic attraction between a covalently bonded hydrogen atom and an atom with an unshared pair of electrons.

   c. Adenine forms two hydrogen bonds with thymine, while guanine forms three hydrogen bonds with cytosine.

   d. Although these hydrogen bonds are weak when they are alone, two or three thousand hydrogen bonds are capable of providing great stability to the helix.

5. The arrangement of sugars and bases along the axis also provides stability to the structure.

   a. Watson and Crick made the structure of DNA so that the **hydrophobic**, "water-fearing," nitrogenous bases are stacked on the inside of the axis away from the water.

   b. They also made the structure where the **hydrophilic**, "water-loving," sugar-phosphate backbone is on the outside of the axis.

   c. These two properties add great stability to the structure.

6. In a second publication in *Nature*, Watson and Crick reported more findings about DNA.

   a. First, they reported that a specific mode of replication occurred in DNA and that it was **semiconservative**.

b. They also reported that the storage of genetic information in was in the sequences of the bases and that mutation or genetic change would result from the alteration of these bases.
c. They received the Nobel Prize in Physiology and Medicine in 1962, for their work with the structure of DNA.

VIII. Other forms of DNA

A. Transparency 32, Page 202, Figure 9-14.
B. When Watson and Crick made their analysis, there were two known forms of DNA, **A-DNA** and **B-DNA**.
   1. Watson and Crick's analysis was on the studies of the B-form, done by Franklin.
   2. The B-form is present under aqueous, low-salt conditions and is believed to be the biologically significant conformation.
C. Recently, **single-crystal X-ray analysis** has been used to study the structure of DNA; this analysis allows us to see every atom, which means that much greater structural detail is available.
D. The A-DNA is prevalent under high salt or dehydration conditions.
   1. A-DNA is slightly more compact than the B-DNA.
   2. It is also a right-handed helix.
   3. The bases are also titled and displaced laterally in relation to the axis of the helix.
   4. It is doubtful that the A-form occurs under biological conditions.
E. Another form of DNA is the Z-form, which was discovered in 1979 by Andrew Wang, Alexander Rich, and their colleagues.
   1. It is a left-handed helix, which contains the Watson-Crick base pairs.
   2. It is 18 angstroms in diameter, which means that there are approximately 12 base pairs per turn.
   3. It assumes a zigzag conformation, and the major groove present in B-DNA is nearly eliminated.
   4. It is still not clear what functional purposes Z-DNA has.

IX. The Structure of RNA

A. RNA, or ribonucleic acid, is a second type of nucleic acid.

B. Its structure is both similar and different from the structure of DNA.
   1. First of all, the building block nucleotides are linked into polynucleotide chains, but the sugar ribose replaces deoxyribose.
   2. Also in RNA, the base uracil replaces the base thymine.
   3. RNA is usually single-stranded.
      a. Under some circumstances, RNA molecules can fold back on themselves and form double-stranded regions.
      b. Also, in some animal viruses that have RNA as their genetic material contain double-stranded helices.

C. There are three forms of RNA, ribosomal RNA (rRNA), messenger RNA (mRNA), and transfer RNA (tRNA).
   1. These different forms of RNA all originate from complementary copies of one of the strands of DNA, during transcription.
   2. Each class of RNA can be characterized by its size, sedimentation behavior, and genetic function.
      a. The sedimentation behavior depends on the molecule's density, mass, and shape, and its measure is called the **Svedberg coefficient (*S*)**.
      b. The higher the *S* values the greater the molecular weight.
   3. Ribosomal RNA is generally the largest of these molecules.
      a. It constitutes approximately 80 percent of the RNA in a cell.
      b. It is a very important structural component of ribosomes.
      c. It functions as a nonspecific workbench for the synthesis of proteins, during the process of translation.
   4. Messenger RNAs are molecules that carry the genetic information from the DNA of the gene to the ribosome, where translation occurs.
      a. These vary greatly in length.
      b. Precursors of many mRNAs, called **primary transcripts**, may demonstrate values considerably higher.
   5. Transfer RNA is the smallest of RNA molecules.
      a. They carry amino acids to the ribosome during translation.

b. Their small size is essential to their function because more than one tRNA molecule interacts simultaneously with the ribosome.

6. There are still other forms of RNA as well.
   a. **Small nuclear RNA (snRNA)** participates in processing mRNAs.
   b. **Telomerase RNA** is involved in DNA replication at the ends of the chromosomes.
   c. **Antisense RNA** is involved in gene regulation.

X. Hydrogen Bonds and the Analysis of Nucleic Acids

A. When DNA is isolated and subjected to slow heating, the double helix is denatured and unwinds.
   1. When it is cooled the DNA strands begin to reattach, a process called **annealing**.
   2. This is one way of seeing how related two different organisms are; that is, how complementary the strands are.

B. During the unwinding, the viscosity of DNA decreases while the UV absorption increases.

C. By measuring the temperature and UV absorption, one can create a graph with data points, which form a curve.
   1. The midpoint of each is curve is called the **melting temperature (T_m)**, where 50 percent of the strands are unwound.
   2. The molecule with the higher T_m has a higher percentage of G-C base pairs than A-T base pairs, since G-C shares three hydrogen bonds, while A-T share only two hydrogen bonds.

D. The property of denaturation/renaturation is known as **molecular hybridization**.
   1. Molecular hybridization is possible between DNA strands from different species and between DNA and RNA strands.
   2. For example, an RNA molecule will hybridize with a segment of DNA from which it was transcribed, or with a DNA molecule from a different species provided that its nucleotide sequence is nearly the same.

E. ***In situ molecular hybridization*** can be performed using the DNA present in cytological preparations.

1. The first step in this process is taking mitotic or interphase cells and fixing them to slides.
2. Single-stranded DNA or RNA is then added, and hybridization is monitored.
3. The nucleic acid that is added can either be radioactive or contain a fluorescent label to allow for its detection.
4. In **fluorescent *in situ* hybridization**, fluorescence is used to identify chromosomes.
   a. A "probe" consisting of a short fragment of DNA that is complementary to DNA present in the centromere regions has been hybridized, in transparency 33, Page 204, Figure 9-16.
   b. Fluorescence occurs only in the centromere regions, thus identifying each chromosome.

F. **Reassociation kinetics**, the analysis of the rate of reassociation of complementary single strands of DNA, was first refined by Roy Britten and David Kohne.
   1. First, DNA is fragmented into small pieces.
   2. Then, the DNA is heated to allow it to dissociate into single strands, and then they are slowly cooled.
   3. The fragments are able to bombard each other, and if they are complementary, they will reassociate and become double stranded again.
   4. The results of such a procedure are plotted on a graph of DNA fragments against a logarithmic scale of normalized time; a function referred to as $\mathbf{C_o t}$.
   5. C_o is equal to the initial concentration of DNA single strands and t is equal to time.
   6. The **half reaction time**, the time when one-half of the DNA exists as double stranded fragments, can be measured using this technique, and the $\mathbf{C_o t_{1/2}}$ varies directly with the total length of DNA.
   7. Britten and Kohne examined DNA from calf thymus tissue.
      a. Based on their findings, they hypothesized that the rapidly reassociating fraction might represent repetitive sequences that are present many times in the calf's genome.

b. Multiple copies of the same sequence are more likely to make matches, and to make the matches more quickly than single copies.

c. These copies are collectively called **repetitive DNA**.

- DNA sequences are repeated over a million times, where longer sequences are repeated only a few times, in some cases.
- Repetitive DNA was one of the first clues that much of the DNA in eukaryotes is not contained in genes that encode proteins.

Lecture Outline

Chapter Ten: DNA-Replication and Synthesis

I. The Mode of DNA Replication

A. Transparency 34, Page 212, Figure 10-1.

B. Watson and Crick had an idea of replication

1. Template strand
2. Unwound
3. Polynucleotide chains
4. **Semiconservative replication**

C. Two other forms

1. **Conservative replication**
2. **Dispersive replication**

D. Matthew Meselson and Franklin Stahl

1. Transparency 35, Page 213, Figure 10-2
2. *E. coli* in $^{15}NH_4Cl$
3. ^{15}N
4. After many generations
5. **Sedimentation equilibrium centrifugation**
6. $^{14}NH_4Cl$
7. Time 0
8. Sedimentation equilibrium centrifugation
9. See page 213, Figure 10-3 in text
10. One generation cycle
11. Two generations

E. J. Herbert Taylor, Philip Woods, and Walter Hughes

1. *Vicia faba*
2. Examined chromosomes
3. ^{3}H-thymidine
4. **Autoradiography**
   a. Photographic
   b. Develop the slide

c. Dark spots or "grains"

5. One generation
6. Colchicine
7. Look at page 214, Figure 10-4 in the text
8. Acceptance of the semiconservative mode of replication

F. **Origin of replication**

1. **Replicon**
2. Circular chromosome
   a. One point
   b. ***oriC***
      1. 245 base pairs
      2. Replicon

G. **Unidirectional** or **bi-directional replication**

1. **Replication fork**.
2. Look at page 215, Figure 10-5 in the text
3. Both directions
4. Merging of replication forks
5. ***ter***

H. Eukaryotes

1. Multiple origins
2. Numerous replicating events
3. Merging of replication forks
4. Complex structure of chromosomes

II. Synthesis of DNA in Microorganisms

A. Arthur Kornberg

1. Cell-free system.
2. **DNA polymerase I**
3. Two major requirements
   a. All four deoxyribonucleoside triphosphates
   b. Template DNA
   c. See page 216, Figure 10-7 in the text.

4. 928 amino acids

B. Fidelity of enzyme
   1. Indirect methods
   2. Composition of the nitrogenous bases
      a. Table 10.1 in the text
      b. Base composition
      c. Replicated faithfully

C. Still not convinced
   1. Rate of synthesis
   2. Efficiency
   3. Degrade and synthesize

D. Another approach
   1. **Biologically active DNA**
   2. Mehran Goulian, Kornberg, and Robert Sinsheimer
      a. Phage ϕX174
      b. Isolated and allowed to infect
      c. New mature phages produced

E. Paula DeLucia and John Cairns
   1. Mutant strain of *E. coli*
   2. "Repair" damaged DNA
      a. UV radiation
      b. Two conclusions:
         1. One other enzyme
         2. Secondary function
   3. **DNA polymerases I and II**
      a. Table 10.2 on page 217
      b. **Primer**
      c. 5' – 3'
         1. Figure 10-8 on page 218 in the text
         2. New nucleotides
         3. Exposed 3'-OH group

d. Large complex proteins

e. **Exonuclease activity**

f. Polymerase III

1. Proofread, excise, and repair errors

2. **Holoenzyme**

- "Core" enzyme
- α and ε subunits

g. γ complex

1. "Loading" the enzyme

2. Energy requiring

h. β subunit

i. τ subunit

1. Large complex

2. **Replisome**.

j. Polymerase I

1. Gaps

2. Removing primer

3. Proofread

k. RNase

1. Remove RNA primer

2. Prior to polymerase I

l. Polymerase II

1. Repair synthesis

2. External factors

III. DNA Synthesis: A Model

A. For correct Synthesis

1. Unwind the helix

2. Reduce tension

3. Synthesis of a primer

4. Continuous synthesis and discontinuous synthesis

5. Remove primers

6. Ligation

B. *E. coli*

1. Transparency 36, Page 219, Figure 10-9
2. **9mers** and **13mers**
3. **DnaA**
   a. Subunits
   b. **DnaB** and **DnaC**
   c. **Helicases**
4. **Single-stranded binding proteins (SSBPs)**
   a. **Supercoiling**
   b. **DNA gyrase**; **DNA topoisomerases**

C. Initiation

1. DNA polymerase III
2. No free 3' end
3. RNA primer
4. Short segment of RNA
   a. Transparency 37, page 220, Figure 10-10
   b. **Primase**
   c. DNA polymerase III
   d. DNA polymerase I

D. Simultaneous synthesis

1. **Continuous DNA synthesis** on **leading strand**
2. **Lagging strand** with **discontinuous DNA synthesis**
3. Reiji and Tuneko
   a. Discovered small fragments
      1. **Okazaki fragments**
      2. Longer DNA strands
   b. Primers must be removed and gaps must be filled
      1. DNA polymerase I
      2. **DNA ligase**
   c. Supporting observations

E. Simultaneous synthesis at replication fork on both DNA strands
   1. Transparency 38, page 221, Figure 10-11
   2. Loop structure
   3. Released by the enzyme

F. Not always perfect
   1. Noncomplementary nucleotide
   2. **3'-5' exonuclease activity**
      a. Detect, pause, and excise
      b. Synthesis proceeds
   3. **Exonuclease proofreading**
   4. ε subunit

G. Summary of DNA synthesis; transparency 39, page 222, Figure 10-12
   1. Helicases unwind
   2. Replication fork moves, supercoiling, topoisomerases
   3. Primase
   4. DNA polymerase III
   5. Continuous and discontinuous synthesis
   6. Primers removed, DNA polymerase I, DNA ligase
   7. Okazaki fragments
   8. Proofreading
   9. Result, semiconservative replication

IV. Genetic Control of Replication

A. Genetic analysis
   1. Mutant strains
   2. **Temperature-sensitive mutations**
   3. Table 10.4 on page 224 of the text

V. Eukaryotic DNA synthesis

A. More complex
   1. 50 times more DNA per cell
   2. Complexity of cell

B. **Histones**

1. **Nucleosome**
2. Dissociated prior to DNA initiation

C. DNA polymerases (α, β, γ, δ, and ε)
1. α and δ forms
2. β form
3. Same fundamental requirements:
a. Four deoxyribonucleoside triphosphates
b. Template
c. Primer

D. Bi-directional
1. More points of origin
2. Mammals
3. *Drosophila*

E. More DNA polymerase molecules
1. *E. coli*
2. α form
3. S phase of interphase

F. Rate of synthesis
1. *E. coli* versus eukaryotes
2. Smaller replicons

G. **Semidiscontinuous**

H. Chromosome structure
1. Linear chromosomes
2. Telomeric region
3. Transparency 40, page 225, Figure 10-13
4. Lagging strand
a. Gap
b. No free 3'-OH end
c. Shortening of chromosome
d. **Telomerase**
1. Transparency 41, page 225, Figure 10-14

2. *Tetrahymena*
3. TTGGGG
4. Adds several copies.
5. "Hairpin loop,"
   - Creates free 3'-OH end
   - Loss of DNA is avoided
6. Elizabeth Blackburn and Carol Greider
   - Other DNA termini
   - **Ribonucleoprotein**

VI. DNA Recombination

A. **Genetic recombination**

B. Robin Holliday and Harold L. K. Whitehouse

1. Figure 10-15 in the text on page 227 (Transparency 42)
2. Two paired DNA duplexes
   a. Single-stranded nick
   b. Pair with complements on opposite duplex
   c. Ligase
   d. **Heteroduplex DNA molecules**
3. **Holliday structure**
   a. **Branch migration**
   b. Hydrogen bonds
   c. Increased length
4. **Chi form**
   a. Recombinant duplexes
   b. Arrangement of alleles
5. Evidence to support model
   a. Electron microscopic visualizations, figure 10-15
   b. **RecA protein**

C. **Gene conversion**

Lecture Content

Chapter Ten: DNA-Replication and Synthesis

I. The Mode of DNA Replication

A. Transparency 34, Page 212, Figure 10-1.

B. Following the discovery of the structure of DNA, Watson and Crick had some idea of how it would replicate.

1. Each strand of a DNA double helix would be able to serve as the template strand for synthesis.
2. If the helix were to be unwound, each of the nucleotides along the parental strands would have an affinity for the complementary nucleotides.
3. Then, if the nucleotides were linked covalently into polynucleotide chains along both templates, the result would be the production of two new, but identical double strands of DNA.
4. Each replicated DNA molecule would then consist of one "new" and one "old" strand, hence the term **semiconservative replication**.

C. There are also two other forms of replication that were at once thought to be utilized,.

1. One of those is **conservative replication**.
   a. In conservative replication, the process begins the same as in semiconservative.
   b. The difference between these two is that the two "new" strands would anneal to each other, leaving the two "old" strands to reassociate.
   c. This would result in the original strand of DNA to remain intact.
2. The other possible for is known as **dispersive replication**.
   a. In this form of replication, the parental strands are seen to be dispersed into two new double helices.
   b. Therefore, the result would be that each strand would consist of both old and new DNA.
   c. This method requires cleavage of the parental strands during replication.
   d. This is also the most complex possibility, therefore it is the least likely to occur.

D. In 1958, Matthew Meselson and Franklin Stahl published their findings from their experiments, which strongly supported the idea of semiconservative replication.
   1. Transparency 35, Page 213, Figure 10-2.
   2. They began this experiment by growing *E. coli* for many generations in a medium with $^{15}NH_4Cl$ was the only source of nitrogen.
   3. ^{15}N contains one more neutron than the naturally occurring ^{14}N isotope, but it is not radioactive; therefore, it did not decay.
   4. Following many generations, all nitrogen-containing molecules, including the nitrogenous bases, contained the heavier isotopes in the *E. coli* cells.
   5. The ^{15}N can be distinguished form the ^{14}N by the use of **sedimentation equilibrium centrifugation**.
      a. In sedimentation equilibrium centrifugation, samples are "forced" by centrifugation through a density gradient of a heavy metal salt.
      b. The denser ^{15}N-DNA reaches equilibrium in the gradient at a point that is closer to the bottom than the ^{14}N-DNA, since it is heavier.
   6. Following this method of separation, only uniformly labeled ^{15}N cells were placed on a medium of $^{14}NH_4Cl$, forcing all new synthesis to use the lighter isotope of nitrogen.
   7. The cells were placed in the medium at a time of 0, and were continuously taken out over different periods of time, this was to allow for different amounts of replication to occur.
   8. Following the removal of the sample, sedimentation equilibrium centrifugation was used to determine the density of samples.
   9. See page 213, Figure 10-3 in text.
   10. The first result came from a sample that had time to complete only one generation cycle.
      a. This sample, after being exposed to sedimentation equilibrium centrifugation, displayed one band with intermediate density.
      b. This was consistent with the thought of semiconservative replication, that is that one strand had the heavy isotope and the other had the light isotope.

c. The data also proved that conservative replication was not occurring because the predicted result from that would have been two different bands indicating the different isotopes of nitrogen.

11. Following two cell divisions, the DNA samples showed two density bands.
    a. One of the bands was intermediate, while the other corresponded to the ^{14}N gradient.
    b. Similar results occurred after three and more generations, except that the ^{14}N-band increased in proportion.
    c. If the mode of replication were dispersive, then all generations would demonstrate DNA of an intermediate density.
    d. Therefore, the results of the Meselson-Stahl experiment strongly supported the notion of semiconservative replication.

E. In 1957, one year prior to Meselson and Stahl, J. Herbert Taylor, Philip Woods, and Walter Hughes presented evidence that semiconservative replication also occurred in eukaryotic organisms.
    1. They used the root tip of the broad bean *Vicia faba*, which is an excellent source of dividing cells.
    2. These researchers examined the chromosomes of these cells following the replication of DNA.
    3. They also were able to monitor the replication process by labeling the DNA with ^{3}H-thymidine, which is a radioactive precursor of DNA.
    4. Following the labeling, they used **autoradiography**, a cytological procedure that allows the location of an isotope to be identified within a cell, to monitor the replication.
        a. This process is first done by placing a photographic emulsion over a section of the root tip, and then placed in the dark.
        b. The next step was to develop the slide.
        c. Since the radioisotope emits energy, the emulsion turns black at the approximate point of emission following development.
        d. The result is the presence of dark spots or "grains" on the surface of the section, identifying within the cell the location of the newly synthesized DNA.

5. These root tips were grown for only one generation in the presence of the radioisotope, which were then placed in unlabeled medium.
6. Cell division occurred in this medium, and following each generation, cultures were arrested in metaphase by using colchicine, a chemical that poisons the mitotic spindle fibers.
7. The chromosomes were then examined, look at page 214, Figure 10-4 in the text; the labeled thymidine is only found in the chromatids of the newly synthesized DNA.
8. Together, the Meselson-Stahl experiment and the experiment done by Taylor, Woods, and Hughes, soon led to the acceptance of the semiconservative mode of replication.

F. A question concerning replication is where is the **origin of replication**?
   1. It is important to note that the text defines the length of DNA that is replicated following the initiation of synthesis at a single origin as a unit called the **replicon**.
   2. In bacteria and bacterial viruses, the chromosome is a circle.
      a. There is definitely one point where replication is initiated.
      b. In *E. coli*, this region is called ***oriC***, and has been located.
         - *oriC* consists of 245 base pairs, but only a small number of them are actually necessary to initiate replication.
         - Since the chromosome is circular, the whole chromosome is considered to be the replicon.

G. A second concern is the direction of replication, it could be **unidirectional** or **bi-directional replication**.
   1. At each strand, there must be a point where the strands of the helix unwind, this is called the **replication fork**.
   2. Look at page 215, Figure 10-5 in the text.
   3. In *E. coli*, replication is bi-directional from *oriC*; that is it continues, following initiation, in both directions.

4. There are two replication forks within this chromosome, and they move away from each other at the origin until the merge when semiconservative replication is complete.
5. There is a termination region within *E. coli*, and it is called ***ter***.

H. As compared to replication in bacteria as described above, a major difference occurs in eukaryotes
   1. Replication is bi-directional, as in bacteria, creating two replication forks, but there are multiple origins along each chromosome.
   2. This results in numerous replicating events occurring in the S phase of interphase.
   3. Eventually, all the replication forks merge which completes the replication of the entire chromosome.
   4. There are many more replicating events occurring in the eukaryotes because of the more complex structure of the chromosomes.

II. Synthesis of DNA in Microorganisms

A. Arthur Kornberg and his colleagues were the first to report studies of the enzymology of DNA replication in 1957.
   1. They isolated an enzyme from *E. coli* that was able to direct synthesis of DNA in a cell-free system.
   2. Today, the enzyme that they discovered is known as **DNA polymerase I**, since it was the first one reported.
   3. There are two major requirements for *in vitro* DNA synthesis that Kornberg reported:
      a. All four deoxyribonucleoside triphosphates.
         - If any of the four deoxyribonucleoside triphosphates were omitted from the reaction, the result was no synthesis.
         - If derivatives of these precursor molecules other then the nucleoside triphosphate were used, the result was also no synthesis.
      b. Template DNA.
         - If template DNA were not added, synthesis did occur, but at a very slow rate.

- This template-dependent synthesis directed the specific enzyme, appeared to be the type that is required for the semiconservative replication.
- See page 216, Figure 10-7 in the text.

4. This enzyme has since been discovered to consist of 928 amino acids on a single polypeptide.

B. Following the discovery of how DNA was synthesized, Kornberg wanted to demonstrate the accuracy, or fidelity, with which the enzyme had replicated the DNA template.
   1. Since it was only 1957, and the nucleotide sequences of the template and the product were not known, he had to rely on indirect methods.
   2. One of his approaches was to compare the composition of the nitrogenous bases of the DNA template with those that were recovered from the DNA product.
      a. Table 10.1 in the text describes the base composition analysis of three DNA templates.
      b. The base composition of each product agreed with the template DNAs used.
      c. Along with other data, his research suggested that the templates were replicated faithfully.

C. There were many scientists who were still not convinced that DNA polymerase was the enzyme that replicates DNA, for many reasons.
   1. Determined from observations, the rate of synthesis was much slower in the *in vitro*, as compared with the *in vivo*.
   2. Another reservation was that the enzyme was much more efficient when it came to single-stranded DNA as compared to double-stranded DNA.
   3. A final reservation was that the enzyme could degrade DNA as well as synthesize it.

D. Kornberg decided to pursue another approach to his research to prove that his initial hypothesis was correct.
   1. He felt that if the enzyme could be used to synthesize **biologically active DNA** *in vitro*, then DNA polymerase I must be the major catalyzing force for DNA synthesis within the cell.

2. Biologically active DNA means that the DNA that is synthesized is capable of supporting metabolic activities and directing reproduction of the organism from which it was originally duplicated.
3. In 1967, Mehran Goulian, Kornberg, and Robert Sinsheimer performed an experiment to see if DNA polymerase was in fact the major catalyzing force for DNA synthesis.
   a. They used DNA from the phage ϕX174, and completely copied it using DNA polymerase I *in vitro*.
   b. The new product was not only able to be isolated, but it was shown to infect *E. coli*.
   c. After infecting *E. coli*, new mature phages were produced, thus demonstrating biological activity.

E. In 1969, Paula DeLucia and John Cairns reported a discovery of a mutant strain of *E. coli*, which did not contain DNA polymerase I.
   1. Even though there was an absence of this functional enzyme, replication still occurred.
   2. Other properties of the mutation led DeLucia and Cairns to report that the cells were much more deficient in their ability to "repair" damaged DNA.
      a. For example, nonmutant bacteria are able to repair a great deal of UV-induced damage, whereas the mutant strain was highly sensitive to the UV light.
      b. This observation led to two conclusions:
         - At least one other enzyme must be present in *E. coli* cells that is responsible for replicating DNA *in vivo*.
         - DNA polymerase I may only sever as a secondary function *in vivo*; Kornberg and others then thought that its function was critical to the fidelity of DNA synthesis, but not the one that actually synthesizes DNA.
   3. The other enzymes are now known as **DNA polymerases I and II**.
      a. Table 10.2 on page 217 of the text compares the properties of the three bacterial DNA polymerases.
      b. None of the three polymerases can initiate synthesis, but all can elongate an existing DNA strand, called a **primer**.

c. The elongation occurs by polymerizing nucleotides in the 5' – 3' direction.
   - Look at Figure 10-8 on page 218 in the text for a demonstration of 5'-3' synthesis of DNA.
   - Therefore, each new nucleotide is added at its 5'-phosphate end to the 3'-OH end.
   - Each of the additions creates a new exposed 3'-OH group on the sugar, which is then able to participate in the next reaction.

d. All of the DNA polymerases are large complex proteins, which exhibit molecular weights in excess of 100,000 daltons.

e. All three also possess 3'-5' **exonuclease activity**, which means that they can polymerize in one direction, then the reverse direction, and also excise nucleotides just added.

f. Polymerase III is essential for the polymerization during replication.
   - Its exonuclease activity allows it to proofread, excise, and repair errors made during polymerization.
   - The active form of DNA polymerase III is known as a **holoenzyme**, which consists of two sets (a dimer) of ten separate polypeptide chains, and weighs in excess of 600,000 daltons.
     - The α subunit is the largest subunit, and weighs 140,000 daltons; along with two other subunits, ε and θ, make up the "core" enzyme responsible for the polymerization activity of the holoenzyme.
     - Nucleotide polymerization on template strands is controlled by the α subunit, whereas the ε subunit is responsible for the 3'-5' exonuclease activity.

g. A second group of five subunits, γ, δ, δ', χ, and ψ, forms what is known as the γ complex.
   - This complex is involved in the "loading" of the enzyme onto the template at the replication fork.
   - It is energy requiring; therefore, it requires the hydrolysis of ATP.

h. Dimers of the β subunit serve as donut-shaped clamps, which prevent the core enzyme from falling off the template during polymerization.

i. Finally, the τ subunit holds together the two core polymerases at the replication fork.
   - The τ subunit, along with other proteins, form a complex almost as large as a ribosome.
   - This molecular entity is referred to as a **replisome**.

j. Polymerase I also has a specific function during replication.
   - Gaps are a natural occurrence on one of the two strands during replication as RNA primers are removed.
   - It is thought that polymerase I is responsible for removing the primer as well as for the synthesis that fills in the gaps created during replication.
   - It also is known to proofread the strand during this process.

k. Another enzyme RNase, has been discovered since the work of Kornberg.
   - The function of RNase is to remove the RNA primer.
   - This action occurs before the polymerase I begins to function.

l. Polymerase II has also been discovered to have its own specific function.
   - It is involved in the repair synthesis of DNA.
   - It repairs damages that have been caused by external factors, such as ultraviolet light.

III. DNA Synthesis: A Model

A. There are many processes that must take place in order for DNA to be synthesized correctly.
   1. First of all, a mechanism must exist to unwind the helix, while also stabilizing it in the open configuration.
   2. Then, the increased coiling that occurs further down the helix, which is caused by the unwinding, must be reduced.
   3. A primer must be synthesized so that polymerization can occur under the direction of DNA polymerase III.

4. There must be continuous synthesis in the direction in which the replication fork moves on one strand, while on the other strand, synthesis is discontinuous and in the opposite direction.
5. Next, the RNA primers must be removed prior to the completion of replication, and the gaps must be filled with complementary DNA.
6. Finally, the newly synthesized DNA strand that filled the gaps, must be ligated to the adjacent strand of DNA.

B. The region of the *E. coli*, circular chromosome, where DNA synthesis is initiated, is known as, as said before, *oriC*.
   1. Transparency 36, Page 219, Figure 10-9.
   2. The *oriC* consists of 245 base pairs, which are characterized by repeating sequences of 9 and 13 bases, which are respectively referred to as **9mers** and **13mers**.
   3. **DnaA** is one particular protein that is responsible for the initial step in unwinding the helix.
      a. There are a number of subunits of the DnaA protein, which bind to each of the several 9mers.
      b. This step is essential in aiding the subsequent binding of **DnaB** and **DnaC**, which further open and destabilize the helix.
      c. These proteins all require energy normally supplied by the hydrolysis of ATP, to break the hydrogen bonds and denature the double helix are collectively referred to as **helicases**.
   4. During the process of unwinding, proteins called **single-stranded binding proteins (SSBPs)**, stabilize the conformation.
      a. Coiling tension is a consequence of unwinding the DNA, further down the helix.
      b. Various forms of **supercoiling** often occur, which may take forms of twists and turns in the DNA; it is much like taking a rubber band and while holding one end, twisting the other.
      c. The supercoiling can be relaxed by an enzyme called **DNA gyrase**, which is a member of a large group of proteins together called **DNA topoisomerases**.

- Either single- or double-stranded cuts are made by DNA gyrase, depending on the form of DNA involved.
- The enzyme also increases localized manipulations of DNA strands that have the effect of "undoing" the twists and knots, which are created during this supercoiling process.

C. Once a small portion of the DNA helix is unwound, initiation is able to begin.
   1. DNA polymerase III requires there to be a free 3' end as part of a primer so that it can elongate a polynucleotide chain.
   2. Many scientists wondered how the first nucleotide could be added, since there was not a free 3' end to begin with.
   3. There is now evidence that shows that an RNA primer is involved in initiating DNA synthesis.
   4. It is thought that a short segment of RNA, which is complementary to the DNA, is synthesized on the DNA template first.
      a. Transparency 37, page 220, Figure 10-10.
      b. This segment of RNA is about 5 to 15 nucleotides long, and is made under the direction of **primase**, a form of RNA polymerase.
      c. After the addition of this RNA primer, DNA polymerase III begins to add 5'-deoxyribonucleotides.
      d. The RNA segment is subsequently snipped off and replaced with the corresponding DNA segment.
      e. Both of these steps are thought to be performed by DNA polymerase I.

D. Since, DNA polymerase III synthesizes DNA only in the 5'-3' direction, synthesis of antiparallel strands in one direction along one and in the opposite direction along the other, must occur simultaneously.
   1. As the strand unwinds, only one of the two DNA strands can serve as the template strand for **continuous DNA synthesis**, this is known as the **leading strand**.
   2. The other strand must have many sites of initiation along it, and it is known as the **lagging strand**, which results in **discontinuous DNA synthesis**.

3. Reiji and Tuneko Okazaki discovered evidence to support discontinuous DNA synthesis.
   a. They discovered, while working with *E. coli*, that the some of the newly formed DNA was hydrogen-bonded to the template strand, and consisted of small fragments containing only 1000 to 2000 nucleotides, which also consisted of RNA primers.
      - These segments, called **Okazaki fragments**, must be enzymatically joined.
      - Indeed, the fragments, which are at first a low molecular weight, are converted into longer and longer DNA strands of higher molecular weight.
   b. The RNA primers must be removed from the newly synthesized strand and the gaps must also be filled.
      - DNA polymerase I is responsible for removing the RNA primers and replacing the missing nucleotides, but it does not function to close the gaps which are left over.
      - **DNA ligase**, has been shown to be able to form the phosphodiester bond, which is the last step in sealing the gap between these discontinuously synthesized strands.
   c. There has been observations to support this thought in a ligase-deficient mutant strain (*lig*) of *E. coli*, where Okazaki fragments accumulate in large amounts and are never joined adequately.

E. It is believed, that a mechanism exists to allow for simultaneous synthesis at the replication fork on both strands of DNA.
   1. Transparency 38, page 221, Figure 10-11.
   2. The lagging-strand of DNA is able to form a loop structure, which inverts its sequence without changing the direction of synthesis, which must always be from 5'-3'.
   3. Following every 100 to 200 base pairs on the Okazaki fragments, the lagging-strand duplex is released by the enzyme, which allows for a new loop to develop, and the process is repeated.

F. Although the action of DNA polymerase is very accurate, the synthesis is not always perfect.
   1. Every once in a while, a noncomplementary nucleotide is inserted incorrectly.
   2. Both DNA polymerases I and III posses the ability of **3'-5' exonuclease activity**.
      a. This activity allows the polymerases to detect, pause, and excise a mismatched nucleotide, but in the 3'-5' direction.
      b. Once the error is removed, synthesis can proceed again in the 5'-3' direction.
   3. This process is known as **exonuclease proofreading**, and is used to increase the accuracy of synthesis.
   4. The ε subunit, of the holoenzyme form of DNA polymerase III, is the subunit, which is responsible for this process.

G. Below, are steps that summarize DNA synthesis; transparency 39, page 222, Figure 10-12.
   1. Helicases unwind, or denature, the helix at the origin of synthesis, which creates a replication fork; also, SSBPs stabilize the newly created single strands of DNA.
   2. The moving of the replication fork causes the increase coiling of the helix, the tension created by the coiling, is then released by the action of the topoisomerases, such as DNA gyrase, by cutting the DNA strands and then resealing them.
   3. An RNA primer, synthesized by primase, is used to initiate synthesis of the DNA strand, which occurs concurrently on both template strands.
   4. DNA polymerase III is responsible for polymerizing the complementary DNA strands by elongating the existing primer in the 5'-3' direction, simultaneously.
   5. Synthesis is continuous on the leading strand, and discontinuous on the lagging strand forming Okazaki fragments.
   6. The RNA primers are then removed and the resulting gaps are filled in by DNA polymerase I, and are finally closed by DNA ligase.
   7. The Okazaki fragments present on the lagging strand are then joined by DNA ligase as well.

8. Proofreading is occurring under the direction of DNA polymerase III while synthesis is proceeding, and the result is semiconservative replication.

IV. Genetic Control of Replication

A. Many of the details we know about DNA replication are from genetic analysis of different bacteria and viruses.
   1. Much of the information has actually come from mutant strains of these.
   2. These strains can be most easily studied if they are **temperature-sensitive mutations**, that is the mutation is expressed under specific conditions (restrictive temperature) and are not expressed under other conditions (permissive temperature).
      a. A mutation that might be lethal, can be maintained under the permissive temperature, and cells from that culture can be studied under the restrictive temperature.
      b. Investigations such as these, have the potential to provide valuable information into the product and the function of the normal, nonmutant gene.
   3. Table 10.4 on page 224 of the text, shows the enzyme product or the role in replication for a variety of genes from *E. coli.*
      a. There are numerous mutations in genes which specify the subunits of polymerases I, II, and III.
      b. There are also genes, which have been identified to encode for products for the origin of synthesis, helix unwinding, stabilization, initiation, and priming.

V. Eukaryotic DNA synthesis

A. Eukaryotic DNA synthesis is much more complex than prokaryotic synthesis.
   1. The Eukaryotes have approximately 50 times more DNA per cell as the prokaryotes do.
   2. The general complexity of the eukaryotic cell is also different than that of the prokaryotic cell.

B. Eukaryotic cells contain **histones**, which are major proteins within the DNA.
   1. These histones bond to the negatively charged phosphates and form a repeating structure known as the **nucleosome**.

2. The histones must be dissociated prior to DNA initiation, but must reassociate within the daughter cells following cell replication.

C. Eukaryotic cells have five kinds of DNA polymerases, called α, β, γ, δ, and ε.
   1. The α and δ forms seem to be the major enzymes involved in the replication of nuclear DNA.
   2. The β form is thought to be involved in DNA repair, while the γ form is the only DNA polymerase found in mitochondria.
   3. Eukaryotic DNA polymerases posses the same fundamental requirements:
      a. Four deoxyribonucleoside triphosphates.
      b. A template.
      c. A primer.

D. Eukaryotic DNA, as mentioned earlier, is bi-directional, creating two replication forks from each point of origin.
   1. Since there is so much more DNA present in the eukaryotic cells, as compared to the prokaryotic cells, there are many more points of origin present.
   2. In mammals, there are about 25,000 replicons present in the genome which each consist of 100,000 to 200,000 bases.
   3. In *Drosophila*, there are about 3500 replicons present each with an average of 40,000 bases.

E. There are also many more DNA polymerase molecules present in eukaryotic cells to accommodate the larger number of points of origin.
   1. *E. coli* only have about 15 copies of DNA polymerase III per cell.
   2. There may be up to 50,000 copies of the α form of DNA polymerase in the eukaryotic cells.
   3. Consequently, most replicons may be replicated in the S phase of interphase.

F. Synthesis of DNA is also slower in the eukaryotic cells.
   1. *E. coli* add approximately 100 kb to a growing chain per minute, while eukaryotes only range from 0.5 to 5 kb per minute.
   2. *E. coli* takes approximately 20 to 40 minutes to replicate its chromosome, while *Drosophila*, even with 40 times more DNA, can accomplish this task in 3 minutes.

3. Therefore, the presence of smaller replicons compensate for the slower rate of DNA synthesis in eukaryotes.

G. It is not yet proven if the large amount of DNA on the leading strand can be completed continuously as in the prokaryotes, hence the term **semidiscontinuous**.

H. Another major difference between the eukaryotic and prokaryotic cells, is the chromosome structure.

1. The chromosome is usually circular in the prokaryotes, but they are linear in the eukaryotes.
2. The end of the linear chromosome, known as the telomeric region, is a problem when considering replication.
3. Transparency 40, page 225, Figure 10-13.
4. Synthesis is not usually a problem at the end of the leading strand, but there is a problem at the end of the lagging strand.
   a. When the RNA primer is removed from the lagging strand, there is a gap created.
   b. Normally, the gap would be filled by adding a nucleotide to the existing 3'-OH group, but here is where the problem is, there is no free 3'-OH end.
   c. Theoretically, each successive round of replication would cause a shortening of the chromosome.
   d. The discovery of the enzyme **telomerase**, present in eukaryotes, is how this problem is solved.
      - Transparency 41, page 225, Figure 10-14.
      - In the protozoan *Tetrahymena*, all telomeres end with a sequence of 5'-TTGGGG-3'.
      - This enzyme is capable of adding repeats of TTGGGG to the ends of the molecules that already contain this sequence.
      - Telomerase adds several copies of the 6-nucleotide repeat to the 3'-end of the lagging strand.
      - The repeats are then able to form a characteristic "hairpin loop," which is stabilized by unique hydrogen bonding between guanine residues.

- This "hairpin loop" creates a free 3'-OH end, which can then serve as a substrate for the DNA polymerase I to fill in the gap.
- If the hairpin is then cleaved off, the potential loss of DNA is avoided.

5. Elizabeth Blackburn and Carol Greider, isolated and studied the telomerase enzyme extensively.
   a. They discovered that the enzyme adds the same TTGGGG sequence to other DNA termini as well.
   b. They also discovered that the enzyme contains a short piece of RNA that is essential to catalytic activity, this functional enzyme is this a **ribonucleoprotein**.
      - The RNA contains 159 bases which also include the sequence 5'-CCCCAA-3', the complementary strand to the sequence it synthesizes.
      - This RNA enzyme acts much like reverse transcriptase, in that it synthesizes a DNA complement from an RNA strand.

VI. DNA Recombination

A. **Genetic recombination** is the genetic exchange between any two homologous double-stranded DNA molecules, whether they be viral or bacterial chromosomes, or even eukaryotic homologs during meiosis.

B. There are several different models used to explain crossing over, and they are all based on the independent work of both Robin Holliday and Harold L. K. Whitehouse in 1964.
   1. One of those models is illustrated in figure 10-15 in the text on page 227 (Transparency 42).
   2. It begins with two paired DNA duplexes.
      a. Each of these duplexes experiences a single-stranded nick.
      b. This nick is placed at identical positions by an endonuclease; then, the ends are displaced and subsequently pair with their complements on the opposite duplex.
      c. Next, the ligase comes around and seals the loose ends together.
      d. Finally, there is a creation of hybrid duplexes also known as **heteroduplex DNA molecules**.

3. The exchange creates what is called a cross-bridged or **Holliday structure**.
   a. The position of this cross-bridge can move down the chromosomes as a result of **branch migration**.
   b. This can occur because of the zipperlike action as the hydrogen bonds break and then re-form between the complementary bases of the displaced strands.
   c. The migration does cause an increased length in the heteroduplex DNA on both homologs.
4. If then the duplexes separate and the bottom portions then rotate 180°, an intermediate planar structure is formed, which is known as a **chi form**.
   a. If the two previously uninvolved strands are then nicked with the endonuclease, and ligation occurs, recombinant duplexes are created.
   b. The arrangement of the alleles is altered due to this recombination.
5. There is evidence to support this model.
   a. There are electron microscopic visualizations of the chi-form, from bacteria where four duplex arms are joined at a single point of exchange, as seen in figure 10-15.
   b. Also, *E. coli* has the **RecA protein**, which promotes the exchange of reciprocal single-stranded DNA molecules.
   c. This protein also promotes the hydrogen bond formation during the strand displacement process.

C. **Gene conversion** is characterized by a genetic exchange ratio that involves two closely linked genes that is nonreciprocal.
   1. It was called gene conversion because it appeared that one allele had somehow been "converted" to another allele during and event in which genetic exchange also occurred.
   2. This is now considered to be a consequence of the process of DNA recombination.

Lecture Outline

Chapter Three: Mendelian Genetics

I. Gregor Johann Mendel

A. Johann Mendel

B. Mendel's experimental approach

1. The pea plant
2. Unit characters
   a. Tall and dwarf
   b. Other characters
3. "True breeding"
4. Basis for the transmission of hereditary traits

II. The monohybrid cross

A. **Monohybrid cross**

1. Transparency 11 (Page 45, Figure 3-1)
2. **P_1** or **parental generation**
3. **F_1** or the **first filial generation**
4. **F_2** or **second filial generation**
5. The first monohybrid cross
6. Other crosses: **reciprocal crosses**
7. **Unit factors**

B. Mendel's first three postulates, or principles of inheritance

1. Unit factors in pairs
   a. Genetic characteristics
   b. Specific unit factor
      1. Possible combinations
      2. Determination
2. Dominance/recessiveness
   a. Unlike unit factors
      1. Controlled by the dominant unit factor
      2. Present in pairs
3. Segregation

a. Segregate randomly

1. Like unit factors
2. Unlike unit factors
3. Explanation for Mendel's results
4. Example
   - The P_1 tall plants
   - F_1 plants
   - Gametes
5. Outcomes

C. Modern genetic terminology

1. Transparency 12 (Page 47, Figure 3-2)
2. **Phenotype**
3. Genes
4. **Alleles**
5. Symbol of the trait
   a. **Genotype**
   b. Example
6. **Homozygous**
7. **Heterozygous**

D. Punnett squares

1. Transparency 13 (Page 48, Figure 3-3)
2. **Punnett square**
3. Assigning of the gametes

E. The test cross: one character

1. **Test cross**
2. Dominant X recessive

III. The dihybrid cross

A. Transparency 14 (Page 49, Figure 3-5)

B. **Dihybrid cross ; two-factor cross**

1. Example
2. Offspring

C. Mendel's fourth postulate: Independent Assortment

1. Transparency 15 (Page 49, Figure 3-6)
2. **Product law**
3. **Independent assortment**
4. Figure 3-7 in the text shows an example of independent assortment
   a. Probabilities for the progeny
   b. **Mendel's 9:3:3:1 dihybrid ratio**

IV. The trihybrid cross

A. Transparency 16 (Page 52, Figure 3-8)

B. **Trihybrid cross**; **three-factor cross**

C. Processes of segregation and independent assortment

D. Example

E. The forked-line method or branch diagram

1. **Forked-line method**, or **branch diagram**
   a. AA X aa
      1. F_1 individuals
      - F_2 generation
      - BB X bb and CC X cc
   b. Law of independent assortment
   c. Example of a branch diagram is on page 52, figure 3-9

V. The rediscovery of Mendel's work

A. Thirty-five years

1. **Continuous variation**
2. **Discontinuous variation**
3. Walter Flemming
4. Hugo de Vries, Karl Correns, and Erich Tschermak
   a. De Vries's work
   b. Correns' and Tschermak's work
5. Walter Sutton and Theodor Boveri
6. **Chromosomal theory of inheritance**

B. Unit factors, genes, and homologous chromosomes

1. Unit factors are genes
2. Homologous chromosomes
   a. **Maternal parent**
   b. **Paternal parent**
3. Genetic information
   a. **Genes**
   b. **Locus**
   c. **Alleles**

VI. Independent assortment and genetic variation

A. Genetic variation

B. Possible gamete combinations
   1. For example
   2. Humans

VII. Probability and genetic events

A. Range of probabilities

B. **Product law**

C. **Sum law**

D. Large sample sizes

VIII. Evaluating genetic data: Chi-Square analysis

A. Certain assumptions

B. **Chance deviation**

C. Example

D. **Null hypothesis**
   1. Rejected or fail to be rejected
   2. When rejected
   3. Fails to be rejected

E. **Chi-square (X^2) analysis**
   1. Transparency (Page 56, Table 5.1)
   2. X^2
   3. The formula:

$$X^2 = \frac{\Sigma(o - e)^2}{e}$$

4. Interpret the X^2 value
    a. **Degrees of freedom (*df*)**
    b. Example
5. **Probability** value, **(p)**
    a. Table on page 57 of the text
    b. Steps
6. Understanding *p*
    a. Example
    b. The nearer to 1.0
    c. Another interpretation
    d. Standard
        1. 0.05.
        2. Less than 0.05

IX. Human Pedigrees

A. **Pedigree**

1. Transparency 18 (Page 58, Figure 3-12)
2. Gene-controlling traits
3. There are many different symbols used within a pedigree
    a. Circles
    b. Squares
    c. Diamond
    d. Shaded
    e. Parents
    f. Offspring
    g. Arrangement
    h. Twins
        1. **Monozygotic twins**
        2. **Dizygotic twins**

Lecture Content

Chapter Three: Mendelian Genetics

I. Gregor Johann Mendel

A. Johann Mendel was one of the most influential scientists of genetics.

1. Mendel was born in 1822, to a peasant family in a European village.
2. He studied philosophy for several years after graduating from high school.
3. In 1843, he was admitted to the Augustinian Monastery of St. Thomas in Brno.
4. In 1849, he was relieved of pastoral duties and received a teaching appointment.
5. From 1851-1853, he attended the University of Vienna, and studied physics and botany.
6. In late 1853, he returned to Brno and for the next fifteen years taught physics and natural science.
7. It was in 1856 that Mendel performed his first set of hybridization experiments with the garden pea.
8. In 1868, he was elected abbot of his religious chapter, and the research phase of his career was over.
9. Mendel died in 1884 from chronic kidney disease and heart failure.

B. Mendel's experimental approach was somewhat advanced for his time.

1. Mendel chose a wonderful organism for experimentation, the pea plant.
   a. The pea plant is easy to grow and hybridize artificially.
   b. It reproduces well and grows to maturity in one season.
2. Mendel worked with seven different unit characters, which are visible features that were each represented by two contrasting traits.
   a. For example, for the unit character of stem height, he had only *tall* and *dwarf* plants.
   b. His other characters included seed shape and color, pod shape and color, and pod and flower arrangement.
3. He would breed the flowers that he received from the local merchant for a few generations to ensure that they were "true breeding," meaning that the traits appeared the same generation after generation.

4. There are some other factors besides his choice of organism that may have given Mendel success where others had failed.
   a. One other factor was that he restricted his examination to one or very few pairs of contrasting traits in each of his experiments.
   b. Another factor was that Mendel kept accurate quantitative records on all his experiments.
5. Through his experiments, Mendel discovered the basis for the transmission of hereditary traits.

II. The Monohybrid Cross

A. The **monohybrid cross** was one of the simplest crosses performed by Mendel.
   1. Transparency 12, page 45, figure 3-1.
   2. To perform a monohybrid cross, Mendel mated individuals from two parent strains, each of which exhibited one of the two contrasting forms of the character under study.
   3. The original parents are called the $\mathbf{P_1}$ or **parental generation**.
   4. The offspring of the parental generation are called the $\mathbf{F_1}$ or the **first filial generation**.
   5. The individuals from the selfing of the F_1 generation are called the $\mathbf{F_2}$ or **second filial generation**.
   6. The first monohybrid cross Mendel performed was between *tall* and *dwarf* plants.
      a. When Mendel crossed the tall and dwarf plants, the F_1 generations were all tall.
      b. When the members of the F_1 generation were selfed, Mendel observed the following data, 787 of the 1064 F_2 generation were tall, while 277 of the 1064 were dwarf.
      c. Much of the genetic data is recorded in ratio format; therefore, the ratio for this experiment would be 2.8:1.0 or about 3:1.
   7. Mendel performed other crosses using different characters.
      a. The outcomes of the other crosses were similar to the tall/dwarf one just described.

b. All the F_1 offspring were identical to one of the parents, while the F_2 generation was an approximate ratio of 3:1.

8. In each of the experiments, the inheritance patterns were similar regardless of which P_1 plant served as the source of pollen, and which served as the source of ovum.
   a. These types of crosses are called **reciprocal crosses**, because they can be done using either plant as the sperm or egg.
   b. Mendel's monohybrid crosses were therefore not sex-dependent.
9. To explain his results, Mendel proposed the existence of **unit factors** for each trait, which served as the basic units of heredity and were passed unchanged from generation to generation.

B. Mendel's first three postulates, or principles of inheritance.
   1. Unit factors occur in pairs.
      a. Genetic characteristics are controlled by unit factors that exist in pairs in individual organisms.
      b. In the monohybrid cross involving the tall and dwarf stems, a specific unit factor exists for each trait and they occur in pairs.
         - Three combinations are possible: two factors for tallness, two factors for dwarfness, or one for each factor.
         - Every individual contains one of these combinations, which determine the height of the stem.
   2. Dominance/recessiveness are two forms that the unit factors conform to.
      a. When there are two unlike unit factors, which are responsible for one character, one unit factor is dominant over the other, which is said to be recessive.
         - The trait that is expressed in the F1 generation is controlled by the dominant unit factor; therefore, the trait that is not expressed is said to be the recessive unit factor.
         - This relationship of dominance/recessiveness only pertains to unlike unit factors that are present in pairs.

b. In Mendel's first experiment, the tall stems are said to be dominant, while the dwarf stems are said to be recessive.

3. Segregation of the chromosomes occurs during meiosis.
   a. During the formation of gametes, the paired unit factors segregate randomly so that each gamete receives one or the other unit factor with equal probability.
   b. If an individual contains a pair of like unit factors, then the gametes will each receive that unit factor.
   c. If an individual has a pair of unlike unit factors, then each gamete has a fifty-percent chance of receiving either one.
4. These postulates provide an explanation for the results of Mendel's monohybrid crosses.
   a. To illustrate this point, we will use Mendel's cross of the tall and dwarf plants.
      - The P_1 tall plants all contained like unit factors, as did the P_1 dwarf plants; therefore, the gametes of the tall plants each received a tall unit factor, and the gametes for the dwarf plants each received a dwarf unit factor.
      - Following fertilization, all the F_1 plants received two unit factors, one from each parent, a tall one and a dwarf one.
      - Because the tall is dominant to the dwarf, all the F_1 generation plants were tall.
      - When the F_1 plants formed gametes, according to the segregation postulate, each gamete randomly received either the tall or the dwarf unit factor.
        - Following the fertilization of the F_1 plants, there were only four possible outcomes:
          (1) tall/tall (2) tall/dwarf (3) dwarf/tall (4) dwarf/dwarf
        - Outcomes 1,2, and 3 will produce tall plants because tall is dominant to dwarf, while only outcome 4 will produce dwarf plants. This follows the 3:1 ratio that Mendel observed in his experiments.

C. Mendel's words have been modified into the modern genetic terminology.
   1. Transparency 13, page 47, figure 3-2.

2. The **phenotype** of an individual is expressed as the physical appearance.
3. Unit factors are now called genes.
4. The phenotype is determined from the alternative forms of genes called **alleles**.
5. We usually use the first letter of the trait in question to symbolize the trait.
   a. We use the uppercase of that letter to show that it is dominant.
   b. And we use the lowercase of the letter to show that it is recessive.
      - If we let d stand for the dwarf unit factor, then D would be the tall unit factor.
      - By putting the two factors together, such as DD,Dd, and dd, we are symbolizing the genetic makeup also referred to as the **genotype**.
   c. The genotype allows us to know what the phenotype is, for example DD would equal tall/tall, or a tall plant.
6. When both alleles are the same (DD) the individual is said to be **homozygous**, or a homozygote.
7. When the alleles are different (Dd) the individual is said to be **heterozygous**, or a **heterozygote**.

D. Punnett squares.
   1. Transparency 14, page 48, figure 3-3.
   2. Reginald G. Punnett devised an easy approach to visualize the genotypes and phenotypes resulting from a combination of gametes, the **Punnett square**.
   3. Each of the possible gametes is assigned to a column or a row. The vertical column represents the female, while the horizontal column represents the male.
   4. The new generation is predicted by combining the male and female gametic information into the boxes.
      a. This process accounts for all possible random fertilization events.
      b. The genotypes and phenotypes of the offspring can be ascertained by reading the entries in the boxes.
      c. Figure 3-3 in the text shows a case of the 3:1 phenotypic ratio, and the 1:2:1 genotypic ratio of the F_2 generation.

E. The test cross, devised by Mendel, uses one character or trait within the cross.

1. Mendel devised an easy method to discover the genotype of an individual, called a **test cross**.
2. To perform one of these, the organism expressing the dominant trait is crossed with a homozygous recessive organism.
3. If the dominant organism is homozygous (DD), and is crossed with an organism with the genotype of dd the resulting progeny will be Dd and Dd, all tall.
4. If the dominant organism is heterozygous (Dd) and is crossed with a dd, the resulting progeny will be Dd and dd, 1:1, tall:dwarf.

III. The Dihybrid Cross

A. Transparency 15, page 49, figure 3-5.

B. A **dihybrid cross** is when two contrasting traits are examined simultaneously, this is also known as a **two-factor cross**.
   1. For example, Mendel crossed pea plants having yellow round seeds with green wrinkled seeds.
   2. The offspring were yellow and round.
   3. Therefore, yellow is dominant to green, and round is dominant to wrinkled.
      a. Then, the F_1 individuals were self-crossed.
      b. The results were approximately 9/16 of the F_2 plants express yellow and round, 3/16 expresses yellow and wrinkled, 3/16 express green and round, and 1/16 express green and wrinkled.

C. Mendel's fourth postulate is known as Independent Assortment.
   1. Transparency 16, page 49, figure 3-6.
   2. It is easier to understand a dihybrid cross when it is thought of as two individual monohybrid crosses.
   3. Because it is evident that the two pairs of contrasting traits are inherited independently, a prediction can be made of the frequencies of the F_2 phenotypes by applying the **product law** of probabilities: **When two independent event occur simultaneously, the combined probability of the two outcomes is equal to the produce of their individual probabilities of occurrence.**

a. For example, since the probability of an F_2 plant being yellow is ¾, and its probability of being round is also ¾, the probability of the plant being yellow and round is (3/4)(3/4), or 9/16.
b. Another example would be for an F_2 plant to be yellow and wrinkled. Its probability of being yellow is ¾, while its probability of being wrinkled is ¼; therefore, the probability that the plant will be yellow and wrinkled is (3/4)(1/4), or 3/16.
c. These calculations make it apparent why the F_1 and F_2 results are identical whether or not the initial cross is yellow, round, bred with green, wrinkled, or if yellow, wrinkled, is bred with green, round.

4. It was based on the results of many experiments like the one above that Mendel proposed a fourth postulate, **independent assortment**: **During gamete formation, segregating pairs of unit factors assort independently of each other**.
   a. This means that every gamete receives one member of every pair of unit factors.
   b. For one pair of unit factors, does not influence the segregation of any other pair.
5. Figure 3-7 in the text shows an example of independent assortment.
   a. In this example, the probabilities for the progeny are 9/16 yellow and round, 3/16 yellow and wrinkled, 3/16 green and round, 1/16 yellow and round.
   b. This ratio is now designated **Mendel's 9:3:3:1 dihybrid ratio**.

IV. The Trihybrid Cross

A. Transparency 17, page 52, figure 3-8.
B. A **trihybrid cross**, also known as a **three-factor cross**, utilizes three contrasting unit factors.
C. Mendel demonstrated that the identical processes of segregation and independent assortment apply for a trihybrid cross as well.
D. An example would be using a theoretical genotype such as A/a, B/b, and C/c.
   1. In the cross between AABBCC and aabbcc individuals, all the F_1 are heterozygous, or AaBbCc, resulting in the phenotypic expression of ABC.

2. If we were to cross the F_1 generation, there would be eight gametes possible, and sixty-four separate boxes in the Punnett square.

E. The forked-line method or branch diagram.

1. The **forked-line method**, or **branch diagram**, relies on the simple application of the laws of probability established for the dihybrid cross.
   a. When the monohybrid cross AA X aa is made, we know two things:
      - All the F_1 individuals will have the genotype Aa and express the phenotype A.
      - The F_2 generation with consist of individuals with either the A phenotype or the a phenotype in the ratio of 3:1.
      - We can assume that the same would follow for crosses such as BB X bb and CC X cc.
   b. The proportions of organisms that express each of the phenotypic variations can be predicted assuming that they follow the law of independent assortment.
   c. Again, the law of probabilities is applied.
      - The predicted ratio is 27:9:9:9:3:3:3:1.
      - This same process can be used for any case with any number of genes.
   d. An example of a branch diagram is on page 52, figure 3-9.

V. The Rediscovery of Mendel's Work

A. Mendel's work, while being published and cited, basically went unnoticed for thirty-five years.

1. His conclusions did not fit well with existing theories involving the cause of variation.
2. Many individuals believed in **continuous variation**, whereby offspring were a blend of their parent's phenotypes.
3. Mendel on the other hand, believed in **discontinuous variation**, or that variation was due to discrete or particular units.
4. In 1879, Walter Flemming discovered chromosomes, and was able to describe their behavior during cell division, which soon became an integral part of the ideas of inheritance, hence Mendel's work was rediscovered.

5. Hybridization experiments similar to Mendel's were performed independently by three botanists: Hugo de Vries, Karl Correns, and Erich Tschermak.
   a. De Vries's work demonstrated the principle of segregation.
   b. Correns' and Tschermak's work also produced conclusions similar to Mendel's.
6. In 1902, Walter Sutton and Theodor Boveri independently published papers concerning their discoveries of the behavior of chromosomes during meiosis with Mendelian principles of segregation and independent assortment.
7. Sutton and Boveri are credited with the initiation of the **chromosomal theory of inheritance**.

B. Unit factors, genes, and homologous chromosomes.
   1. Unit factors are really genes located on homologous chromosomes.
   2. To understand the principle of independent assortment, we must distinguish between homologous chromosomes.
      a. One member is derived from the **maternal parent**.
      b. The other is derived from the **paternal parent**.
   3. It is logical to assume that there are more genes than there are chromosomes; therefore, each homolog must carry genetic information for more than just one trait.
      a. A chromosome is composed of a large number of linearly ordered information-containing units called **genes**.
      b. The location on a chromosome where any particular gene is located is called a **locus**.
      c. The different forms taken by a given gene, called **alleles**, contain slightly different genetic information.

VI. Independent Assortment and Genetic Variation

A. Genetic variation results because the two members of any homologous chromosome pair are rarely, if ever, genetically identical.
B. The number of possible gamete combinations is 2^n, where n is the haploid number.
   1. For example, if the haploid number is 4, then there are 2^4, or 16, different gamete combinations.

2. This might not be high, but when you consider humans, whose haploid number is 23, then there are 2^{23} or over 8 million possible types of gamete combinations.

VII. Probability And Genetic Events

A. Probabilities range from 0, when an event will not occur, to 1, when an event will definitely occur.

B. When there are two or more events occurring independently, but at the same time, we can calculate the probability that they will occur together, using the **product law**.

1. For example, if you were to take a penny (P) and a nickel (N) at the same time and examined all combinations of heads (H) and tails (T), there are four possible outcomes:

$$(P_H: N_H) = (1/2)(1/2) = ¼$$
$$(P_T: N_H) = (1/2)(1/2) = ¼$$
$$(P_H: N_T) = (1/2)(1/2) = ¼$$
$$(P_T: N_T) = (1/2)(1/2) = ¼$$

a. The probability of obtaining a head or tail is ½, and is unrelated to the outcome of the toss of the other coin.

b. Therefore, when the probabilities are multiplied together, you get ¼.

2. If we want to know the possible outcomes of two events, which are independent of each other, but can be accomplished in more than one way, we apply the **sum law**.

a. For example, if we wanted to toss our penny and nickel again, but this time we wanted one to be heads and the other to be tails, we would use the sum law.

b. There are two ways that this can occur, the penny can be heads or the nickel can be heads, both have the probability of occurring of ¼, so the overall probability would be:

$$(1/4) + (1/4) = ½$$

3. It is important to remember that probability predictions are made on large sample sizes, not small ones.

VIII. Evaluating Genetic Data: Chi-Square analysis

A. There are certain assumptions, which must be made when using Mendel's ratio predictions.
   1. Each allele is dominant or recessive.
   2. Segregation is operative.
   3. Independent assortment occurs.
   4. Fertilization is random.

B. The last three are influenced by chance events and subject to random fluctuation, also known as **chance deviation**.

C. An example would be tossing a coin in the air, many times, and recording the number of heads and tails observed.
   1. The expected ratio is 1:1, since there is a 50% chance for both heads and tails.
   2. The impact of the chance deviation increases when the total number of tosses is reduced.

D. When performing a genetics experiment, one assumes that the date will fit into a given ratio, called a **null hypothesis**.
   1. The null hypothesis may be either rejected or fail to be rejected.
   2. When it is rejected, then the observed deviation is not attributed to chance alone.
   3. If the null hypothesis fails to be rejected, then the deviations are attributed to chance.

E. A test to assess the null hypothesis, is the **chi-square (X^2) analysis**.
   1. Transparency 18, page 56, table 5.1.
   2. This test takes into account the observed deviation in each component of an expected ratio as well as the sample size and reduces them to a single numeric value.
   3. The value, X^2 then estimates the frequency that the observation deviation might occur strictly as a result of chance.
   4. The formula for the chi-square test is :

$$X^2 = \sum \frac{(o-e)^2}{e}$$

      a. The o is this equation is the observed value, whereas the e is the expected value.

b. Σ, represents the sum of the calculated values for each category of the ratio.

5. The final step in this analysis is to interpret the X^2 value.
   a. First, a value must be determined for the **degrees of freedom (*df*)**, which is equal to $n-1$, where n is the number of categories into which each data number point may fall.
   b. For the ratio 3:1, $n = 2$, this number must be taken into consideration because the greater the number of categories, the greater the chance of deviation.
6. Then, the chi-square value must be interpreted in terms of a corresponding **probability** value, **(p)**.
   a. The *p* value is located on a chi-square table on page 57 of the text.
   b. There are a few simple steps that must be followed to determine the value of *p*.
      - First, the X^2 value must be located on the horizontal axis.
      - Then, follow up the graph to the appropriate value of *df*.
      - Next, follow with your finger, to the Y-axis.
      - Finally, estimate the corresponding *p* value.
7. The most important part of the chi-square to test is not only to determine the *p* value, but to understand what it means.
   a. For example, if the *p* value is 0.26, then think about it equaling 26%.
   b. This means, that the probability is 26% that the observed deviation can be attributed to chance.
   c. The nearer the *p* value is to 1.0, the closer the data are to the predicted or ideal ratio.
   d. Another way to interpret these results is that if the experiment were repeated many times, 26% of the trials would be expected to exhibit chance deviation as great or greater than that seen in the initial trial.
   e. This analysis gives a relative standard that is set as the basis for either rejecting or failing to reject the null hypothesis.
      - This standard is usually a probability value of 0.05.

- If the p value is less than 0.05, then there is a less than 5% chance that the observed deviation could be obtained from chance alone, or vice versa.

IX. Human Pedigrees

A. The pattern of inheritance of a specific phenotype in humans can be studied by using a **pedigree**.
   1. Transparency 19, page 58, figure 3-12.
   2. A pedigree is a family tree that indicates the phenotype of the trait in question.
   3. By analyzing the pedigree, predictions can be made on how the gene-controlling trait is inherited.
   4. There are many different symbols used within a pedigree.
      a. Circles represent females, while squares represent males.
      b. If the sex if unknown, then a diamond is used.
      c. If an individual expresses a certain trait, then the symbol is shaded, if it is known that the individual is heterozygous, then the symbol is shaded on the left half of it.
      d. Parents are connected with horizontal lines, and their offspring are designated with vertical lines.
      e. The offspring are called **sibs**, and a horizontal **sibship** line connects them.
      f. The sibs are arranged from left to right in birth order, and are labeled with Arabic numerals, while each generation is labeled with a Roman numeral.
      g. Twins are identified with a diagonal line from the sibship line.
         - **Monozygotic twins** are represented with a connecting line.
         - **Dizygotic twins** lack this connection.

Lecture Outline

Chapter Four: Modification of Mendelian Ratios

I. Potential Function of an Allele

A. Function of an Allele

1. **Alleles**
2. **Wild-type allele**
3. **Mutation**

II. Symbols for Alleles

A. A simple review of the notations for alleles

1. Recessive allele and dominant allele
2. Mendel's pea plants

B. Another system

1. Recessive and dominant alleles
2. Wild-type trait
3. Body color in the fruit fly *Drosophila*
   a. *Ebony* and gray colors
   b. Three possible genotypes for a diploid fly:

e^+ / e^+ gray homozygote (wild type)

e^+ / e gray homozygote (wild type)

e / e ebony homozygote (mutant)

   c. Advantage to using this system

III. Incomplete or Partial Dominance

A. **Incomplete** or **partial dominance**

1. An example
2. Red and white flower color
3. Results of the cross
   a. F_2 generation
   b. No dominance
   c. Labeling the alleles

IV. Codominance

A. **Codominance**

B. **MN blood group**

1. Glycoprotein
2. One or both

C. Control of MN system

1. Three different combinations possible

Genotype	*Phenotype*
$L^M L^M$	M
$L^M L^N$	MN
$L^N L^N$	N

2. Mating between two MN parents

V. Multiple Alleles

A. **Multiple allelism**

1. Populations.
2. Diploid organisms
3. **ABO blood group**
   a. Karl Landsteiner
   b. **Antigens**
   c. Chromosome 9
   d. Codominant mode of inheritance
   e. Antisera
   f. A antigen (A phenotype), the B antigen (B phenotype), bother the A and B antigens (AB phenotype), or neither antigen (O phenotype).
   g. 1924
   h. Symbols for this system
      1. **Isoagglutinogen**
      - I^A, I^B, and I^O
      - Genotypic possibilities:

Genotype	*Antigen*	*Phenotype*
$I^A I^A$	A	A
$I^A I^O$	A	A
$I^B I^B$	B	B
$I^B I^O$	B	B
$I^A I^B$	A and B	AB
$I^O I^O$	Neither	O

2. **Bombay phenotype**
3. Eye color of *Drosophila*

VI. Lethal Alleles

A. Heterozygous state
   1. **Recessive lethal allele**
   2. Death of individual
B. Dominant with respect to phenotype, recessive with respect to genotype
   1. Yellow coat color in mice
   2. Agouti (wild-type)
   3. Transparency 20, Page 72, Figure 4-3
C. **Dominant lethals**
   1. Lethal effect
   2. **Huntington's Disease**
      a. Adulthood
      b. Nervous and motor degeneration

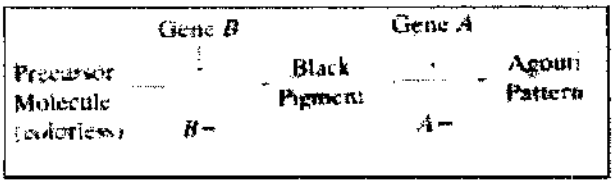
VII. Combinations of Two Gene Pairs

A. **Discrete**, or **discontinuous, phenotypes**
B. **Gene interaction**
   1. Development of a common phenotype
   2. Insect's complex eye
C. **Discontinuous variation**
D. **Continuous variation**

VII. Epistasis

A. **Epistasis**

1. Best examples of gene interaction
2. Different conditions.
   a. Homozygous recessive form
      1. **Epistatic**
      2. **Hypostatic**
3. ABO blood group
   a. Homozygous condition (*hh*)
   b. H-genotype
   c. (Transparency 21, Page 74, Figure 4-5)
4. Assumptions
5. Other examples of genes interacting and recombining
   a. Coat color in mice
      1. Agouti
   b. Another type of epistasis
      1. Fruit color squash



      2. White fruit
      3. *A* allele absent (*aa*)
      4. Cross between two heterozygotes (*AaBb*)
6. Novel phenotypes

VIII. Complementation Analysis and Alleles

A. **Complementation analysis**

B. Wingless *Drosophila*

1. Two alternative outcomes and interpretations.
   a. Case 1
   b. Case 2
2. **Complementation**

3. **No complementation**
4. **Complementation group**

IX. Genes on the X Chromosome

A. **X-linkage**
   1. X and Y chromosomes
   2. In *Drosophila*

B. Thomas H. Morgan
   1. Eye color
   2. Eye color was clearly related to the sex of the parent carrying the mutant allele
   3. F_1 and F_2 data
   4. **X-linked**
   5. **Hemizygous**
   6. **Crisscross pattern of inheritance**

C. Humans and X-linked genes
   1. **Pedigree** (Transparency 23, page 81, figure 4-11)
   2. Lesch-Nyhan syndrome
   3. Circumstances associated with recessive autosomal disorders
      a. **Duchenne muscular dystrophy**
      b. Transparency 24, Page 82, Table 4.2

X. Sex-Limited and Sex-Influenced Inheritance

A. Inheritance affected by the sex of the organism

B. Domestic fowl
   1. Cock feathering
   2. Hen feather
   3. **Sex-limited inheritance**

C. **Sex-influenced inheritance**: **pattern baldness**

Lecture Content

Chapter four: Modification of Mendelian Ratios

I. Potential Function of an Allele

A. **Alleles** are alternative forms of the same gene, which contains modified genetic information and often specifying an altered gene product.
   1. For example, in humans there are well over 100 known alleles of the genes that specify the protein portions of hemoglobin.
   2. These alleles store information for the synthesis of a polypeptide chain of hemoglobin, but each allele specifies a modification of the chemical composition.
   3. A **wild-type allele** occurs most frequently in a population and is arbitrarily designated as normal.

B. A **mutation** is a source of new alleles.
   1. A new phenotype *may* result from a mutation.
   2. The mutation represents a separate allele, which may reduce or enhance rather than eliminate the functional capacity of the gene product, hence it could possible be a "good" or "bad" mutation.

II. Symbols for Alleles

A. A simple review of the notations for alleles.
   1. For a recessive allele, we use the lower case form of the first letter of the phenotype, whereas for the dominant allele we use the uppercase form of the letter.
   2. For example, in Mendel's pea plants, there were tall and dwarf plants, D was used for tall (the dominant genotype), while d was used for dwarf (the recessive genotype).

B. Another system was developed using *Drosophila*, to discriminate between the wild-type and the mutant traits.
   1. In this system, if the trait is recessive, the lowercase form of the letter is used; if it is dominant the uppercase form of the initial letter is used.
   2. The contrasting wild-type trait is denoted by the same letter, but uses a + as a superscript.

3. An example would be body color in the fruit fly *Drosophila*.
   a. *Ebony* is a recessive body color mutation in the fly; gray is the wild-type color.
   b. Using this system, *ebony* is denoted by the symbol *e*, while gray is denoted by e^+.
   c. Therefore, a diploid fly can have three possible genotypes:

      e^+/e^+ gray homozygote (wild type)

      e^+/e gray homozygote (wild type)

      e/e ebony homozygote (mutant)

   d. The slash is used to designate that these two alleles represent the same locus on two homologous chromosomes.
   e. An advantage to using this system, is that the "+" sign can be used to designate the wild-type allele.
   f. For example,

      +/+ gray homozygote (wild type)

      +/*e* gray homozygote (wild type)

      e/*e* ebony homozygote (mutant)

C. It is useful to recognize, when considering each cross, all other genes, which are not under consideration, are assumed to have no effect on the inheritance patterns described.

III. Incomplete or Partial Dominance

A. **Incomplete** or **partial dominance** is based on the observation of intermediate phenotypes generated by a cross between parents with contrasting traits.
   1. An example would be, with flowers such as snapdragons, if red and white colored flowers were crossed, the resulting phenotype of the F_1 generation would be pink.
   2. It is obvious that neither the red nor the white flower color is dominant, and that some of the red pigment shows up in the offspring producing a pink color.
   3. Since this phenotype is under the control of a single pair of alleles, and neither is dominant, the results of the F_1 (pink) X F_1 (pink) can be predicted.

a. The genotypic ratio of the F_2 generation is identical to that of Mendel's monohybrid cross.
b. Because there is no dominance, however, the phenotypic ratio is identical to the genotypic ratio.
c. In a cross like this, it is common to label the alleles as R^1 and R^2 to denote the red and white alleles.

IV. Codominance

A. **Codominance** is when there are two alleles responsible for the production of two distinct, detectable gene products.
B. The **MN blood group**, present in humans, is an example that illustrates this phenomenon and is characterized by a glycoprotein, which is present on the surface of the red blood cell.
   1. Two forms of this glycoprotein exist, designated M and N.
   2. Any individual can exhibit either one or both of them.
C. The MN system is under the control of an autosomal locus found on chromosome 4 and two alleles designated L^M and L^N.
   1. Since humans are diploid, there are three different combinations possible and each results in a distinct blood type.

Genotype	*Phenotype*
$L^M L^M$	M
$L^M L^N$	MN
$L^N L^N$	N

   2. Mating between two MN parents can produce offspring with all three blood types: $L^M L^N$ X $L^M L^N$

$\downarrow$

¼ $L^M L^M$

½ $L^M L^N$

¼ $L^N L^N$

V. Multiple Alleles

A. **Multiple allelism** is when there are three or more alleles of the same gene.

1. The concept of multiple alleles can only be studied within populations.
2. A diploid organism has two homologous gene loci that may be occupied by different alleles of the same gene; however, among members of a species, many alternative forms of the same gene may exist.
3. The **ABO blood group** in humans illustrates the simplest form of multiple allelism with three alternative alleles.
   a. Karl Landsteiner discovered this group in the early 1900s.
   b. The ABO system is characterized by the presence of **antigens** on the surface of red blood cells.
   c. These antigens are under control by a gene located on chromosome 9.
   d. One combination of alleles in the ABO system exhibits a codominant mode of inheritance.
   e. Individuals are tested using antisera containing antibodies against the A or B antigen, and four different phenotypes are revealed.
   f. An individual has either the A antigen (A phenotype), the B antigen (B phenotype), bother the A and B antigens (AB phenotype), or neither antigen (O phenotype).
   g. In 1924, it was hypothesized that these phenotypes were inherited as the result of three alleles for a single gene.
   h. For this system, we use the symbols I^A, I^B, and I^O for the three alleles.
      - The *I* designation stands for **isoagglutinogen**, which is another term for antigen.
      - I^A and I^B stand for their appropriate A and B antigens, while I^O is an allele that does not produce any antigens.
      - The various genotypic possibilities can be listed and the appropriate phenotype assigned to each:

Genotype	*Antigen*	*Phenotype*
$I^A I^A$	A	A
$I^A I^O$	A	A
$I^B I^B$	B	B
$I^B I^O$	B	B
$I^A I^B$	A and B	AB
$I^O I^O$	Neither	O

4. The **Bombay phenotype** is a mutation of the **H substance** and results in type O blood.
   a. The A and B antigens are based on terminal sugars of a carbohydrate group, and are both derived from the precursor molecule known as the H substance.
   b. This condition was first noted in a woman in Bombay, who had the recessive mutation *h* and had O blood, even though her father was type AB.
   c. This mutation causes the H substance to be incompletely formed, and there is inadequate substrate for the enzyme that normally adds either terminal sugar.
5. Another example of multiple allelism is found in the eye color of *Drosophila*.
   a. Thomas H. Morgan and Calvin Bridges discovered the recessive mutation that causes white eyes in *Drosophila*, in 1912.
   b. It was also noted that there are over 100 alleles that may occupy this locus.

VI. Lethal Alleles

A. The heterozygous state can sometimes tolerate mutations that result in the synthesis of a gene product that is nonfunctional.
   1. When such a mutation is present in the homozygous state, however, it is known as the **recessive lethal allele**.
   2. The time of death of an individual with the recessive lethal allele, depends on when the product is essential, in development or in adulthood.

B. There are situations where such an allele behaves dominant with respect to phenotype, even though it is recessive with respect to genotype.
   1. An example would be the yellow coat color in mice.

2. The yellow coat varies from the normal agouti (wild-type), and when crossed shows unusual results.
3. Transparency 20, page 72, figure 4-3.

C. There are also alleles that act as **dominant lethals**.
   1. With a dominant lethal, only one copy is necessary for the lethal effect.
   2. An example would be **Huntington's Disease** in humans.
      a. The effect is not seen until adulthood.
      b. The individual experiences gradual nervous and motor degeneration, until he/she dies.
      c. Since this disease has such a late onset, many affected individuals have already had a family, and each child has a fifty percent chance of being inflicted with it as well.

VII. Combinations of Two Gene Pairs

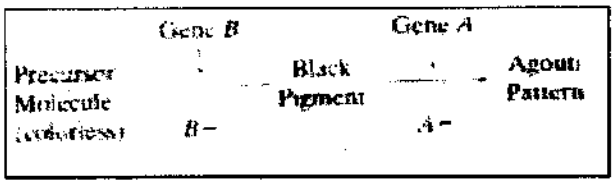
A. After the rediscovery of Mendel's work, it is was discovered that individual characteristics that display **discrete**, or **discontinuous, phenotypes** are often under the control of more than one gene.
B. The concept of **gene interaction** does not mean that two or more genes, or even their products interact directly.
   1. The cellular function of the numerous gene products is related to the development of a common phenotype.
   2. An example would be that of an insect's complex eye.
      a. The development of the eye can be envisioned as occurring as the result of a "complex cascade of developmental events leading to its formation.
      b. This process also illustrates the developmental concept of epigenesis.
C. **Discontinuous variation** is when discrete phenotypic categories are produced that vary from one another.
D. **Continuous variation** is when phenotypic categories vary in a quantitative way.

VII. Epistasis

A. **Epistasis** occurs when the expression of one gene or gene pair masks the expression of another gene pair.

1. Epistasis is one of the best examples of gene interaction leading to discontinuous variation.
2. This phenomenon of epistasis might occur under different conditions.
   a. One example would be if the homozygous recessive form was present, and that locus might prevent or override the expression of other alleles at the second locus.
      - The alleles at the first locus, in this case, are said to be **epistatic** to those at the second locus.
      - The alleles at the second locus are said to be **hypostatic** to those at the first locus.
3. Another example involves the human ABO blood group.
   a. The homozygous condition (*hh*) masks the expression of both I^A and I^B alleles.
   b. Only the individuals with the H-genotype can from and express the A or B antigen.
   c. There is an example of outcomes of matings between individuals who are heterozygous at both loci (Transparency 21, page 74, figure 4-5).
4. As we discuss other examples, it is important to realize the assumptions that we make when considering gene interactions:
   a. Distinct phenotypic classes are produced, each discernible from all others, and they illustrate discontinuous variation.
   b. The genes that are considered are not linked on the same chromosome; therefore, they assort independently during gamete formation.
   c. When speaking of a dominant allele, the uppercase letter is used to distinguish it from the recessive allele (lowercase), and a (-) mark means that it could be either dominant or recessive, as in the case of A- or B-.
   d. All parental crosses involve homozygous individuals; therefore, all the first generation is heterozygous.
   e. The second generation produced is always the one under analysis, and fall into four main categories: 9/16 A-B-, 3/16 A-bb, 3/16aaB-, and 1/16 aabb (Transparency 22, page 75, figure 4-6).

5. There are many other examples of genes interacting and recombining, resulting in modifications of the 9:3:3:1 ratio.
   a. The first example is the inheritance of coat color in mice.
      - Agouti is the normal wild-type color of mice and is dominant to black, which is caused by a recessive mutation *a*.
      - If the mouse is homozygous for a recessive mutation *b* at a separate locus, the pigment is eliminated all together, and the result is an albino mouse.
      - A cross between agouti (AABB) and albino (aabb) yields all AbBb progeny, but when the F_1 generation our crossed, the results are 9/16 agouti, 4/16 albino, and 3/16 black.
   b. The gene interaction yielding the observed 9:3:4 F_2 can be seen as a two step process.



      - If the B allele is present, black pigment can be made from a colorless substance.
      - If the A allele is also present, the black pigment is distributed in such a fashion that the agouti color becomes the phenotype.
      - If the *aa* genotype occurs, then the hair remains black, whereas if the *bb* genotype occurs, the mouse is albino.
   c. Another type of epistasis occurs when a dominant allele at one genetic locus masks the expression of the alleles at a second locus.
      - An example of this would be the fruit color in summer squash.
      - The dominant allele *A* results in white fruit, regardless of what alleles are present at other loci.
      - If the *A* allele is absent (*aa*), the *BB* or *Bb*, results in a yellow color, while the *bb* results in a green color.
      - A cross between two heterozygotes (*AaBb*) yields interesting results.
        - 9/16 are *A-B-* and are thus white

- 3/16 bear the genotype *A-bb*, and are also white.
- 3/16 are yellow (*aaB-*).
- 1/16 are green (*aabb*).
- When combined, this ratio is 12:3:1.

6. There are also novel phenotypes, for example, the inheritance of fruit shape in the summer squash.
   a. When a plant with a disc-shaped fruit (*AABB*) is crossed with a plant with long fruit (*aabb*), the F_1 generation all have disc shaped fruit.
   b. The F_2 progeny however, result in a novel shape, a sphere.
      - The F_2 generation exhibits a modified 9:6:1 ratio.
      - 9/16 disk, 6/16 sphere, and 1/16 are long.

VIII. Complementation Analysis and Alleles

A. **Complementation analysis** is an easy approach to utilize to determine if specific noted mutations are within the same gene.

B. For instance, scientists from both the United States and Canada, found specific mutations which occur *Drosophila* to be wingless, but the question was, was were they the same mutation on the same gene.
   1. There are two alternative outcomes and interpretations.
      a. Case 1: All offspring develop normal wings.
         - Interpretation: The two recessive mutations are in separate genes, and are not alleles of one another.
         - Following the cross, all the F_1 flies became heterozygous for both.
      b. Case 2: All offspring fail to develop wings.
         - Interpretation: The two mutations affect the same gene and are alleles of one another.
         - All offspring are homozygous for the mutations in the genes, and fail to develop any wings.
   2. In case 1, **complementation** occurs, or they are nonallelic.
   3. In case 2, **no complementation** occurs, because the two mutations affect the same gene.

4. All mutations determined to be present in any single gene are said to fall in the same **complementation group**; all mutations in the same complementation group complement mutations in all other groups.

IX. Genes on the X Chromosome

A. Another mode of neo-Mendelian inheritance is known as **X-linkage**.
   1. One of the sexes in many animals and in a few plant species, contain a pair of unlike chromosomes, the X and the Y.
   2. In *Drosophila*, like in humans, females have two copies of the X chromosome; therefore, the males who have the Y chromosome are subject to the transmission and expression of the genes located on the X.

B. One of the first X-linkage cases was documented by Thomas H. Morgan, while working with *Drosophila*, around 1920.
   1. He discovered this while working with the eye color.
   2. The normal wild-type red eye color is dominant to white.
   3. Morgan noticed that the white eye color was clearly related to the sex of the parent carrying the mutant allele.
   4. When crosses were performed, the F_1 and F_2 data were very similar regardless of which parent exhibited the recessive mutant trait.
   5. Morgan's analysis resulted in his conclusion that the *white* allele was located on the X chromosome, and is therefore called **X-linked**.
   6. Morgan hypothesized that the recessive allele for the white eye is located on the X chromosome, but its corresponding locus is absent from the Y chromosome.
      a. Therefore, females have two possible gene sites, whereas males only have one.
      b. Whatever alleles are present on the nonhomologous part of the X chromosome, will be expressed.
      c. Since males cannot be termed either homozygous or heterozygous with respect to X-linked genes, they are termed **hemizygous**.
   7. A **crisscross pattern of inheritance** is present when a mother is homozygous recessive for an X-linked gene, and passes it to all of her sons.

C. There are also many different disorders and specific phenotypes present in humans that are due to X-linked genes.
   1. These X-linked traits may be easily identified in a **pedigree** because of the crisscross pattern of inheritance (Transparency 23, page 81, figure 4-11).
   2. Genes controlling two forms of hemophilia and one form of muscular dystrophy are located on the X chromosome, as well as Lesch-Nyhan syndrome.
   3. There are certain circumstances associated with recessive autosomal disorders.
      a. If an X-linked disorder debilitates or is lethal to the affected individual prior to reproductive maturation, the disorder occurs exclusively in males.
      b. Heterozygous females also pass the allele to one-half of their daughters, who become carriers, but do not develop the disorder.
      c. An example of this is **Duchenne muscular dystrophy**, which has an onset prior to age 6, and is usually lethal prior to age 20.
      d. Transparency 24, page 82, table 4.2.

X. Sex-Limited and Sex-Influenced Inheritance

A. The following topic involves inheritance affected by the sex of the organism, but not necessarily by the genes on the X chromosome.

B. An example would be neck and tail plumage in domestic fowl.
   1. Cock feathering is longer, more curved and pointed.
   2. Hen feather is shorter and more rounded.
   3. This demonstrates **sex-limited inheritance**.

C. Cases of **sex-influenced inheritance** include **pattern baldness** in humans.
   1. Autosomal genes are responsible for the contrasting phenotypes displayed by both males and females, but the expression of the genes is dependent on the hormone constitution of the individual.
   2. Even though females may display pattern baldness, the phenotype is much more prevalent in males.

Lecture Outline

Chapter Five: Quantitative Genetics

I. Quantitative Inheritance

A. Sir Francis Galton

1. F_1 generation
2. F_2 generation
3. Continuous variation
4. **Quantitative inheritance**.

B. Josef Gottlieb Kolreuter

1. F_1 generation
2. F_2 generation
   a. Intermediate height
   b. Figure 5-1 in text

C. William Bateson and Gudny Yule

1. **Multiple-factor**, or **multiple-gene hypothesis**
2. Quantitative way

D. 1920s

1. Edward M. East
2. Corollas
3. True breeding
4. F_1 generation
5. F_2 generation
6. Bell-shaped curve, Page 93, figure 5-2
7. F_3 generation
8. Continuous phenotype

E. Major points for multiple-factor hypothesis

1. Characters exhibit continuous variation.
2. *Polygenic*
3. **Additive** or **nonadditive alleles**
4. Total effect of each additive allele
5. Substantial phenotypic variation

6. Large numbers of progeny

F. Herman Nilsson-Ehle

1. Grain color in wheat
2. Crossed red grain with white grain
   a. F_1 generation
   b. F_2 generation
   c. Closer examination of F_2 generation
      1. P_1 parents
      2. F_1 generation
      3. The F_2 generation
3. Conclusions
4. 1:4:6:4:1 ratio
5. Page 95, figure 5-4 in textbook

G. Number of genes involved

1. Formula for number of gene pairs involved (*n*)

$$\frac{1}{4^n} = \text{ratio of } F_2 \text{ individuals expressing either extreme phenotype}$$

2. Example

$$\frac{1}{4^n} = \frac{1}{16}$$

$$\frac{1}{4^2} = \frac{1}{16}$$

$$n = 2$$

3. Low number of gene pairs, use $2n + 1$
   a. $2n + 1$ equals total number of possible phenotypes
   b. Example

H. Polygenic control

II. Analysis of Polygenic Traits

A. Quantitative measurements

1. Transparency 43, Page 97, Figures 5-5 and 5-6
2. Frequency diagram

3. Experimental validity of the data.
4. Galton
5. Three purposes for statistical analysis
   a. **Descriptive summary** of sample
   b. **Statistical inference**
   c. Compare two or more data sets
6. Statistical methods
7. **Mean**
   a. **Central tendency**
   b. Formula

$$\overline{X} = \frac{\Sigma X_i}{n}$$

   c. $\overline{X}$ = mean, ΣX_i = sum of all the individual values, and n =number of individual samples
   d. Figure 5-6 in text
   e. **Frequency distribution**
8. **Variance**
   a. Divergence from mean
   b. Formula

$$s^2 = \frac{\Sigma (X_i - \overline{X})^2}{n - 1}$$

   c. (Σ)= sum of squared differences between each measured value $\overline{(X_i)}$, mean (X) is divided by one less than the total sample size $(n - 1)$
   d. Large population size

$$s^2 = \frac{\Sigma X_i - n \overline{X}^2}{n - 1}$$

9. **Standard deviation**
   a. Square root of variance
   b. Formula

$$s = \sqrt{\overline{s^2}}$$

   c. Table 5.2 in the text

d. 95% found within two standard deviations

e. Also interpreted as a probability

10. **Standard error of the mean**

a. Formula

$$S_{\bar{X}} = \frac{s}{\sqrt{n}}$$

b. Always smaller than standard deviation

11. Example

a. Transparency 44, page 99, Table 5.3

b. Fruit weight in tomatoes

c. Inbred strains

d. F_1 generation

e. F_2 generation

f. Mean value for F_1 generation

$$\bar{X} = \frac{\Sigma X_i}{n} = (626/52) = 12.04$$

g. Mean value for F_2 generation

$$\bar{X} = \frac{\Sigma X_i}{n} = (872/72) = 12.11$$

h. Average weight for both generations

i. Variance

1. Transparency 45, page 99, Table 5.4

2. Formula

$$s^2 = \frac{n \Sigma f(x^2) - (\Sigma fx)^2}{n(n-1)}$$

3. The results for F_1 generation and F_2 generations

4. Converted to standard deviation

5. Conclusion

6. Gene pairs associated with fruit weight

- Phenotype of F_2 offspring
- Results

III. Heritability

A. Causes of variation

1. **Genetic background**
2. Environmental conditions on phenotypic variability
3. Due to genetic factors
4. Due to nongenetic factors

B. **Heritability index (H^2)**

1. **Broad-sense heritability**, H^2, **phenotypic variance** (V_P)
2. Sum of three components
   a. **Environmental variance** (V_E)
   b. **Genetic variance** (V_G)
   c. Interaction of genetics and environment (V_{GE})
   d. Formula

$$V_P = V_E + V_G + V_{GE}$$

   e. V_{GE} is often excluded

$$V_P = V_E + V_G$$

   f. Broad heritability

$$H_2 = \frac{V_G}{V_P}$$

   g. H_2 values
   h. Broad-sense heritability estimates are not very accurate

C. **Narrow-sense heritability**

1. **Additive variance** (V_A), Formula

$$V_G = V_A + V_D + V_I$$

2. **Dominance variance** (V_D)
3. **Interactive variance** (V_I), usually omitted from the equation
4. Formula

$$h^2 = \frac{V_A}{V_P}$$

Since $V_P = V_E + V_G$ and $V_G = V_A + V_D$, we obtain the formula:

$$h^2 = \frac{V_A}{V_E + V_A + V_D}$$

D. Impact of selection

1. Simplified way

a. Measurements of the central tendencies from

1. Parental population with bell-shaped distribution (M)
2. "Selected" segment of the parental (M1)
3. Offspring (M2)

b. Formula

$$M2 = M + h^2(M1 - M)$$

c. Solving for h^2 leads to this formula

$$h^2 = \frac{M2 - M}{M1 - M}$$

d. Simplified even more

1. **Response** (R) [M2 – M], **Selection differential** (S) [M1 – M]
2. Formula

$$h^2 = \frac{R}{S}$$

2. Example

a. Interbreed the selected population; estimate the potential for artificial selection on kernel size

b. Formula

$$h^2 = \frac{20 - 30}{15 - 30}$$

$$h^2 = \frac{-10}{-15} = 0.67$$

c. Conclusion

d. Table 5.5 in text

e. Heritability is low for certain traits

E. Heredity-versus-environment

1. **Monozygotic**, or **identical twins**

a. Exactly the same

b. Rearing of twins

c. Good study subjects

1. Traits with strong genetic component

2. Traits affected by environment more genetics

2. **Dizygotic**, or **fraternal twins**

a. Not genetically identical

b. Not great study subjects alone

3. **Concordant** versus **discordant**

4. High concordance value

a. Measles

b. Eye color

5. Compare monozygotic and dizygotic twins

IV. Mapping Quantitative Trait Loci

A. **Chromosome mapping**

B. **Quantitative trait loci (QTLs)**

C. Example, *Drosophila*

1. Select resistant and nonresistant flies

a. Cross with flies carrying markers

b. Transparency 46, Page 109, Figure 5-8

c. Tested for survival

d. Results

2. Position of genes

a. QTLs

b. **Restriction fragment length polymorphisms (RFLPs)**

D. Example, tomato

1. Markers on twelve chromosomes

2. Cross plants with extreme, opposite polygenically controlled phenotypes

3. See if RFLP marker and QTL demonstrate an association together

4. Cosegregate

5. Genetic map

E. Locations of QTLs for agricultural characteristics

Lecture Content

Chapter Five: Quantitative Genetics

I. Quantitative Inheritance

A. Sir Francis Galton investigated the diameter of sweet peas.
   1. F_1 plants produced from a cross of large peas with small peas, were all of intermediate diameters.
   2. The F_2 generation produced peas of various sizes, including small, large, and in-between diameters.
   3. This example shows that the F_1 generations were an intermediate blend of the parental generation, and the F_2 generations exhibited continuous variation of phenotypic expressions.
   4. This genetic explanation of continuous variation is what serves as the foundation for the current understanding of the field of **quantitative inheritance**.

B. Josef Gottlieb Kolreuter encountered one of the first cases of continuous variation when he crossed tall and dwarf tobacco plants.
   1. All of the resulting F_1 generation plants exhibited intermediate heights.
   2. The F_2 generation plants had heights that were as tall and as short as the original parental generation, as well as many intermediate heights.
      a. The majority of the F_2 generation plants were intermediate, while only a few exhibited heights of the P_1 generation.
      b. Look at figure 5-1 in the text, these are histograms that show the relative frequency of individuals expressing the various phenotypic heights from this cross.

C. In the early 1900s, William Bateson and Gudny Yule suggested that there were a large number of factors or genes which could account for the observed variations.
   1. They proposed their idea as the **multiple-factor**, or **multiple-gene hypothesis**.
   2. This implied that there were many factors or genes, which contributed to the phenotype in a cumulative or quantitative way.

D. By the 1920s, there were many experiments performed that supported this hypothesis.

1. Edward M. East, who made crosses between the tobacco plant *Nicotiana longiflora*, performed one such experiment.
2. Strain A in his experiment, had fused inner petals, which were shorter than the ones in strain B.
3. Both strain A and strain B were true breeding.
4. The F_1 generation displayed corollas that were intermediate in length as compared to the P_1 generation.
   a. The corolla length of the P_1 generation ranged from 40 mm to 94 mm.
   b. The corolla length of the F_1 generation were on average 64 mm.
5. The F_2 generation displayed more variation in the corolla lengths, ranging from 52 mm to 82 mm.
6. When the data are graphed, a bell-shaped curve results, look at page 93, figure 5-2.
7. East experimented with the corolla lengths by creating an F_3 generation, these data is also plotted on the graphs in figure 5-2.
8. The results of East's experiments demonstrated that the variation in corolla length, which seemed continuous, could be explained by the multiple-factor hypothesis.

E. There are a few major points for the multiple-factor hypothesis:
   1. Measuring, weighing, counting, etc. can usually quantify the characters that exhibit continuous variation.
   2. The hereditary influence can be accounted for by the fact that there are two or more pairs of genes, which act on the phenotype in an additive way, and because there are many genes, which could be involved, the inheritance is often called *polygenic*.
   3. Each of the gene loci may be occupied by either an **additive allele**, which contributes a set amount to the phenotype, or a **nonadditive allele**, which does not contribute quantitatively to the phenotype.
   4. The total effect of each additive allele at each locus is approximately equivalent to all other additive alleles at other gene sites.

5. Substantial phenotypic variation is produced from the genes controlling a single character.
6. Large numbers of progeny are required for the studies of polygenic traits.

F. Herman Nilsson-Ehle's experiments in the early twentieth century illustrated the major points of the multiple-factor hypothesis.
   1. Nilsson-Ehle performed experiments involving grain color in wheat.
   2. In one of his experiments, he crossed red grain with white grain.
      a. The F_1 generation expressed an intermediate phenotype.
      b. The F_2 generation had approximately 15/16 of the progeny with some degree of red grain, and only 1/16 with white grain.
         - Since the ratio occurred in sixteenths, we can hypothesize that there are two gene pairs that control the phenotype.
         - We can also hypothesize that these segregate independently from one another in a Mendelian fashion.
      c. When Nilsson-Ehle closely examined the F_2 generation, he noticed that there were four different shades of red grain.
         - Within this cross, both P_1 parents were homozygous; the red parent contained only additive alleles (expressed as uppercase), while the white parent contained only nonadditive alleles (expressed as lowercase).
         - The F_1 generation was heterozygous, and contained only two additive alleles, which caused them to express an intermediate phenotype.
         - The F_2 generation had 4, 3, 2, 1, or 0 additive alleles.
           - The wheat with 0 additive alleles (1/16) was white like one of the P_1 parents.
           - The wheat with 4 additive alleles was red like the other P_1 parent.
           - Plants that consisted of 3, 2, or 1 additive alleles, constituted the other three categories of red color, with most (6/16) containing 2 additive alleles, just like the F_1 plants.

3. Nilsson-Ehle's experiment showed that continuous variation could be explained in a Mendelian fashion, and that continuous variation resulted from the multiple-factor hypothesis.
4. If two gene pairs are involved, like in Nilsson-Ehle's experiment, the predicted ratio for the F_2 phenotypic categories is a 1:4:6:4:1 ratio.
5. Look at page 95, figure 5-4 in the textbook, this figure shows the expected ratios in crosses that involve up to five gene pairs.

G. When polygenic traits are controlled by additive effects, scientists usually want to know the number of genes that are involved.

1. When the ratio of F_2 individuals resembling either of the two most extreme phenotypes can be determined, then the number of gene pairs involved (n) can be calculated with this simple formula:

$$\frac{1}{4^n} = \text{ratio of } F_2 \text{ individuals expressing either extreme phenotype}$$

2. For example, in Nilsson-Ehle's experiment, 1/16 of the F_2 were white like the P_1 classes; therefore, the equation would look like:

$$\frac{1}{4^n} = \frac{1}{16}$$

$$\frac{1}{4^2} = \frac{1}{16}$$

$$n = 2$$

3. When the number of gene pairs is low, it is sometimes easier to use the $(2n + 1)$ rule.
   a. In this equation, n still equals the number of gene pairs, and $2n + 1$ will equal the total number of possible phenotypes.
   b. For example, when n=2, $2n + 1$=5, therefore there are five possible categories for the phenotypic variation: 4, 3, 2, 1, and 0 additive alleles.

H. Polygenic control is believed to serve as the mode of inheritance for a vast number of traits among animals as well as plants.

1. Height, weight, stature, beef and milk production in cattle, egg production in chickens, and size and grain yield in crops are all thought to be under polygenic control.
2. The genotype, which is fixed at fertilization, establishes the potential range in which a phenotype may fall, but it is important to realize that the environment can determine how much of that potential is utilized.

II. Analysis of Polygenic Traits

A. The analysis of any given polygenic trait involves the quantitative measurements from many offspring derived from many crosses.
   1. Transparency 43, page 97, figures 5-5 and 5-6.
   2. The results from the crosses can be expressed as a frequency diagram that will often demonstrate a normal (bell-shaped) curve.
   3. Statistical techniques must be employed so that an assessment can be made concerning the experimental validity of the data.
   4. Galton, early in this century, devised these techniques, in the field of study known as **biometry**.
   5. The statistical analysis serves three purposes:
      a. One purpose is so the data can be mathematically reduced to provide a **descriptive summary** of the sample.
      b. Data from a small, but random sample can be utilized to infer information about groups larger than those from which the original data were obtained, this is known as **statistical inference**.
      c. Finally, two or more sets of data can be compared to determine whether they represent significantly different populations of measurements.
   6. The most useful statistical methods for the analysis of traits include the mean, variance, standard deviation, and standard error of the mean.
   7. The **mean** is the arithmetic average of a set of measurements or data.
      a. When the distribution of two sets of phenotypic measurements is graphed, it seems to cluster around a central value, called **central tendency**.
      b. The mean can be calculated as:

$$\overline{X} = \frac{\Sigma X_i}{n}$$

c. In this equation, $\overline{X}$ is the mean, ΣX_i represents the sum of all the individual values in the sample, and n is the number of individual samples.

d. As seen in figure 5-6, a symmetrical distribution may be clustered near the mean, or the values may have the same mean, but be distributed widely around it.

e. The contradicting conditions are referred to as the **frequency distribution**, the variation that is possible, creates the need for methods to describe the sample measurements statistically.

8. **Variance** is used as an estimate of the variation present in an infinitely large population.

a. This shows the degree to which values within this distribution diverge from the mean.

b. The variance can be calculated by this formula:

$$s^2 = \frac{\Sigma (X_i - \overline{X})^2}{n - 1}$$

c. In this formula, the sum (Σ) of the squared differences between each measured value (X_i) and the mean ($\overline{X}$) is divided by one less than the total sample size ($n - 1$).

d. When there is a large population size, we can convert the equation to avoid the numerous subtraction functions to :

$$s^2 = \frac{\Sigma X_i - n\overline{X}^2}{n - 1}$$

e. This estimation of variance has been very useful in determining the degree of genetic control of traits when the immediate environment also influences the phenotype.

9. The **standard deviation** must be calculated to express the variation in the original units of measurement.

a. Since variance is calculated as a squared value, the units are also squared; therefore the standard deviation, which is just the square root of the variance, must be calculated.

b. The formula for standard deviation is:

$$s = \sqrt{s^2}$$

c. Table 5.2 in the text, shows the percentage of the individual values within a normal distribution, as well as the different multiples of the standard deviation.

d. The mean plus or minus one standard deviation includes 68% of the values within the sample, and over 95% are found within two standard deviations.

e. The standard deviation can also be interpreted as a probability; for example, there is a 68% probability that a measured value that is picked at random will fall within that range.

10. The **standard error of the mean**, estimates how much the means of other similar samples from the same population could vary.

a. The standard error of the mean is a measure of the accuracy of the sample mean, and can be calculate with the following formula:

$$S_{\overline{X}} = \frac{s}{\sqrt{n}}$$

b. Since the equation divides *s* by the square root of *n*, it is always smaller than the standard deviation.

11. The following is an example on how to analyze a quantitative character.

a. Transparency 44, page 99, Table 5.3.

b. First, we are going to be using fruit weight in tomatoes.

c. The first strain is a highly inbred strain that produces tomatoes averaging 18 oz. in weight, while the second strain, another highly inbred strain, produces tomatoes averaging 6 oz. in weight.

d. These two strains are crossed, and the resulting F_1 generation produce fruit ranging in weight from 10 oz. to 14 oz.

e. The F_2 generation contains individuals, which produce fruit that range from 6 oz. to 18 oz.

f. The mean value for the fruit weight for the F_1 generation is :

$$\overline{X} = \frac{\Sigma X_i}{n} = (626/52) = 12.04$$

g. The mean value for the fruit weight of the F_2 generation is:

$$\overline{X} = \frac{\Sigma X_i}{n} = (872/72) = 12.11$$

h. Therefore, the average weight for fruit in the F_1 generation is 12.04 oz, whereas in the F_2 generation it is 12.11 oz.

i. We need to calculate the variance to assist us in quantifying the amount of variation present in each generation.

- Transparency 45, page 99, Table 5.4.
- In this case, a number of observations were grouped together into representative classes; therefore we must use this equation:

$$s^2 = \frac{n \Sigma f(x^2) - (\Sigma fx)^2}{n(n-1)}$$

- The results of this formula are shown on Table 5.4, the value for the F_1 generation is 1.29, while the value for the F_2 generation is 4.27.
- When the values are converted to standard deviation, they become 1.13 and 2.06, respectively; therefore, the distribution for the F_1 generation can be stated as 12.04 ± 1.13, and the distribution for the F_2 generation can be stated as 12.11 ± 2.06.
- This analysis indicates that the mean weight of the F_1 generation is exactly the same as the mean weight for the F_2 generation, but that the F_2 generation shows more variability.
- For this example, if we assume that each parental strain is homozygous for the additive or nonadditive alleles that are involved with fruit weight, we are able to estimate the number of gene pairs associated with fruit weight in their strains.

- Since only 1/72 of the F_2 offspring had a phenotype that overlapped with one of the parental strains, the formula $1/4^n = 1/72$ indicates that n must be in-between 3 and 4.
- This leads to the conclusion that there are at least 3, possibly 4, genes controlling fruit weight in these strains of tomato plants.

III. Heritability

A. There have been many experiments on both plants and animals to test the causes of variation, since it possible that a trait can be measured quantitatively.
   1. One possibility is to use inbred strains containing individuals of a relatively homogenous **genetic background**.
   2. These experiments test the effects of the range of prevailing environmental conditions on phenotypic variability.
   3. It is thought that variations present between different inbred strains reared in a constant environment are due to genetic factors.
   4. Variations that are observed between individuals of the same strain reared under different environmental conditions are due to nongenetic factors, which are generally characterized as "environmental."

B. The **heritability index (H^2)**, can be calculated using an analysis of variance among individuals of a known genetic relationship, can reveal the relative importance of genetic versus environmental factors.
   1. The **broad-sense heritability**, H^2 measures the degree of **phenotypic variance** (V_P) that is due to variation in genetic factors for a single population.
   2. This calculation does not determine the proportion of the total phenotype attributed to genetic factors, it does however estimate the proportion of observed variation in the phenotype attributed to genetic factors as compared to environmental factors.
   3. The phenotypic variance is actually the sum of three different components:
      a. **Environmental variance** (V_E)
      b. **Genetic variance** (V_G)
      c. The variance which results from the interaction of genetics and environment (V_{GE}).

d. The phenotypic variance is therefore expressed as :

$$V_P = V_E + V_G + V_{GE}$$

e. Since the V_{GE} is often negligible, it is often excluded from the equation, and what is left is:

$$V_P = V_E + V_G$$

f. Broad heritability expresses the proportion of variance due to the genetic component, and the formula for that is:

$$H_2 = \frac{V_G}{V_P}$$

g. An H_2 value that approaches 1.0, indicates that there has been little impact on the variance from the environment; however, an H_2 value that approaches 0, indicates that the environment is almost solely responsible for the variation seen with that particular population.

h. It is not possible to obtain an exact value for H_2 because the H_2 might change for a certain character under a greater or lesser degree of environmental variability; therefore, the broad-sense heritability estimates are not very accurate.

C. Another type of calculation, **narrow-sense heritability**, has been designed for a more practical use.

1. This uses a different estimate based on a subcomponent of V_G referred to as **additive variance** (V_A):

$$V_G = V_A + V_D + V_I$$

2. The V_A represents additive variance, which results from the average effect on additive components of genes.
3. The V_D represents **dominance variance**, which is a deviation from the additive components that result when phenotypic expression in heterozygotes is not exactly intermediate between the two homozygotes.
4. The V_I represents **interactive variance**, which is a deviation from the additive components that occur when two or more loci behave epistatically, this number is often negligible, therefore it is often omitted from the equation.

5. When V_G is partitioned into V_A and V_D a new assessment of heritability, h^2, or narrow-sense heritability is calculated.

$$h^2 = \frac{V_A}{V_P}$$

Since $V_P = V_E + V_G$ and $V_G = V_A + V_D$, we obtain the formula:

$$h^2 = \frac{V_A}{V_E + V_A + V_D}$$

D. The impact of selection altering a population leads us to predict that there will be a relatively high h^2 value.

1. Measuring the different values required for the calculation of h^2 can be very tedious; therefore, a more simplified way has been derived.
   a. This approach involves measurements of the central tendencies, or the means, of a trait from
      - A parental population that exhibits a bell-shaped distribution (M).
      - A "selected" segment of the parental population that expresses the most desirable quantitative phenotypes (M1).
      - The offspring that result from interbreeding the selected M1 group (M2).
   b. The relationship of the three means and h^2 can be calculated with this formula:

$$M2 = M + h^2(M1 - M)$$

   c. Solving this equation for h^2 leads to this formula:

$$h^2 = \frac{M2 - M}{M1 - M}$$

   d. The above equation can be simplified even more once the relationships are established.
      - By defining M2 – M as the **response** (R) and M1 – M as the **selection differential** (S), the h^2 reflects the ratio of the response observed to the total response that is possible.
      - The equation for the simplified form is:

$$h^2 = \frac{R}{S}$$

2. For an example, we could use the diameters of corn kernels.

a. If there were a population with a mean diameter (M) of 30mm, a larger diameter than desired, and from that population we selected the smallest kernels, with a mean diameter (M1) that equaled 15mm, we could interbreed that population and estimate the potential for artificial selection on kernel size.

b. The equation would be:

$$h^2 = \frac{20 - 30}{15 - 30}$$

$$h^2 = \frac{-10}{-15} = 0.67$$

c. The conclusion would be that the selection potential for kernel size is relatively high.

d. Table 5.5 in the text shows estimates of heritability for traits in various organisms.

e. In general, heritability is low for traits, which are essential to an organism's survival, because the genetic component has to be largely optimized during evolution.

- Egg production, litter size, and conception rates are examples of some of these essential traits.
- Traits such as body weight, tail length, and wing length show higher heritability rates.

E. Human twins are very useful for studying heredity-versus-environment.

1. Twins that are derived from the splitting of a single egg are called **monozygotic**, or **identical twins**.

a. Monozygotic twins are exactly the same with respect to genetics.

b. Although most monozygotic twins are reared together, there are some instances when they are split and reared in separate homes.

c. When they are reared in separate homes, they become great study subjects.

- Traits that remain similar between identical twins reared apart are thought to have a strong genetic component.
- Traits that are different between identical twins reared separately are considered to be affected by the environment more so than by genetics.

2. Twins that originate from two separately fertilized eggs are termed **dizygotic**, or **fraternal twins**.
   a. Fraternal twins are no more genetically similar than two siblings born from different conceptions.
   b. It is because of this that they do not make good subjects to study heritability-versus-environment.
3. Twins are said to be **concordant** for a trait if they both possess the trait or if neither posses the trait, and they are said to be **discordant** if one possess the trait and the other one does not.
4. If the concordance value is high, around 90 to 100 percent, it is usually suggested that the trait has a strong genetic component.
   a. There are some instances, for example measles, where even though there is a high concordance value, it is more likely to have an environmental factor acting on it, in this case a virus.
   b. For a trait such as eye color, we would expect monozygotic twins to have a high concordance value because it is known that eye color is genetic.
5. It is more meaningful to compare concordance values between monozygotic and dizygotic twins.
   a. If the values are significantly higher in monozygotic twins, as compared to dizygotic twins, it can be assumed that there is a strong genetic component with regard to this trait.
   b. We would assume that traits, which are controlled genetically, would result in higher concordance values in monozygotic twins, who are genetically identical, as compared to dizygotic twins, who are genetically similar, but not identical.

IV. Mapping Quantitative Trait Loci

A. **Chromosome mapping** is an exercise that initially relies on the localization of genes of interest on a particular chromosome.
B. This is a very important skill for geneticists to find the location of genes controlling the expression of quantitative traits, also called **quantitative trait loci (QTLs)**.

C. An example would be analyzing the phenotypic trait of resistance to DDT, an insecticide, in *Drosophila*.
    1. First, strains that were resistant to DDT were selected, as well as strains that were not resistant to DDT.
        a. These two strains were crossed with flies carrying markers on each of the *Drosophila's* four chromosomes.
        b. Transparency 46, Page 109, Figure 5-8.
        c. Following a variety of crosses, the various chromosome combinations were tested for survival when exposed to DDT.
        d. Each chromosome in *Drosophila* contains genes that contribute to resistance, as indicated by the results, in other words, the genes that control DDT resistance are scattered throughout the genome of *Drosophila*.
    2. The position of the genes on the chromosomes can be mapped because of the molecular markers, which were present on the chromosomes.
        a. The QTLs are determined relative to the position of the known markers.
        b. These markers are known as **restriction fragment length polymorphisms (RFLPs)**.

D. Another example concerns the tomato.
    1. Hundreds of DNA marker locations are known on the tomato's twelve chromosomes.
    2. Analysis is done by crossing plants with extreme, but opposite polygenically controlled phenotypes, looking for cosegregation of specific RFLPs along with the phenotype of the trait of interest.
    3. If an RFLP marker and a QTL, which is responsible for the specific trait, are closely linked on a chromosome, they are more likely to demonstrate an association together.
    4. When both the marker and the phenotype are expressed together through many generations, they are said to cosegregate.
    5. A genetic map is created when numerous QTLs are located.

E. The determination of the locations of QTLs for agriculturally important characteristics has permitted an efficient way of selecting and manipulating certain organisms.

Lecture Outline

Chapter Six: Linkage and Chromosome Mapping

I. Chromosomes and Genes

A. Many genes per chromosome

1. **Linked**
2. **Linkage**
3. Transferring
   a. Transmitted as one unit
   b. **Crossing over**
   c. **Recombination**
4. Distance between genes
   a. **Chromosome maps**
   b. Relative locations of genes

II. Linkage Versus Independent Assortment

A. Transparency 47, page 112, Figure 6-1

1. Figure 6-1
   a. Independent assortment
   b. No linkage exhibited
   c. Four different gametes
2. Genes linked on same chromosome
   a. No crossing over
   b. Two different gametes.
   c. **Complete linkage**
   d. Rare occurrence
3. Crossing over of two linked genes
   a. Two nonsister chromatids
   b. **Recombinant** or **crossover gametes**
4. Frequency of crossing over
   a. **Complete linkage**
   b. Small distance
   c. Increasing distance

5. No more than 50 percent recombinant gametes

B. Unique phenotype in F_2 generation

1. Example in *Drosophila*
2. *Brown (bw)* eyes and *heavy (hv)* wing veins, and *red (bw^+)* and *thin veins (hv^-)*
3. The cross
4. Expression of linked genes
5. P_1 generation

$$P_1 = \underset{\text{brown, thin}}{\frac{bw\ hv}{bw\ hv}} \qquad \underset{\text{red, heavy}}{\frac{bw^+\ hv}{bw^+\ hv}}$$

6. F_1 generation

$$F_1 = \underset{\text{red, thin}}{\frac{bw\ hv^+}{bw^+\ hv}}$$

7. F_1 gametes
8. Small number of progeny
9. Test cross

C. **Linkage group**

III. Incomplete Linkage, Crossing Over, and Chromosome Mapping

A. Thomas H. Morgan and Alfred H. Sturtevant

1. Transparency 48, page 115, Figure 6-3
2. Morgan's cross
   a. F_1 females and F_1 males
   b. F_1 generation
3. Another X-linked cross by Morgan
4. F.A. Janssens
   a. Chiasmata
   b. Points of overlap
   c. Points of exchange
   d. Exchange between two X chromosomes
5. **Crossing over**

B. Mapping sequence of genes

1. Sturtevant
2. Frequencies of crossing over
3. Distance between two genes
   a. Constructing a map
   b. Map unit
4. Other genes on X chromosome
   a. Sturtevant and Calvin Bridges
   b. 1923,
5. **Chromosomal theory of inheritance**

C. Limited number of cross over events
   1. **Single crossover** event
   2. Figure 6-5 in the text
      a. Nonsister chromatids
      b. Recombinant gametes
   3. 50 percent of potential gametes formed
      a. Percentage of tetrads involved in exchange
      b. No more than 50 percent

D. **Double crossovers**
   1. Figure 6-7 in the text
   2. Three genes must be involved
   3. Two separate and independent events
      a. Estimation of single crossovers
      b. Estimation of double crossovers
         - Example
         - Probability
         - Figure 6-7 in the textbook

E. Three-point mapping in *Drosophila* (Transparency 49, page 119, Figure 6-8)
   1. Three criteria
      a. Heterozygous loci
      b. Phenotype must reflect genotype
      c. Sufficient number of offspring

2. Figure 6-8 in the text
3. P_1 generation males and P_1 generation females
4. F_1 generation produces females and males
   a. Phenotype of the females
   b. F_1 males
5. **Noncrossover phenotypes**
6. **Reciprocal class**
7. Easily recognized
8. **Double-crossover phenotypes**
9. Four remaining phenotypic classes
10. Creating a map
    a. *y* and *w* genes
    b. *w* and *ec* genes
    c. The map

```
___y_____w_________ec__//____
|- 1.56 -| - - 4.06 - - -|
```

F. Determining the gene sequence if unknown
   1. Sequence is not always known.
   2. Three possible orders
      a. (I) *w-y-ec* (*y* is in the middle)
      b. (II) *y-ec-w* (*ec* is in the middle)
      c. (III) *y-w-ec* (*e* is in the middle)
   3. Certain steps
      a. Arrangement of the alleles
      b. Observed double-crossover phenotype
      c. Try the other two orders
   4. Figure 6-9 in the text
   5. Heterozygote could look like

$$\mathbf{I}\quad \frac{w\ \ y\ \ ec}{w^{+}\ y^{+}\ ec^{+}}$$

      a. Cross of P_1 generation
      b. P_1 female and P_1 male

c. Double crossover, map would look like

$$\underline{w \quad y^- \quad ec} \text{ and } \underline{w^- \quad y \quad ec^+}$$

6. Other two orders

$$\textbf{II} \ \frac{y \quad ec \quad w}{y^- \quad ec^- \quad w^+} \quad \text{or} \quad \textbf{III} \ \frac{y \quad w \quad ec}{y^+ \quad w^- \quad ec^-}$$

G. A mapping problem in maize (corn)

1. Transparency 50, page 122, Figure 6-10
2. Autosomal genes
3. + symbol
4. Same three criteria
5. Chromosome number 5; Example
   a. Female plant
   b. Male plant
6. Gametes divided into groups
   a. No crossing over **(NCO)**
   b. One of two possible single-crossover events **(SCO)**
   c. A double crossover event **(DCO)**
7. Two noncrossover classes
   a. They are

$$\underline{+ \quad v \quad m} \text{ and } \underline{pr \quad + \quad +}$$

   b. Unaffected by recombination
8. Yield observed double-crossover phenotypes
   a. Known arrangement of alleles

$$\frac{+ \quad v \quad bm}{pr \quad + \quad +}$$

   b. Other two orders

$$\frac{+ \quad bm \quad v}{pr \quad + \quad +} \qquad \frac{v \quad + \quad bm}{+ \quad pr \quad +}$$

   c. Latter arrangement
9. Map distance between alleles
   a. Figure 6-11 (e)
   b. Final map

v pr bm
-22.3-|----43.4----|

H. Impossible to detect all crossover events

1. Example
2. Even number crossover events

I. **Interference**

1. **Expected double crossovers**
   a. DCO
   b. In figure 6-10 and 6-11 in the text
   c. Expected DCO

$$DCO_{exp} = (0.223) \times (0.434) = 0.097 = 9.7\%$$

2. Expected DCOs larger than the observed number
   a. **Coefficient of coincidence** (*C*)

$$C = \frac{\text{observed DCO}}{\text{expected DCO}}$$

   b. The maize cross

$$C = \frac{0.078}{0.097} = 0.804$$

   c. Inference (*I*)

$$I = 1.0 - C$$

   d. The maize cross

$$I = 1.0 - 0.804 = 0.196$$

- No double crossovers occur
- **Positive inference**
- **Negative inference**

3. Eukaryotic systems
   a. Inhibition of second crossover event
   b. Close genes
   c. Mechanical stress

J. Figure 6-13 in textbook

IV. Somatic Cell Hybridization and Human Gene Mapping

A. Pedigree analysis

1. Difficulty in human pedigrees
2. **Lod score method**
   a. Two or more traits without linkage
   b. Calculate probability
   c. Ratio of probabilities
   d. Limited by the family data
3. **Somatic cell hybridization**
   a. Georges Barsky
   b. Two cells fused together
   c. Mouse cells
   d. **Heterokaryon**
   e. Hybrids can be isolated
   f. Two significant changes
      - **Synkaryon** forms
      - Chromosomes are randomly lost
      - Full complement of mouse chromosomes
   g. Production of human gene product
   h. Panel of cell lines
   i. **Synteny testing**
   j. Figure 6-14, for example
      - Cell line 23
      - Cell line 34
      - Cell line 41
   k. Gene C
   l. Gene D
      - Where is it located?
      - Answer – Chromosome 1.
4. Figure 6-15
5. Recombinant DNA technology with pedigree analysis

V. The Use of Haploid Organisms in Linkage and Mapping Studies

A. Single-celled eukaryotes

B. Small haploid organisms
   1. Cultured and manipulated easily
   2. Single allele for each gene

C. Meiotic structures are isolated
   1. **Tetrad**
   2. **Tetrad analysis**

D. Figure 6-16 in the text
   1. **Mapping the centromere**
   2. Ascospores
   3. No crossover events
      a. **First division segregation**
      b. Crossover events can alter pattern
      c. **Second division segregation**
      d. Six possible combinations of genotypes

First Division Segregation

a	*a*	–	+
+	–	*a*	*a*

Second Division Segregation

a	+	*a*	+
+	*a*	–	*a*
+	*a*	*a*	+
a	–	+	*a*

   4. Calculation for distance between gene and centromere

$$d = \tfrac{1}{2}\,\frac{\text{(second division segregant asci)}}{\text{(total asci)}}$$

      a. Distance (*d*)
      b. One-half the number of the second segregant asci
   5. Example involving *Neurospora*

VI. Other Aspects of Genetic Exchange

A. Curt Stern, Harriet Creighton, and Barbara McClintock
   1. Creighton and McClintock's
   2. Figure 6-17 illustrates the markers
   3. The cross

a. Many offspring

b. Results of experiments

B. Genetic exchange in mitosis

1. Reciprocal exchanges

2. **Sister chromatid exchanges**, or SCEs

3. Modern staining techniques

a. Presence of **bromodeoxyuridine** (BUdR)

b. Following two generations

c. Differential staining technique

d. **Harlequin chromosomes**

Lecture Content

Chapter Six: Linkage and Chromosome Mapping

I. Chromosomes and Genes

A. There are a large number of genes on each chromosome.

1. Genes that are located on the same chromosome are said to be **linked**.
2. Linked genes demonstrate **linkage** in a genetic cross.
3. The gene is the transferring unit during meiosis, and they are not free to undergo independent assortment.
   a. In theory, the alleles at all loci on one chromosome should be transmitted as one unit.
   b. However, during meiosis a reciprocal exchange often takes place between chromosomes, this is called **crossing over**.
   c. Crossing over results in the **recombination**, or a reshuffling, of the alleles between homologs.
4. The degree to which crossing over between any two loci on a chromosome is proportional to the distance between them.
   a. This correlation serves as the basis for the construction of **chromosome maps**.
   b. Chromosome maps provide relative locations of genes on their respective chromosome.

II. Linkage Versus Independent Assortment

A. Transparency 47, page 112, Figure 6-1.

1. Figure 6-1 shows linkage with and without crossing over.
   a. The result of independent assortment of two pairs of nonhomologous chromosomes is represented in this part of the figure.
   b. The figure illustrates the nonhomologous chromosomes with one heterozygous gene pair, and with no linkage exhibited.
   c. The final result is four genetically different gametes.
2. An example with the same genes linked on the same chromosome is also represented here.

a. Even though these genes are linked on the same chromosome, there is no crossing over represented.
b. The result would be only two genetically different gametes.
c. This is known as **complete linkage**, where the results are the production of only **parental** or **noncrossover** gametes.
d. Complete linkage rarely occurs in nature; it is only in theory that this would result.

3. The next part of the figure, part c, illustrates crossing over between two linked genes.
   a. This crossover only involves the two nonsister chromatids, and not all four.
   b. The result of this action is two new allele combinations, which are referred to as **recombinant** or **crossover gametes**.
4. The frequency with which crossing over occurs is proportional to the distance, which separates the loci.
   a. Two randomly selected genes could be so close to each other on a chromosome that the crossover events would be too infrequent to detect, this is called **complete linkage**, and results in the production of parental gametes only.
   b. If a small distance were between two genes, the result would be the production of only a few recombinant gametes and mostly parental gametes.
   c. As the distance between two loci increases, the proportion of recombinant gametes increases, and the proportion of the parental gametes decreases.
5. Linked genes, whose distance is considered to be very far apart, will never produce more than 50 percent recombinant gametes.

B. A unique phenotype in the F_2 generation results when an organism that expresses the wild-type of two genes that have complete linkage, is crossed with an organism that expresses the mutant form of the alleles.
   1. An example would be in the organism *Drosophila*, considering the two closely linked genes for eye color and wing venation.
   2. The mutant forms of these genes are *brown (bw)* eyes and *heavy (hv)* wing veins, whereas the wild-type form is *red* (bw^+) and *thin veins* (hv^+).

3. The cross would consist of flies with mutant brown eyes and normal then veins with flies of normal red eyes and mutant heavy veins.
4. Linked genes can be expressed by placing their allele designations above and below either a single or double horizontal line.
5. Therefore, the P_1 generation could be represented as:

$$P_1 = \underset{\text{brown, thin}}{\frac{bw\ hv^+}{bw\ hv^+}} \quad \underset{\text{red, heavy}}{\frac{bw^+\ hv}{bw^+\ hv}}$$

6. In the F_1 generation, each of the flies receives a chromosome from each parent; therefore, all flies are heterozygous in the F_1 generation, and would be represented as:

$$F_1 = \underset{\text{red, thin}}{\frac{bw\ hv^+}{bw^+\ hv}}$$

7. The F_1 generation produces only parental gametes; therefore the F_2 generation produces a 1:2:1 phenotypic and genotypic ratio.
   a. ¼ of the progeny in the F_2 generation will exhibit brown eyes and thin veins.
   b. ½ of the progeny in the F_2 generation will exhibit wild-type traits, red eyes and thin veins.
   c. ¼ of the progeny of the F_2 generation will exhibit red eyes and thick veins.
8. Complete linkage is usually observed only when the genes are close together and the number of progeny is relative small.
9. If a test cross were performed with the F_1 flies, a ratio of 1:1 (brown, thin and red, thick) would have been produced since the alleles are completely linked.

C. A **linkage group** can be established when a large number of mutant genes on the same chromosome are investigated thoroughly.

III. Incomplete Linkage, Crossing Over, and Chromosome Mapping

A. Thomas H. Morgan and Alfred H. Sturtevant were the first to explain the phenomenon of crossing over.
   1. Transparency 48, page 115, Figure 6-3.

2. As can be seen by the transparency, Morgan crossed female flies with the mutant *yellow* body (*y*) and *white* eyes (*w*) with males of who were wild-type (*gray* bodies and *red* eyes).
   a. The F_1 females were all wild-type, while the F_1 males exhibited both the mutant traits.
   b. When the F_1 generation was crossed, the F_2 generation produced 98.7 percent with parental phenotypes, consisting of either *yellow*-bodied, *white*-eyed, or wild-type flies.
   c. The remaining 1.3 percent of the flies were *yellow*-bodied with *red* eyes or *gray*-bodied with *white* eyes.
3. Morgan performed other crosses involving X-linked genes, and the results were even more puzzling.
   a. One of the other crosses that he performed involved *white*-eye, ***miniature*** wing mutants.
   b. The F_2 generation consisted of only 62.8 percent expressing the parental phenotypes, while the other 37.2 percent appeared as if the mutant genes had been separated during gamete formation.
4. Morgan was perplexed by his discovery; therefore, he looked to the work of F.A. Janssens, a scientist who had made many cytological observations before this time.
   a. Janssens observed homologous chromosomes forming chiasmata during synapses.
   b. He also reported that points of overlap were evident at these chiasmata.
   c. Morgan then proposed that these chiasmata could represent points of exchange.
   d. Morgan made many different crosses involving the X chromosome, and he suggested that if exchange occurred between the two X chromosomes of the F_1 females, it would in fact lead to the observed results.
      - He proposed that these exchanges would lead to 1.3 percent recombinant gametes in the *yellow-white* cross, and 32.7 percent in the *white-miniature* cross.

- Morgan soon concluded that linked genes on any chromosome existed in linear form, and that a variable amount of exchange could occur between any two genes on that chromosome.

5. Morgan also proposed that the closer together the genes were located on a chromosome, the less likely it is that genetic exchange would occur between them.
6. Morgan derived the term **crossing over** to describe the actual physical exchange leading to recombination.

B. Sturtevant was the first to realize that by using his mentor's proposal, he could map the sequence of the linked genes.

1. Sturtevant compiled more data on the crosses between *yellow*, *white*, and *miniature* mutants.
2. The frequencies of crossing over were as follows:

(1) *yellow*, *white* 0.5%
(2) *white*, *miniature* 34.5%
(3) *yellow*, *miniature* 35.4%

   a. Sturtevant noticed that the sum of 1 and 2 approximately equaled the percentage of 3.
   b. He realized that the *yellow* and *white* genes were close together because their recombination frequency was low, and that these genes are both relatively far from the *miniature* gene because the combinations of these two with the *miniature* gene resulted in a high recombination frequency.
   c. From his data, Sturtevant he concluded that the order of the genes on the chromosome are *yellow-white-miniature*.

3. Sturtevant realized that the frequency of exchange could be taken as an estimate of the distance between two genes along the chromosome.
   a. He then constructed a map of these three genes on the X chromosome.
   b. Sturtevant made a map unit equal one- percent recombination between the two genes, and referred to map units as centimorgans (cM), in honor of Morgan's work.

- The distance between the *yellow* and *white* could be 0.5 map unit, while the distance between the *yellow* and the *miniature* would be 35.4 map units.
- It follows that the distance between the *white* and *miniature* would be 35.4 – 0.5 or 34.9, which is close to the recombination frequency between these two genes.

4. Sturtevant began to study two other genes on the X chromosome, and soon created a map containing all five genes.
   a. Sturtevant and Calvin Bridges, a colleague, began to do research for autosomal linkage in *Drosophila*.
   b. By 1923, they had clearly shown that recombination was not restricted to the X chromosome, but it could in fact been seen on the autosomal chromosomes as well.
5. Sturtevant's work helped to strengthen to the **chromosomal theory of inheritance**, which was still being disputed at that time.

C. During meiosis, there is a limited number of cross over events that occur.
   1. The closer two genes are located, the less likely it is that a **single crossover** event will occur between them, while the further apart they are, the more likely a single crossover event will occur.
   2. Look at figure 6-5 in the text.
      a. A crossover event occurs between two nonsister chromatids, but not between the two loci; therefore, the crossover goes undetected because no recombinant gametes are produced.
      b. On the other hand, in part (b) of the figure, a crossover event occurs between the two loci and recombinant gametes are produced.
   3. Even if a single crossover event occurs 100 percent of the time, only 50 percent of the potential gametes will be formed.
      a. The percentage of tetrads involved in an exchange between two loci is twice as great as the percentage of recombinant gametes produced.
      b. The theoretical limit of recombination due to crossing over is therefore only 50 percent.

D. **Double crossovers** occur from double exchanges of genetic material.
   1. Figure 6-7 in the text, illustrates a double crossover event.
   2. In order for a double crossover to be studied, three genes must be involved in the investigation, and the alleles for each gene must be heterozygous.
   3. Two separate and independent events or exchanges must occur simultaneously in order to produce a double crossover.
      a. Single crossovers can be estimated by the distance between the two genes on the chromosome.
      b. For a double crossover, you must take the probability of two independent events occurring simultaneously, which is equal to the product of the individual probabilities.
         - For example, consider the probability of crossover gametes occurring from a single exchange between A and B to be 20 percent, p=0.02, and between B and C to be 30 percent, p=.30.
         - The probability of recovering a double crossover gamete from the exchanges between A and B and B and C, would be (0.20)(0.30) = 0.06, or 6 percent.
         - Therefore, the frequency of gametes occurring from a double crossover is always much lower than the frequency of either single crossover gametes occurring.
         - This example is illustrated in figure 6-7 in the textbook.

E. An experiment has been performed and is illustrated involving three-point mapping in *Drosophila* (Transparency 49, page 119, Figure 6-8).
   1. Three criteria must be met for a successful mapping cross:
      a. All loci under investigation within the organism must be heterozygous.
      b. Each phenotypic class must reflect the genotype of the gametes of the parents producing it.
      c. There must be a sufficient number of offspring in the mapping experiment to recover all crossover classes.

2. The three sex-linked recessive mutant genes- *yellow* body color, *white* eye color, and *echinus* eye shape are considered in the experiment which is illustrated in figure 6-8 in the text.
   a. The first assumption that must be made is the order of the genes on the chromosome.
   b. Here, the order is assumed to be *y-w-ec*, if this is not correct, analysis of the data will show it.
3. The P_1 generation males are all hemizygous for all three wild-type alleles, while the P_1 generation females are all homozygous for the recessive mutant alleles.
4. The F_1 generation produces females, which are all heterozygous at the three loci, while the males, because of their Y chromosome, are all hemizygous at all three loci.
   a. The phenotype of the females in the F_1 generation is wild type.
   b. The phenotype of the females fulfills the first requirement for mapping three linked genes.
   c. The second criterion is met by the virtue of the gametes formed by the F_1 males, which would be either an X chromosome with all three mutant alleles, or a Y chromosome, which is genetically inert for the three loci being considered.
      - The genotype of the gamete produced by the F_1 female will be expressed phenotypically in the F_2 male and female offspring.
      - Therefore, all F_1 noncrossover and crossover gametes can be detected through the phenotypes of the F_2 generation.
5. The **noncrossover phenotypes** are expressed by the genotype, which contains one or the other of the X chromosomes unaffected by crossing over.
6. The genotypes of the two parental gametes and the phenotypes of the two F_2 phenotypes complement one another.
   a. In other words, if one is completely mutant, the other is wild type.
   b. Therefore, these are called **reciprocal classes** of gametes and phenotypes.
7. The two noncrossover phenotypes are the most easily recognized.
   a. One reason is because they are present in the greatest proportion.

b. The flies that express *yellow*, *white*, and *echinus* phenotypes, and those that are wild type for all three categories, constitute 94.44 percent of the F_2 generation, these are noted as classes 1 and 2.

8. The **double-crossover phenotypes** are also easily recognized.
   a. This group represents two independent, but simultaneous single-crossover events.
   b. The two reciprocal phenotypes are the mutant traits *yellow* and *echinus*, but normal eye color, noted as class 7, and the mutant trait *white*, but normal body color and eye shape, noted as class 8.
   c. Together, these represent only 0.06 percent of the F_2 generation.
9. There are four remaining phenotypic classes all result from single crossover events, represent two different categories.
   a. One of these categories involves a single crossover event between the *yellow* and *white* loci, which constitutes 1.50 percent of the F_2 generation, noted as classes 3 and 4.
   b. The other category consists of a single crossover event occurring between the *white* and *echinus* loci, which constitutes 4.00 percent of the F_2 generation, noted as classes 5 and 6.
10. Since the data has all been recorded, a map separating the three loci can be created.
   a. For the *y* and *w* genes, which are included in classes 3, 4, 7, and 8 the total would be 1.5 % + 0.06 %, or 1.56 map units.
   b. The distance between *w* and *ec* is equal to the sum of the percentages of classes 5, 6, 7, and 8, which is 4.00% + 0.06%, or 4.06 map units.
   c. The map according to this data, would look like:

```
___y_____w_________ec__//____
|- 1.56 -|- - 4.06 - - -|
```

F. There is a straightforward method of determining the gene sequence if the sequence is unknown.

1. In the previous example, the gene sequence was assumed to be *y-w-ec*, but this is not always known.
2. There are three possible orders for the three genes to be:
   a. (I) *w-y-ec* (*y* is in the middle).
   b. (II) *y-ec-w* (*ec* is in the middle).
   c. (III) *y-w-ec* (*e* is in the middle).
3. There are certain steps that must be followed in order to determine the gene sequence.
   a. First, the arrangement of the alleles along each homologue of the heterozygous parent giving rise to noncrossover and crossover gametes must be determined, this was the F_1 female in the above example.
   b. Second, it must be determined whether the double-crossover event will produce the observed double-crossover phenotype within that arrangement.
   c. If the order does not produce the predicted phenotype, try the other two orders.
4. Figure 6-9 in the text illustrates these steps using the cross previously discussed.
5. If it is assumed that *y* is between *w* and *ec*, the arrangement of alleles in the heterozygote would look like:

$$\mathbf{I}\quad \frac{w\ \ y\ \ ec}{w^{-}\ \ y^{-}\ \ ec^{-}}$$

   a. This is known because of the way the P_1 generation was crossed.
   b. The P_1 female contributed an X chromosome bearing the *w*, *y*, and *ec* alleles, while the P_1 male contributed an X chromosome bearing the w^+, y^+, and the ec^- alleles.
   c. If a double crossover event were to occur with the above arrangement, the resulting gametes would be:

$$\underline{w\ \ y^{-}\ \ ec} \text{ and } \underline{w^{+}\ \ y\ \ ec^{+}}$$

      - If *y* is in the middle, following fertilization, offspring would be produced that would exhibit *white, echinus* and that exhibit *yellow*.
      - Instead, offspring were produced that exhibited *yellow, echinus* and *white*; therefore, this order is incorrect.

6. If the other two orders are considered, we would have the following allele orders:

$$\textbf{II}\ \frac{y\ \ ec\ \ w}{y^{+}\ \ ec^{+}\ \ w^{+}} \qquad \text{or} \qquad \textbf{III}\ \frac{y\ \ w\ \ ec}{y^{+}\ \ w^{+}\ \ ec^{+}}$$

   a. Immediately, we can see that arrangement II provide the predicted double-crossover phenotypes that do not correspond to the observed double-crossover phenotypes; therefore, this order is also incorrect.
   b. Arrangement III, on the other hand, will produce the observed phenotypes – *yellow, echinus* flies and *white* flies; therefore, this order is correct and the *w* gene is in the middle.

G. Next, we will consider a mapping problem in maize (corn).
   1. Transparency 50, page 122, Figure 6-10.
   2. Here, autosomal genes will be considered, whereas in the *Drosophila*, we were looking at sex-linked genes.
   3. In this example, the + symbol will be used to identify the wild type alleles.
   4. Even though these are autosomal genes, the same three criteria must be met as they were in the cross involving the *Drosophila*.
   5. The recessive mutant genes in maize are *bm (brown midrib), v (virescent seedlings),* and *pr (purple aleurone)*, which are linked on chromosome number 5.
      a. Assume that the female plant is heterozygous for all three traits, the arrangement of alleles on this heterozygote is not known, the sequence of genes is not known, and the map distances between the genes is not known.
      b. The male is homozygous for all three of the mutant alleles.
   6. In this example, the offspring have been divided into groups of two for each pair of reciprocal phenotypic classes, which are derived from either:
      a. No crossing over **(NCO)**.
      b. One of two possible single-crossover events **(SCO)**.
      c. A double crossover event **(DCO)**.
   7. First, determine the two noncrossover classes, those that occur at the highest frequency.

a. In this example, they are:

$$\underline{+\ v\ m} \text{ and } \underline{pr\ +\ +}$$

b. These homologues are unaffected by any recombination event when they segregate into gametes.

8. Next, make sure that the assumed arrangement of alleles will yield the observed double-crossover phenotypes.

a. The known arrangement of alleles is:

$$\frac{+\ v\ bm}{pr\ +\ +}$$

b. The other two orders, keeping the same arrangement would be:

$$\frac{+\ bm\ v}{pr\ +\ +} \qquad \frac{v\ +\ bm}{+\ pr\ +}$$

c. Only the latter arrangement will yield the observed double-crossover classes; therefore, the *pr* gene is in the middle.

9. To determine the map distance between the alleles, it must be remembered that the distance is calculated on the basis of all detectable recombinational events occurring between two genes.

a. Figure 6-11 (e) illustrates that the phenotypes $\underline{v\ pr\ +}$ and $\underline{+\ +\ bm}$, result from single crossovers between the *v* and *pr* loci, accounting for 14.5% of the offspring.

b. If the percent of double crossovers (7.8%) is added to the percent of single crossovers (14.5%), the total distance between the *v* and the *pr* loci would be 22.3 map units.

c. The phenotypes $\underline{v\ +\ +}$ and $\underline{+\ pr\ bm}$, result from single crossovers between the *pr* and *bm* loci 35.6%; with the addition of the double-crossover classes (7.8%), the distance between the *pr* and *bm* loci would be 43.4 map units.

d. Therefore, the final map looks like:

$$\underline{V\quad pr\quad bm}$$
$$|\text{-}22.3\text{-}|\text{----}43.4\text{----}|$$

H. There are times when mapping experiments are not very accurate, this can occur because it is impossible to detect all crossover events.

1. An example would be a double exchange between two loci, if the original order of the alleles is recovered, there is no evidence of a crossover.
2. This can go for all even number crossover events.
3. The farther apart the two genes are, the greater the probability that crossovers will occur undetected.

I. **Interference** is the reduction of the expected number of double crossovers, which occurs when genes are very close to one another.

1. The percentage of **expected double crossovers** is predicted by multiplying the percentage of the total crossovers between each pair of genes, this is in theory of course.
   a. A DOC represents two simultaneous single crossover events.
   b. In figure 6-10 and 6-11 in the text, the expected double crossover would be calculated like this:

$$DCO_{exp} = (0.223)\ X\ (0.434) = 0.097 = 9.7\%$$

2. In general, the expected number of DCOs is larger than the observed number.
   a. The concept of interference explains why.
   b. This is calculated by taking the ratio of observed DCOs to expected DCOs, which is called the **coefficient of coincidence** (*C*):

$$C = \frac{\text{observed DCO}}{\text{expected DCO}}$$

   c. The maize cross gives:

$$C = \frac{0.078}{0.097} = 0.804$$

   d. Once C is calculated, the inference (*I*) can be quantified by using this equation

$$I = 1.0 - C$$

   e. The maize cross gives:

$$I = 1.0 - 0.804 = 0.196$$

- If no double crossovers occur, and inference is complete, I = 1.0.
- If there are fewer observed DCOs than expected, *I* is a positive number and is called **positive inference**.
- If there are more observed DCOs than expected, *I* is a negative number and is called **negative inference**.

3. Positive inference is most often observed in eukaryotic systems.
   a. It could be that a crossover event in one region is inhibiting a crossover event in another region.
   b. More positive inference is observed, when the genes are close together.
   c. It is thought that there might be a mechanical stress imposed on chromatids during crossing over, and that one chiasma inhibits the formation of second chiasma in the neighboring region.

J. Figure 6-13 in the textbook shows partial maps for each of the four chromosomes in *Drosophila*.

IV. Somatic Cell Hybridization and Human Gene Mapping

A. Pedigree analysis was the method for the earliest linkage studies in humans.
   1. Difficulty arises when studying human pedigrees when two genes of interest are separated on a chromosome in such that recombinant gametes are formed because they obscure linkage in the pedigree.
   2. In 1947, J.B.S. Haldane and C.A. Smith devised the **lod score method**, which assesses the probability that a particular pedigree involving two traits reflects linkage or not; it was later refined by Newton Morton in 1955.
      a. The first step is to take the probability of the family data concerning two or more traits that conform to the transmission of traits without linkage.
      b. Then, the probability is calculated that the identical family data following these same traits result from linkage along with a specified recombination frequency.
      c. Next, the ratio of these probabilities expresses the "odds" for and against linkage.
      d. The lod score method is not always accurate because it is limited by the family data.
   3. In the 1960s, a new technique called **somatic cell hybridization**, was developed and helped assign human genes to their correct chromosome.
      a. This method was first discovered by Georges Barsky.
      b. The method relies on the fact that two cells can, in culture, be fused together to form a single hybrid cell.

c. Barsky was using mouse cells when he discovered this, but it soon became apparent that cells from other organisms could be used in this method as well.
d. The initial cell type formed is called a **heterokaryon**, which contains two cell nuclei in a common cytoplasm.
e. If this technique is performed properly, human cells can be fused with mouse cells and the hybrids can be isolated.
f. During the formation of this heterokaryon, two significant changes occur.
   - First, the two separate nuclei fuse to form what is called a **synkaryon**.
   - Then, as the generations pass, chromosomes from one of the parental species are randomly lost.
   - In the case of human and mouse cells, the human chromosomes are randomly lost until there is a full complement of mouse chromosomes and only a few human chromosomes in the synkaryon.
g. The experiment is fairly straightforward, if the synkaryon produces a specific human gene product, then the gene responsible for it must be located on one of the chromosomes left in the synkaryon.
h. A panel of cell lines that each contains several remaining human chromosomes is often utilized.
i. **Synteny testing** is the correlation of the presence or absence of each chromosome with the presence or absence of each gene product.
j. For example, look at figure 6-14, where four gene products (A, B, C, and D) are tested in relationship to eight human chromosomes; concentrate on product A.
   - Cell line 23, does not produce product A and since chromosomes 1, 2, 3, and 4 are present in cell line 23, they can be excluded from the possibility of containing gene A.
   - Cell line 34, which contains chromosomes 5 and 6, but not 7 and 8, does produce product A; therefore, it is known that gene A must be on either chromosome 5 or chromosome 6.

- Cell line 41 also produces product A, and this cell line contains chromosome 5, but not chromosome 6; therefore it can be concluded that gene A is located somewhere on chromosome 5.

k. Gene C poses an interesting situation, data shows that it is not contained on any of the first seven chromosomes, but there is not enough data to support its residence on chromosome 8; therefore, it is thought to be on chromosome 8, but more data is needed to know for sure.

l. What about gene D?

- Where is it located?
- Answer – Chromosome 1.

4. Figure 6-15 in the textbook shows many gene assignments that have been made for the human chromosome number 1 and the X chromosome.
5. Using recombinant DNA technology with pedigree analysis, researchers have been able to assign the positions for the genes responsible for Huntington disease, cystic fibrosis, and neurofibromatosis.

V. The Use of Haploid Organisms in Linkage and Mapping Studies

A. *Chlamydomonas* and *Neurospora* are examples of single-celled eukaryotes that are haploid during the vegetative stages of their life cycles.

B. Small haploid organisms provide an advantage to genetic studies.
   1. They can be cultured and manipulated easily.
   2. They contain only a single allele for each gene.

C. Crosses are made between these organisms, and then the meiotic structures are isolated following fertilization.
   1. All four of the meiotic products give rise to spores, where each structure is called a **tetrad**.
   2. **Tetrad analysis** is when these structures are isolated, grown, and analyzed separately from other such tetrads.

D. Figure 6-16 in the text shows the analyzed data of single genes in *Neurospora*.
   1. This data can be used to calculate the map distance of genes in relation to the centromere, a process called **mapping the centromere**.

2. Once the meiotic products are formed, they proceed through meiosis and produce eight ordered products called ascospores.
3. If there are no crossover events between the gene under study and the centromere, the pattern of the ascospores, in this example, is (*aaaa*++++).
   a. This pattern is representative of **first division segregation**.
   b. A crossover event can alter this pattern, (*aa*++*aa*++) and (++*aaaa*++), or (++*aa*++*aa*) and (*aa*++++aa)
   c. All four of these patters reflect **second division segregation**, since the two alleles are not separated until the second meiotic division.
   d. There are six combinations of genotypes possible:

First Division Segregation

a	*a*	+	+
+	+	*a*	*a*

Second Division Segregation

a	+	*a*	+
+	*a*	+	*a*
+	*a*	*a*	+
a	+	+	*a*

4. In order for the distance between the gene and the centromere to be calculated, a large number of ascospores must be counted and then used in this formula:

$$d = \frac{1}{2}\,\frac{\text{(second division segregant asci)}}{\text{(total asci)}}$$

   a. The distance (*d*) is the percentage of recombination.
   b. The distance is also only one-half the number of the second segregant asci.
5. Assume that *a* represents albino, and + represents wild type in *Neurospora*.
   a. Assume also, that after the cross was made between these two genetic types, the data observed is 65 first division segregants and 70 second division segregants.
   b. The distance between *a* and the centromere is:

$$D = \tfrac{1}{2}(70/35) = 0.259, \text{ or about 26 map units.}$$

   c. As the distance increases to 50 units, all asci should reflect second division segregation, at least in theory.

VI. Other Aspects of Genetic Exchange

A. Curt Stern, Harriet Creighton, and Barbara McClintock have individually demonstrated that an actual physical exchange between homologous chromosomes does take place in crossing over.

1. In Creighton and McClintock's work examined two linked genes in maize on chromosome number 9.
   a. One of sets of alleles were *colorless (c)* and *colored (c)*, which controlled the color of the endosperm.
   b. The other gene that was studied had the alleles of *starchy (Wx)* and *waxy (wx)*, which control the carbohydrate characteristics of the endosperm.
2. Figure 6-17 illustrates the markers, which were found on a corn plant that was heterozygous at both loci, as well as containing two unique cytological markers on one of the homologues.
   a. One of these markers was a densely stained knob at the end of one of the chromosomes.
   b. The other marker was a translocated piece of chromosome 8 at the other end.
3. This unique plant was crossed to one that was homozygous for the color alleles and heterozygous for the endosperm alleles.
   a. They obtained many different offspring from this cross, but there two that they were more interested in.
   b. The first one was a plant with the genotype for colorless, waxy phenotype.
   c. After this plant was examined for the cytological markers, the scientists were happy with what they discovered.
   d. If genetic crossing over is accompanied by a physical exchange between homologs, then the translocated chromosome should still be preset, but the knob should not; this was exactly what they found.
   e. The second plant had a phenotype consisting of colored and starchy endosperm.
      - This phenotype could occur from nonrecombinant gametes or from crossover gametes.

- If the latter were the case, then the plant should contain the dense knob. but should not contain the translocated piece of chromosome; again this is what they found.

4. Their discoveries, as well as similar findings by Stern who used *Drosophila*, leave no doubt that there is actual physical exchange during crossing over.

B. In mitosis, since there are not homologous chromosomes pairing up, it was wondered if crossing over could take place between the sister chromatids.
   1. In fact, many experiments have shown that reciprocal exchanges, which are similar to crossovers, do occur during mitosis between sister chromatids.
   2. These are called **sister chromatid exchanges**, or SCEs.
   3. There are several modern staining techniques to identify SCEs.
      a. One technique is to allow cells to replicate for two generations in the presence of a thymidine analog **bromodeoxyuridine** (BUdR).
      b. After the two generations, each pair of sister chromatids has one member with one BUdR "labeled" strand, and on with both strands labeled.
      c. They are then exposed to a differential staining technique, where pairs with both chromatids "labeled" stain less brightly than pairs with only one "labeled" chromatid.
      d. This technique allows the chromosomes to easily be distinguishable, and due to the patchy appearance of the chromatids, they often referred to as **Harlequin chromosomes**.

Lecture Outline

Chapter Seven: Extranuclear Inheritance

I. Maternal Effect

A. **Maternal effect**

B. Egg cytoplasm

C. The products

D. Ephestia pigmentation

1. *Ephestia kuehniella*
2. Wild type
3. Mutation
4. Figure 7-1 illustrates different crosses
   a. If male is heterozygous parent
   b. If female is heterozygous parent
5. Explanation

E. *Limnaea peregra*

1. Permanent effect
2. Two different strains
   a. Left handed or sinistrally coiled
   b. Right handed or dextrally coiled
3. Hermaphroditic
4. Figure 7-2 illustrates coiling pattern
5. Orientation of mitotic spindle fibers
   a. Maternal genes actively control spindle fiber orientation
   b. Dextral allele = right-handed coiling
   c. Sinistral is a classical mutation

F. Edward B. Lewis, Christiane Nusslein-Volhard, and Eric Wieschaus

1. Products are stored into oocyte prior to fertilization
2. They specify molecular gradients in early embryo
3. *bicoid* (*bcd*) gene
   a. Development of anterior part of fly
   b. If maternal parent is homozygous

c. If maternal parent has at least one wild-type allele

d. Concept of maternal effect

II. Organelle Heredity

A. Discovery of DNA in other organelles

B. Mitochondria and chloroplasts

1. Function of organelles

2. More than one transferred to progeny

C. Carl Correns

1. *Mirabilis jalapa*

2. Table 7.1

3. Ovules from green branch produce green branches

4. Cytoplasm

D. Marcus M. Rhoades

1. Cytoplasm and nuclear gene

2. ***iojap* (*ij*)**

a. Wild type (*Ij*)

b. Heterozygous mutation (*ij/ij*)

3. Transparency 51, page 143, Figure 7-3

a. Female (*ij/ij*) crossed with male (*Ij/Ij*)

b. Male (*ij/ij*) crossed with female (*Ij/Ij*)

c. Inheritance pattern is influenced by maternal parent

4. Self-fertilization

a. Striped plant

b. Green plant

c. Conclusion

E. *Chlamydomonas reinhardi*

1. Eukaryotic with one large chloroplast and many mitochondria

2. Ruth Sager

3. Single chloroplasts fuse

a. Genetic information derived from *mt* parent only

b. mt- chloroplast degenerated

F. Mary B. and Hershel K. Mitchell
   1. **Poky**
   2. Crosses between wild type and poky
      a. Female poky and male wild type
      b. Male poky and female wild type
   3. Phase in the life cycle of fungi
      a. Hyphae fuse
      b. **Heterokaryon**
      c. The cytoplasm
      d. **Conidia**
   4. Fusion of poky and wild type hyphae
   5. An explanation for this occurrence
      a. Poky mitochondria replicate more rapidly and "wash out" wild type
      b. Poky mitochondrion produces substance which is inactivating
      c. **Suppressive mutation**

G. *Saccharomyces cerevisiae*
   1. Boris Ephrussi and co-workers
   2. ***Petite*** mutations
      a. Deficient in cellular respiration
      b. Anaerobe
   3. Transparency 52, page 145, figure 7-4
      a. **Segregational *petites***
      b. Cytoplasmic transmission
         1. **Neutral *petites*** crossed with wild type
            - Neutral mutants lack mtDNA
            - Wild type is effective source of normal mitochondria
         2. **Suppressive *petites*** crossed with wild type
            - Result is all mutant cells
            - *Petite* behaves as dominant
            - Deletions of mtDNA
      c. Two separate hypotheses

1. Mutant mtDNA replicates much faster and "takes over"
2. Recombination occurs

III. Mitochondrial DNA and Human Diseases

A. Human mitochondrial DNA
   1. Circular DNA
   2. 16,569 base pairs
   3. Inherited from maternal parent
   4. Gene products
      a. 13 proteins
      b. 22 different transfer RNAs (tRNAs)
      c. 2 ribosomal RNAs (rRNAs)

B. Large number of mitochondria from maternal parent
   1. Mitochondria very dispersed initially
   2. **Heteroplasmy**
   3. Criteria for a human disorder to be attributed to genetically altered mitochondria
      a. Maternal inheritance expressed
      b. Deficiency in the bioenergetic function
      c. Documentation of specific genetic mutation

C. Several cases
   1. **Myclonic epilepsy and dragged red fiber disease (MERRF)**
      a. Only present if mother is affected
      b. Deafness and dementia in addition to seizures
      c. Muscle fibers and mitochondria are abnormal in appearance
      d. Mitochondria are void of internal cristae
      e. Mutation within the genes encoding a transfer RNA
   2. **Leber's hereditary optic neuropathy (LHON)**
      a. Sudden bilateral blindness
      b. Four different mutations
      c. Subunit of NADH dehydrogenase
      d. Sudden "new" mutation
   3. **Kearns-Sayre syndrome (KSS)**

a. Loss of their vision, as well as hearing, and display heart conditions
b. Progressive symptoms as an adult
c. Deletions at various positions within the mitochondria

4. Hypothesis for aging

IV. Infectious Heredity

A. Tracy Sonneborn

1. **Paramecin**
2. **Kappa**
3. Contain DNA and protein
4. Transparency 53, page 146, figure 7-6
   a. Figure 7-6 illustrates **conjugation**
   b. Kappa and *K* gene can be transmitted
      1. Eight haploid nuclei
      2. Seven eventually degenerate
      3. Diploid state in each cell
      4. **Excongjugates**
5. **Autogamy**
   a. Eight micronuclei
   b. Seven degenerate
   c. Diploid condition
   d. If original cell was heterozygous, nucleus is homozygous
6. Transparency 54, page 147, figure 7-7
   a. Crosses between *KK* and *kk* cells
   b. No cytoplasmic exchange
   c. Cytoplasmic exchange

B. *Drosophila*

1. **CO_2 sensitivity**
   a. Flies killed by CO_2
   b. Passed from infected mothers
   c. Virus named **sigma**
2. **Sex-ratio**

a. *Drosophila bifasciata*
b. Predominantly female offspring
c. Experiment
d. Extranuclear element is responsible for the sex-ratio phenotype
e. Protozoan

V. Genomic Imprinting

A. **Genomic imprinting**
B. Dependent on parental origin
C. Influences genetic expression of offspring
D. Many examples
   1. Huntington disease
   2. Caused by mutation inherited from father
E. Occurrence of imprinting step
   1. Differentially marked egg-forming and sperm-forming tissues
   2. Can be reversed in succeeding generations
F. X chromosome
   1. **Dosage compensation**
   2. Studies in mice
G. Three genes in mice that undergo imprinting
   1. Insulinlike growth factor II (*Igf2*)
      a. Homozygous for nonmutant gene is normal size
      b. Homozygous for mutant gene is smaller
      c. Dependent on parental origin
         1. If it comes from the father
         2. If it comes from the mother
      d. Conclusion
H. Two genetic disorders in humans
   1. Chromosome 15 (15q1)
   2. Caused by deletions
   3. **Prader-Willi syndrome (PWS)**
   4. **Angelman syndrome (AS)**

5. Conclusion
6. Genomic imprinting occurs on specific parts of a chromosome
   a. **DNA methylation**
   b. **DNA methyl transferase**

Lecture Content

Chapter Seven: Extranuclear Inheritance

I. Maternal Effect

A. The **maternal effect**, or maternal influence, is when the offspring's phenotype concerning a particular trait is strongly influenced by the nuclear genotype of the gamete of the maternal parent.

B. The female gamete's genetic information is transcribed, and the genetic products are present in the egg cytoplasm.

C. The products influence the patterns or traits that are established during early development.

D. One example of maternal effect includes Ephestia pigmentation.

1. *Ephestia kuehniella*, is the wild type, in the Mediterranean meal moth, and the larva has a pigmented skin and brown eyes.
2. This wild type is due to a dominant gene A.
3. If a mutation occurs, and the genotype becomes *aa*, then the larva will exhibit red eyes, and little pigmentation.
4. Figure 7-1 in the text shows a variety of examples when the cross *Aa* X *aa* is made, the results are dependent on which parent carries the dominant gene.
   a. If the male is the heterozygous parent, there will be a 1:1 ratio of brown/red-eye present.
   b. If the female is the heterozygous parent, all the larvae will exhibit pigmentation along with brown eyes; one-half of these larvae will eventually develop red eyes, and establish the 1:1 ratio.
5. One explanation for this result is that within the cytoplasm of the female, heterozygous for the *A* gene, kynurenine accumulates prior to the completion of meiosis.
6. This brown pigment can be eventually diluted within the cells of the eyes, as the new pigment is synthesized, resulting in the red eyes as an adult.

E. Another example of maternal effect is seen within the snail, *Limnaea peregra*.

1. This maternal effect represents a permanent rather than a transitory effect on the individual.

2. There are two different strains concerning the coiling of the shell.
   a. One strain has left handed or sinistrally coiled shells, with the genotype *dd*.
   b. Another strain has right handed or dextrally coiled shells, with the genotype *DD* or *Dd*.
3. These snails are hermaphroditic, which means that they can either cross- or self-fertilize, leading to a variety of different mating possibilities.
4. Figure 7-2 in the text illustrates the coiling pattern of many snail progeny.
   a. It can be seen that the genotype of the parent producing the egg, is what determines the coiling pattern of the offspring.
   b. Maternal parents that have *DD* or *Dd* produce only dextral-coiling progeny, whereas maternal parents with *dd* produce only sinistral-coiled offspring.
5. It has been shown that the orientation of the mitotic spindle fibers during the first cleavage division determines the direction of coiling.
   a. The maternal genes acting on the developing egg always control the spindle fiber orientation.
   b. The dextral allele, *D*, produces an active gene product, which causes the right-handed coiling, in fact if ooplasm form dextral eggs is injected into uncleaved sinistral egg, the result will be a dextral pattern.
   c. The sinistral ooplasm, however, has no effect if injected into an uncleaved dextral egg.
   d. It is apparent that the sinistral allele is the result from a classical mutation and produces a nonfunctional gene product.

F. The work of Edward B. Lewis, Christiane Nusslein-Volhard, and Eric Wieschaus in *Drosophila*, has led to a more recent example of maternal effect in embryonic development.
   1. They discovered that products are synthesized by the developing egg and stored into the oocyte prior to fertilization.
   2. Following fertilization, the products specify molecular gradients in the early embryo, and determine spatial organization as the process of development proceeds.
   3. A specific example would be that of the *bicoid* (*bcd*) gene.

a. This gene plays a specific role in the development of the anterior part of the fly.
b. If the maternal parent is homozygous for the mutation, (*bcd*/ *bcd*) then the offspring will fail to develop the anterior areas of the body, which give rise to the head and thorax of the adult fly.
c. If the maternal parent contains at least one wild-type allele, (*bcd*) then the embryo will develop normally.
d. This follows the concept of maternal effect, that the genotype of the female parent, no the genotype of the offspring, determines the phenotype of the offspring.

II. Organelle Heredity

A. Over three decades ago, the discovery of the presence of DNA in other organelles was made.
B. The organelles that contain this "extra" DNA are the mitochondria and the chloroplasts.
   1. The function of these organelles is dependent on gene products of both nuclear and organelle DNA.
   2. The number of organelles that are contributed to each progeny is often more than just one.
C. Carl Correns, in 1908, provided the earliest example of inheritance linked to chloroplast transmission.
   1. Correns worked with *Mirabilis jalapa*, the four o'clock plant, which contained either white, green, or variegated leaves.
   2. Table 7.1 in the text shows that the inheritance in all possible combination patterns is strictly determined by the phenotype of the ovule source.
   3. If ovules from a green branch were used, no matter what the pollen source was from, all the offspring would produce green branches.
   4. Correns concluded that the inheritance was through the cytoplasm of the maternal parent, because the pollen contributes little or no cytoplasm to the zygote, and has no influence on the phenotypes of the progeny.
D. Marcus M. Rhoades studied this subject in maize.

1. Cytoplasm, as well as a nuclear gene, controls the expression of green, colorless, or green-and-colorless striped leaves of maize.
2. The locus is called ***iojap*** (***ij***).
   a. The wild type is designated as *Ij*.
   b. The heterozygous mutation is designated as *ij/ij*, and produce green-and-white striped leaves.
3. Transparency 51, page 143, Figure 7-3 illustrates different crosses within this plant.
   a. If a female with striped (*ij/ij*) is crossed with a male exhibiting green leaves (*Ij/Ij*); the resulting progeny are colorless leaves, striped leaves, and green leaves.
   b. If a male plant with striped leaves (*ij/ij*) is crossed with a female exhibiting green leaves (*Ij/Ij*) all the progeny have green leaves.
   c. The resulting progeny all have the same genotype, (*Ij/ij*), but exhibit different phenotypes; therefore, the inheritance pattern must be strictly influenced by the maternal parent.
4. If the self-fertilized reproduction is observed, the pattern is explained better.
   a. The striped plant, when self-fertilized, gives rise to the progeny with colorless, striped, and green leaves, regardless of its genotype.
   b. If the green plant is self-fertilized, is gives rise to both green and striped progeny, which exhibit a 3:1 ratio.
   c. These results conclude that the mutant chloroplasts are transmitted through the female cytoplasm, regardless of the plant's nuclear genotype.

E. *Chlamydomonas reinhardi* provides an excellent system for the investigation of plastid and mitochondrial inheritance.
   1. This organism is eukaryotic and contains one large chloroplast as well as many mitochondria.
   2. Ruth Sager reported the first cytoplasmic mutant strain, *streptomycin resistant (sr)*, in 1945.
      a. There are two mating types within this alga, mt^+ and mt^- and they appeared to make equal cytoplasmic contributions to the zygote.

b. Sager determined that the *sr* phenotype was transmitted through the *mt* parent only.

3. Following fertilization in this alga, the single chloroplasts of the two mating types fuse.
   a. All of the genetic information of the chloroplasts of the progeny cells are derived from the *mt* parent only.
   b. The genetic information that was present in the mt- chloroplast degenerated following fertilization and meiosis.

F. In 1952, Mary B. and Hershel K. Mitchell discovered a slow growing mutant strain of *Neurospora*, a mold, and called it **poky**.
   1. Further studies have shown that the slow growth is due to an impaired mitochondrial function.
   2. Results of genetic crosses between wild type and poky *Neurosporas* were interesting.
      a. If the female parent is poky and the male parent is wild type, the progeny are poky.
      b. However, if the male parent is poky and the female parent is wild type, the progeny are all normal.
   3. The studies with poky mutants have taken advantage of a unique phase in the life cycle of fungi.
      a. Sometimes, hyphae from separate mycelia fuse and give rise to structures that contain two or more nuclei.
      b. If the nuclei are of different genotypes, then the structure is referred to as a **heterokaryon**.
      c. The cytoplasm also contains mitochondria from both original mycelia.
      d. The heterokaryon may give rise to haploid spores, **conidia**, which may produce new mycelia whose phenotypes can be determined.
   4. Heterokaryons produced from the fusion of poky and wild type hyphae initially show rates of normal growth.
      a. The mycelia that are produced following the conidia formation though, become more and more abnormal until they too exhibit the poky phenotype.

b. This occurs even with both the poky and the wild type mitochondria in the cytoplasm of the hyphae.

5. An explanation of this occurrence could be that the wild type mitochondria support the respiratory needs of the hyphae, but the presence of the poky mitochondria may somehow suppress or prevent the function of the wild type mitochondria.
   a. One possibility is that the poky mitochondria replicate more rapidly and "wash out" the wild type mitochondria.
   b. Another possibility is that the poky mitochondrion produces a substance that inactivates the wild type mitochondria, or interferes with the replication of its DNA.
   c. As a result of this, the poky mitochondria are referred to as **suppressive mutations**.

G. The yeast, *Saccharomyces cerevisiae*, is another organism that has been subjected to many studies concerning mitochondrial mutations.
   1. Boris Ephrussi and his co-workers first discovered the ***petite*** mutation in 1956.
   2. Over the years, there have been many investigations on *petite* mutations.
      a. Every one of the colonies that exhibits this mutation is known to be deficient in cellular respiration involving abnormal electron transport.
      b. Fortunately, this yeast can perform needed functions as an anaerobe, and grow by fermentation of glucose through glycolysis.
   3. Transparency 52, page 145, figure 7-4.
      a. Figure 7-4, shows **segregational *petites***, which means they are a result of nuclear mutations.
      b. The remainders represent cytoplasmic transmission, which produces one of two effects on matings.
         - When **neutral *petites*** are crossed to a wild type, they produce ascospores, which give rise to wild type, normal sized colonies.
           - This is due to the fact that the majority of neutral mutants lack mtDNA completely or have lost a substantial portion of it.

- Therefore, the wild type cell is the effective source of normal mitochondrial function capable of reproduction.

- The final type is known as the **suppressive *petites***, which behave similarly to the poky *Neurospora*.
  - The cross between a mutant and a wild type, give rises to mutant diploid zygotes, which all yield mutant cells.
  - Under these conditions, the *petite* mutation behaves as if it were dominant.
  - These also have deletions of mtDNA, but they are not as severe as the neutral mutation's deletions.

c. There are two separate hypotheses to explain these results.
- One of these is that the mutant mtDNA replicates much faster than the normal DNA, the mutant mitochondria "take over" the phenotype by numbers alone.
- Another explanation is that recombination occurs between the mutant and the wild type mtDNA, which introduces error into the normal mtDNA.

III. Mitochondrial DNA and Human Diseases

A. Human mitochondrial DNA has been extensively studied.
1. It is circular DNA.
2. It contains 16,569 base pairs.
3. It is strictly inherited from the maternal parent.
4. The gene products include:
   a. 13 proteins which are required for oxidative respiration.
   b. 22 different transfer RNAs (tRNAs) which are required for the translating of proteins.
   c. 2 ribosomal RNAs (rRNAs) which are also required for the translating of proteins.

B. The zygote receives a large number of mitochondria from the maternal parent; therefore, it is thought that if only one possesses a mutation, then the cell can still function normally.

1. During the early developmental stages of cell division, the initial populations of mitochondria are very dispersed.
2. The adult cells will therefore exhibit a variable mixture of normal and abnormal organelles if the mutant mitochondria are present, a condition called **heteroplasmy**.
3. There are several criteria that must be met so that a human disorder can be attributed to genetically altered mitochondria.
   a. A maternal rather than a Mendelian pattern of inheritance must be expressed.
   b. The disorder must reflect a deficiency in the bioenergetic function of the organelle.
   c. A documentation of a specific genetic mutation in one of the mitochondrial genes must be present.

C. There are several cases that are known that demonstrate these characteristics.
   1. One example is **Myclonic epilepsy and dragged red fiber disease (MERRF)**.
      a. This disorder is only present in offspring of affected mothers; if the father is affected, the offspring will not exhibit the disorder.
      b. Individuals affected with this disorder express deafness and dementia in addition to seizures.
      c. The muscle fibers and the mitochondria are abnormal in appearance.
      d. The mitochondria are apparently void of any internal cristae.
      e. Within the mitochondria that cause this disorder, there is a mutation within the genes encoding a transfer RNA.
   2. A second example would be **Leber's hereditary optic neuropathy (LHON)**.
      a. This disorder causes sudden bilateral blindness, usually around the age of 27.
      b. There are four different mutations that have been identified in connection with this disorder, and they all disrupt the normal oxidative phosphorylation.
      c. More than one-half of the cases, are due to a mutation that encodes a subunit of NADH dehydrogenase.
      d. An important note is that many of these cases show no prior family history, that is that they are due to a sudden "new" mutation within that individual.
   3. A third example is **Kearns-Sayre syndrome (KSS)**.

a. Individuals affected with this disorder often experience loss of their vision, as well as hearing, and display heart conditions.
b. Many of these patients are symptom free as a child, but display progressive symptoms as an adult.
c. This involves deletions at various positions within the mitochondria.

4. Studies involving mitochondrial DNA have suggested a hypothesis for aging based on the progressive accumulation of mtDNA mutations and the accompanying loss of mitochondrial function.

IV. Infectious Heredity

A. Tracy Sonneborn first described **killer** strains of *Paramecium aurelia*.
   1. These organisms release a cytoplasmic substance called **paramecin** that is toxic, and sometimes lethal to sensitive strains.
   2. Particles called **kappa**, release this substance, and replicate within the Killer cytoplasm.
   3. Kappas contain DNA and protein, and depend for their maintenance on a dominant nuclear gene *K*.
   4. Transparency 53, page 146, figure 7-6.
      a. Figure 7-6 illustrates **conjugation**, the sexual exchange of genetic information.
      b. This is one way that the kappa and *K* gene can be transmitted.
         - First, the two diploid micronuclei are present and undergo meiosis, which results in eight haploid nuclei.
         - Seven of these eight eventually degenerate, while the remaining one undergoes a single mitotic division.
         - Each cell then donates one of the two haploid nuclei to the other, recreating the diploid state in each cell.
         - The two resulting cells are then called **exconjugates**, and are of identical genotype.
   5. **Autogamy** is a similar process that occurs, but only involves a single cell.
      a. Following the process of meiosis of both micronuclei, eight micronuclei are formed.

b. Seven of these degenerate, leaving only one to survive.

c. This nucleus divides and the resulting nuclei fuse to reform the diploid condition.

d. If the original cell was heterozygous, following autogamy, the newly formed diploid nucleus is homozygous.

e. Half of the new cells express one allele and the other half express the other allele, in a population that were originally heterozygous.

6. Transparency 54, page 147, figure 7-7.

a. Figure 7-7 illustrates the progeny of crosses between *KK* and *kk* cells, with and without cytoplasmic exchange.

b. When no cytoplasmic exchange occurs, the resulting cells remain sensitive if there are no kappa particles transmitted.

c. If exchange does occur, the cells will become Killers if the kappa particles are supported by at least one dominant K allele.

B. Two other examples of infectious heredity involve *Drosophila*.

1. One example is **CO_2 sensitivity**.

a. This is where flies, which normally recover from CO_2 anesthetization, become permanently paralyzed and are killed by it.

b. This sensitivity is passed on from infected mothers.

c. Phillip L'Heritier has postulated that the sensitivity is due to the presence of a virus, **sigma**.

d. There is not much known about this virus, sigma; however, it has been seen under the electron microscope.

2. A second example is **sex-ratio**.

a. This was first noticed in *Drosophila bifasciata*.

b. It was shown that if flies were reared at or under 21°C, then the offspring were predominantly female.

c. The injection of ooplasm from sex-ratio females into nonsex-ratio females induced this condition.

d. This suggested that an extranuclear element was responsible for the sex-ratio phenotype.

e. This agent has been shown to be a protozoan, which is lethal primarily to developing male larvae.

V. *Genomic Imprinting*

A. There are many exceptions to Mendelian inheritance, but one of the major exceptions is **genomic imprinting**.

B. This is where the genetic expression varies depending on the parental origin of the chromosome carrying a particular gene.

C. It appears that in some species, there are certain regions of chromosomes that retain memory, or an "imprint" of their parental origin, which in turn influences the genetic expression of the offspring.

D. There are many examples, which have been shown to be caused by this phenomenon.

1. One example is Huntington disease.
2. If there is an early onset of the disease, it is most often caused by a mutant gene inherited from the father.

E. This imprinting step is thought to occur before or during gamete formation.

1. This leads to differentially marked egg-forming and sperm-forming tissues.
2. This process can be reversed in succeeding generations.

F. Another example of genomic imprinting involves the X chromosome.

1. There is a mechanism present, known as **dosage compensation**, which is the random inactivation of either paternal or maternal X chromosome.
2. Studies have shown that in mice, genomic imprinting is complete before embryonic development, causing all the parental X chromosomes to be inactivated.
3. As development continues, the imprint is "released" and random inactivation of either the paternal or maternal X chromosome can occur.

G. In 1991, it was established that there were three genes in mice that undergo imprinting.

1. The first gene is the insulinlike growth factor II (*Igf2*).
   a. A mouse that is homozygous for the nonmutant gene is of normal size.

b. The mouse that is homozygous for the mutant gene is smaller than normal size, and is therefore referred to as a dwarf.

c. The stature of a mouse that is heterozygous depends on the parental origin of the mutant allele.

- If the mutant allele comes from the father, the mouse is of normal size.
- On the other hand, if the mutant allele comes from the mother, the mouse is a dwarf.

d. It was concluded, from these data, that the normal *Igf2* gene is imprinted to function poorly during the course of egg production in females, but function normally when it is passed through males.

H. There are thought to be two distinct genetic disorders in humans that illustrate the phenomenon of genomic imprinting.

1. Both of these disorders are thought to be caused by differential imprinting on the same region of chromosome 15 (15q1).
2. Both cases have shown to be caused by deletions in this region of chromosome 15.
3. The first disorder is known as **Prader-Willi syndrome (PWS)**.
   a. This disorder occurs only when there is an undeleted maternal chromosome remaining.
   b. Symptoms of this disorder include mental retardation, a severe eating disorder, obesity, and diabetes.
4. The second disorder is known as **Angelman syndrome (AS)**.
   a. This disorder is caused by the presence of only the parental chromosome.
   b. This disorder is characterized by metal retardation and puppet-like movements.
5. It can be concluded that region 15q1 is imprinted differently within male and female gametes.
6. There are many questions regarding why genomic imprinting occurs on specific parts of a chromosome, and how is it completed.
   a. It is hypothesized that **DNA methylation** may be the cause of imprinting.

b. This is where methyl groups are added to the carbon atom at position 5 in cytosine, and is the result of the activity of the enzyme **DNA methyl transferase**.

c. DNA methylation is thought to be a reasonable mechanism for establishing a molecular imprint, especially since there is some evidence that a high level of methylation can inhibit gene activity.

Lecture Outline

Chapter Eight: Chromosome Variation and Sex Determination

I. Variation in Chromosome Number: An Overview

A. Variation in chromosome number

1. **Aneuploidy**
   a. **Monosomy**
   b. **Trisomy**
2. **Euploidy**
   a. **Polyploidy**
   b. **Triploidy**
   c. **Tetraploidy**

B. Look at table 8.1 in the text

II. Chromosome Composition and Sex Determination in Humans

A. X and Y chromosome in females and males

1. Maleness causing chromosome
   a. Absence of the second X chromosome
   b. Presence of the second X chromosome
2. Valid in humans, but not in *Drosophila*

B. Two human abnormalities involving sex

1. **Klinefelter syndrome**
   a. Transparency 55, page 157, figure 8-1(a)
   b. Male genitalia and internal ducts
   c. Testes
   d. Feminine sexual development not completely suppressed
   e. 2 out every 1000 births
2. **Turner syndrome**.
   a. Transparency 55, page 157, figure 8-1 (b)
   b. Female genitalia and internal ducts
   c. Characteristics
   d. 1 out of 3000 female births
3. Karyotypes of these syndromes

a. Trisomic with XXY
b. Monosomic with a single X
c. Caused by nondisjunction

4. Convention designating chromosome composition
5. Y chromosome determines maleness in humans
   a. In the absence of the Y
   b. With the presence of the Y
6. Other karyotypes that can produce Turner syndrome
   a. **Mosaics**
   b. 45,X/46,XY, and 45,X/46,XX

C. **47,XXX**

1. Three X chromosomes
2. 1 of every 1200 female births
3. Many cases, female is normal
4. Characteristics for other cases
5. **48,XXXX** and **49,XXXXX**
6. Presence of the additional X chromosome

D. **47,XXY**

1. Scottish maximum security prison
   a. Personality disorders
   b. Subnormal intelligence
   c. Crime
2. Intensely investigated
   a. Above normal height and subnormal intelligence
   b. Higher frequency in penal and mental institutions
   c. Only one constant association
   d. No correlation between extra Y and behavior

E. Male development and Y chromosome

1. Limited homology
2. **Testis-determining factor (TDF)**
   a. Presence of TDF

b. Absence of TDF

3. ***SRY* (sex-determining region Y)**

4. *SRY* gene is important in male development

5. **Transgenic mice**.

a. Transgenic organisms

b. Injection of DNA containing only *Sry*

c. Outcome

III. The X Chromosome and Dosage Compensation

A. **Dosage compensation**

B. Murray L. Barr and Ewart G. Bertram' s, and Keith Moore and Barr

1. Darkly staining body in female cats

a. Absent in male cats

b. Same results in humans

c. Highly condensed structure

2. **Sex chromatin body**, also known as a **Barr body**

3. Ohno

a. Mechanism for dosage compensation

b. One X chromosome is inactive

c. Only one is inactivated

1. Number of Barr bodies present

2. *N*-1 rule

C. Inactivation is completely random

1. **Lyon hypothesis**

a. Coat color of female mice.

b. Also present in female calico cats

c. Do not occur in males

2. One extension is mosaics

a. Maternally derived alleles versus paternally derived alleles

b. **Red-green color blindness** and **anhidrotic ectodermal dysplasia**

1. Transparency 56, page 160, figure 8-5

2. Caused by random inactivation

D. **X-inactivation center (XIC)**

1. Expression of this region
2. ***XIST* (*X-inactivation specific transcript*)**
   a. Comparable regions within mice
   b. Graeme Penny's experiment

IV. Chromosome Composition and Sex Determination in Drosophila

A. Calvin Bridges and *Drosophila*

B. Two phases

1. **Nondisjunction**
   a. $(n+1)$ or $(n-1)$
   b. Fertilized = $(2n+1)$ and $(2n-1)$
2. Progeny of triploid females $(3n)$
   a. Triploid
   b. Look at figure 8-6 in text
   c. Characteristics
   d. Variety of chromosome
3. Ratio of X chromosomes to number of haploid sets of autosomes
   a. Normal (2X:2A) and triploid (3X:3A)
      1. (3X:2A) or 1.5
      2. **Super-females**
      3. **Metafemales**
   b. (XY:2A) and (XO:2A)
      1. (XY:3A)
      2. **Metamale**
   c. Ratios of 0.5 and 1.0
      1. Characteristics
      2. **Intersexes**
4. Conclusions concerning Y chromosome
   a. X chromosome
   b. Absence of X chromosome
   c. **Genic balance theory**

5. Mutant genes involved in sex determination

a. *Transformer (tra)*

1. Homozygous females

2. Homozygous males

b. *Sex-lethal* (*Sxl*)

1. "Master switch"

2. Essential for female development

3. When activation does not occur

V. Aneuploidy

A. Extra or missing chromosomes within diploid

1. Transparency 57, page 163, figure 8-7

2. Primary or secondary nondisjunction

B. Monosomy

1. Not tolerated well in animals

a. **Haplo-IV**

1. Larger chromosomes

2. Lethal alleles

b. Tolerated more in plant kingdom

C. **Partial monosomy**

1. Only part of one chromosome lost

2. **Segmental deletions**

3. Jerome LeJeune

a. Transparency 58, page 163, figure 8-8

b. **Cri-du-chat (cry of the cat) syndrome**

c. Large loss of the short arm on chromosome 5

d. **46,5p-**

1. Characteristics

2. Abnormal growth of the larynx and glottis

3. 1 in every 50,000 live births

D. Trisomy

1. More viable than monosomy

2. Effect
3. Tolerated well within plant kingdom
   a. Transparency 59, page 164, figure 8-9
   b. Jimson weed *Datura*
   c. Twelve possible trisomic conditions
   d. Alters the phenotype of plant's capsule

E. Langdon Down
1. Trisomy of chromosome 21
   a. **Down syndrome**, or **trisomy 21**
   b. Figure 8-10
   c. 3 in every 2000 live births
2. Characteristics
3. Nondisjunction of chromosome 21 during meiosis
   a. Gametes with $n + 1$ composition
   b. Triploid zygote produced
   c. Ovum is most often the source
4. Evidence supporting ovum as main source
   a. Figure 8-11 in text
   b. Increasing mother's age
      1. Primary oocytes are formed at birth.
      2. Eggs held in arrest
   c. **Genetic counseling** is
      1. Information
      2. **Amniocentesis** or **chorionic villus sampling (CVS)**.
   d. Random error

F. Reduced viability
1. **Patau** and **Edwards syndromes, 47,13+** and **47,18+**
2. Spontaneously aborted fetuses
   a. 15 to 20 percent of all conceptions end in spontaneous abortion.
   b. 30 percent due to chromosome anomalies
   c. Many demonstrate aneuploidy

d. David H. Carr

VI. Polyploid and Its Origins

A. More than two multiples of the haploid genome

B. $3n$, $4n$, and $5n$ chromosomes

C. Common in plant species

D. Odd numbers of chromosome sets

E. Origination

1. **Autopolyploidy**
2. **Allopolyploidy**

F. Identical to parent species

1. *AAA* and *AAAA*
2. **Autotriploids**
   a. Failure of all chromosomes to segregate
   b. Two sperm fertilize one ovum
   c. Experiments
3. Tetraploid cell
   a. Look at figure 8-13 in text
   b. Cold or heat shock
   c. **Colchicine**
4. Characteristics of autopolyploid plants
5. Commercial value
   a. Winesap apples
   b. Triploid bananas
6. Strawberry

G. Hybridization of two closely related species

1. Transparency 60, page 168, figure 8-14
2. *AA* crossed with *BB*, results in *AB* hybrid
   a. Sterile
   b. **Allotetraploid**
3. *Gossypium*
   a. 26 chromosomes

b. Old World cotton

c. J.O. Beasley's experiment

4. **Amphidiploid**

5. Somatic cell hybridization

a. Transparency 61, page 169, figure 8-16

b. **Protoplasts**

c. Fusion of protoplasts

VI. Variation in Chromosome Structure and Arrangement: An Overview

A. Structural changes

B. Deletions, duplications, inversions, and translocations

C. Loss or rearrangement of genetic material

D. "Sticky ends"

VII. Deletions

A. **Deletion** , or **deficiency**

1. Small or large part of chromosome

2. **Terminal** and **intercalary deletions**

B. Look at figure 8-17 in textbook

C. **Deficiency loop** or **compensation loop**

VIII. Duplications

A. **Duplication**

B. Compensation loop

C. Look at figure 8-18 in the textbook

D. Unequal crossing over, or replication error

E. Ribosomal RNA

1. Many genes which encode for rRNA

2. **rDNA**, phenomenon called **gene redundancy**

3. Oocytes

a. Nutrients

b. More ribosomes than any other cell

c. **Gene amplification**

4. **Nucleolar organizer region (NOR)**

5. rDNA is selectively replicated
   a. Released from its template
   b. Small nucleoli around each NOR
   c. As many as 1500

F. Duplications and phenotypic variation
   1. *Bar* eye in *Drosophila*
   2. Transparency 62, page 172, figure 8-19
   3. Alfred H. Sturtevant and Thomas H. Morgan
      a. (B^{-}/B^{+})
      b. (B/B^{-})
      c. (B/B)
      d. *Double Bar* (B^{D}/B^{-})
   4. Calvin Bridges and Herman J. Muller
      a. Region 16A on X chromosome
      b. Due to a duplication

G. Susumo Ohno
   1. Unique genes
      a. Cannot mutate
      b. Duplication of unique gene
   2. **Gene families**
      a. DNA sequence homology
      b. Examples

IX. Inversions

A. **Inversion**
   1. Rearranging of sequence of genes
   2. Transparency 63, page 173, figure 8-20
   3. Two breaks
   4. Forms a loop prior to breakage

B. Short or long and may or may not include centromere
   1. **Pericentric inversion**
   2. **Paracentric inversion**

C. Inversion in one of homologous chromosomes
   1. Synapsis during meiosis is not possible
   2. **Inversion heterozygotes**
   3. **Inversion loop**

D. Crossing over is possible
   1. Transparency 64, page 174, figure 8-21
   2. No crossing over occurs
   3. Crossing over does occur
   4. Paracentric inversion
      a. Two parental and two recombinant chromatids produced
      b. **dicentric** (two centromeres), and **acentric** (lacking a centromere)
         1. Acentric chromatid
         2. **Dicentric bridges**
      c. Results
   5. Pericentric inverted chromatid and its noninverted homolog
      a. This example is illustrated in figure 8-21 (b)
      b. No acentric or dicentric chromatids
      c. Inviable embryos
   6. Effect of inversion located in inversion heterozygotes

X. Translocations

A. **Translocation**

B. Occurrence

C. **Reciprocal translocation**
   1. Transparency 65, page 175, figure 8-22
   2. Figure 8-22 (a) illustrates a reciprocal translocation
      a. Nonhomologous arms come close to each other
      b. Only two breaks are required
      c. Four breaks are required

D. Rearrangement of the genetic material

E. Crosslike configuration when pairing
   1. Aberrant gametes not necessarily the result of crossing over

2. Look at homologous centromeres in figure 8-22 (b)
   a. Centromere 1 will migrate with centromere 3 *or* centromere 4
   b. Centromere 2 will migrate with either centromere 3 *or* centromere 4
   c. Four potential products
      1. The 1,4 and 2,3 combinations are balanced
      2. The 1,3 and 2,4 combinations contain duplicated and deleted segments
         - Unbalanced gametes
         - Lethality often results
         - **Semisterility**
         - In humans

F. **Robertsonian translocation**
   1. Common
   2. Larger segments fuse at centromeric region
   3. Familial Down syndrome
      a. Transparency 66, page 176, figure 8-23
      b. **14/21 D/G translocation**
      c. Normal individual
         1. ¼ of gametes will have two copies of chromosome 21
         2. After fertilization
         3. Other potential surviving offspring

XI. Fragile Sites in Humans

A. 1970
   1. "Gap"
   2. **Fragile sites**
   3. Association between one of the sites and mental retardation
   4. *in vitro*

B. **Fragile X syndrome** (or **Martin-Bell syndrome**)
   1. Look at figure 8-24 in text
   2. Inherited mental retardation.
   3. 1 in 1250 males, and 1 in 2500 females
   4. Dominant trait

5. Not fully expressed
6. Characteristics for males
7. A gene spans the entire fragile site
   a. *FMR-1*
   b. **Trinucleotide repeats**
   c. Correlation of number of repeats with expression

C. **Genetic anticipation**
   1. Number of repeats increases through generations
   2. Correlation is not limited to fragile X syndrome

D. *FHIT* (standing for *f*ragile *hi*stidine *t*riad) gene
   1. Derived from lung cancer tumors
   2. *FRA3B*
   3. More fragile in some
   4. **Genetic screening**

Lecture Content

Chapter Eight: Chromosome Variation and Sex Determination

I. Variation in Chromosome Number: An Overview

A. Variation in chromosome number ranges from the addition or subtraction of one or more chromosomes, to the addition of one or more sets of chromosomes.
   1. **Aneuploidy** is when an organisms gains or loses one or more chromosomes, but not a complete set.
      a. **Monosomy** is the loss of a single chromosome.
      b. **Trisomy** is the addition of one chromosome to a diploid genome.
   2. **Euploidy** is the addition of a complete haploid set of chromosomes.
      a. **Polyploidy** is when there are three or more sets of chromosomes within the genome.
      b. **Triploidy** is when there exactly three sets of chromosomes in the genome.
      c. **Tetraploidy** is when there are four sets of chromosomes within the genome.

B. Look at table 8.1 in the text for an organizational chart of these categories.

II. Chromosome Composition and Sex Determination in Humans

A. Females possess two X chromosomes and males possess one X and one Y chromosome in both humans and *Drosophila*.
   1. This fact might lead one to believe that the Y chromosome causes maleness.
      a. However, another possibility is that the absence of the second X chromosome causes maleness.
      b. Or, it could be that the presence of the second X chromosome causes femaleness.
   2. The explanation of Y determining the sex of the organism is valid in humans, but not in *Drosophila*.

B. Around the year 1940, two human abnormalities involving sex were observed.
   1. One of these abnormalities is **Klinefelter syndrome**.
      a. Transparency 55, page 157, figure 8-1(a).
      b. Individuals affected with Klinefelter syndrome generally have male genitalia and internal ducts.

c. The testes are usually underdeveloped in these individuals and generally do not produce sperm.
d. Masculine development does occur, but feminine sexual development is not completely suppressed; slight enlargement of the breasts is common.
e. Klinefelter syndrome occurs in approximately 2 out every 1000 births.

2. The other abnormality is **Turner syndrome**.
   a. Transparency 55, page 157, figure 8-1 (b).
   b. Individuals affected with Turner syndrome have external female genitalia, as well as female internal ducts, but the ovaries are rudimentary.
   c. Characteristics of these individuals include short stature, a webbed neck, and a broad, shielding chest.
   d. Turner syndrome is observed only in about 1 out of 3000 female births, because many 45,X fetuses die *in utero*.
3. The karyotypes of individuals affected with these syndromes were determined to be abnormal with respect to the sex chromosomes in 1959.
   a. Individuals with Klinefelter syndrome are trisomic and have an XXY in addition to the normal 44 autosomes.
   b. Individuals with Turner syndrome are monosomic and have only a single X chromosome in addition to the normal 44 autosomes.
   c. Both of these abnormalities are caused by nondisjunction within the sex chromosomes during meiosis.
4. There is a specific convention used when designating chromosome composition.
   a. The first number indicates the number of chromosomes present in the individual.
   b. The number following the comma indicates the deviation from the normal diploid content.
   c. For example, an individual with Klinefelter syndrome would have the following chromosome composition, **47,XXY**.
   d. An individual with Turner syndrome would this chromosome composition, **45,X**.

5. These karyotypes allowed for the conclusion that the Y chromosome determines maleness in humans.
   a. In the absence of the Y, the individual is female.
   b. With the presence of the Y, even if there are also two Xs, the individual shows masculinization.
6. There are other karyotypes that can produce Turner syndrome.
   a. Individuals with two apparent cell lines often express Turner syndrome; these individuals are called **mosaics**.
   b. The most common chromosome combinations are 45,X/46,XY, and 45,X/46,XX.

C. The karyotype **47,XXX** results in female differentiation.
1. This syndrome involves individuals with three X chromosomes along with the normal 44 autosomes.
2. This occurs in about 1 of every 1200 female births.
3. In many cases, the female with this syndrome is perfectly normal.
4. However, in other cases individuals have characteristics such as underdeveloped secondary sex characteristics, sterility, and mental retardation.
5. There are some cases in which **48,XXXX** and **49,XXXXX** occur, and the symptoms are more pronounced than in 47,XXX.
6. The presence of the additional X chromosome appears to disrupt the balance of genetic information essential for normal female development.

D. Another human trisomy, **47,XYY**, has been discovered and investigated.
1. Patricia Jacobs, in 1965, discovered that 9 of 315 males in a Scottish maximum security prison had this karyotype.
   a. All of the men were above average in height, and suffered personality disorders.
   b. 7 of the 9 were of subnormal intelligence.
   c. All had obviously committed some act of crime.
2. Due to this initial investigation, the phenotype and frequency of 47,XYY has been intensely investigated.

a. The majority of men possessing this phenotype express above normal height and subnormal intelligence.
b. This karyotype is also found in a much higher frequency in penal and mental institutions than in the "public."
c. After much investigation, the only constant association between incarcerated and non-incarcerated individuals with the genotype is that they are over 6 feet tall.
d. It is now clear that there is no correlation between an extra Y chromosome and behavior.

E. There have been studies performed on how the Y chromosome affects male development.
1. The Y chromosome only shares limited homology with loci on the X chromosome, but it does carry genetic information that controls sexual development.
2. The Y chromosome contains genes that are responsible for the **testis-determining factor (TDF)**.
a. TDF is a product that triggers the undifferentiated gonadal tissue of the embryo to develop testes.
b. In the absence of TDF, female development occurs.
3. Investigations have shown that the Y chromosome does in deed carry a gene called ***SRY* (sex-determining region Y)**.
4. There are observations that aid in the argument that the *SRY* gene is important in male development.
a. One observation includes males that contain two X chromosomes and no Y chromosome; however they have *SRY* attached to one of their X chromosomes.
b. Another observation includes females, which contain one X and one Y chromosome, but their Y is missing the *SRY* region.
5. The final observation that aids in the argument of the *SRY* region includes experiments performed on **transgenic mice**.

a. Transgenic organisms are ones that arise from fertilized eggs that have had foreign DNA injected in them and incorporated into the genetic composition.
b. The experiment consisted of injecting DNA containing only *Sry*, the similar region in mice, to fertilized XX eggs.
c. The outcome of this experiment was mostly male mice.

III. The X Chromosome and Dosage Compensation

A. Due to the fact that females have two X chromosomes, while males only have one, there is a question about a possible **dosage compensation** mechanism.
B. Murray L. Barr and Ewart G. Bertram' s experiments with female cats, and Keith Moore and Barr's experiments with humans have demonstrated a genetic mechanism in mammals that compensates for X chromosome dosage disparities.
   1. A darkly staining body was observed by Barr and Bertram in interphase nerve cells of female cats.
      a. This dark staining body was seen to be absent in the same cells of male cats.
      b. The same results were present in humans, the dark staining body was present in females and was absent in males.
      c. This body is a highly condensed structure, which is only about 1 mm in diameter, and lies against the nuclear envelope of interphase cells.
   2. Subsequent experiments suggest that this body, called a **sex chromatin body**, also known as a **Barr body**, is an inactivated X chromosome.
   3. This Barr body, suggested by Ohno, arises from one the two X chromosomes.
      a. This hypothesis is attractive because it explains a mechanism for dosage compensation.
      b. If one X chromosome is inactive, then the dosage of genetic information expressed in both males and females is equivalent.
      c. No matter how many X chromosomes are present, only one of them seem to be inactivated.
         - No Barr bodies are present in Turner syndrome, 45,X; one is seen in Klinefelter syndrome, 47XXY; two are present in 48,XXXX females; and so on.

- The number of Barr bodies follows the simple rule of N-1, where N is the number of X chromosomes present.

C. In 1961, Mary Lyon and Liane Russell independently proposed that the inactivation of an X chromosome is completely random and occurs during an early point in embryonic development.
   1. This explanation, known as the **Lyon hypothesis**, was originally based on observations of female mice containing heterozygous sex-linked coat genes.
      a. The coat color of these female mice was mottled, that is they had large skin patches that expressed the color allele of one X and other patches that expressed the color allele of the opposite X chromosome.
      b. This pattern is also present in female calico cats.
      c. These patterns do not occur in males because they are hemizygous and only have one X-linked coat color allele.
   2. The Lyon hypothesis is generally accepted as valid, but one extension is that female mammals are mosaics for all heterozygous X-linked alleles.
      a. Some parts of the body only express maternally derived alleles, while other parts of the body express only paternally derived alleles.
      b. Two interesting examples are **red-green color blindness** and **anhidrotic ectodermal dysplasia**, which are both X-linked disorders in humans.
         - For red-green color blindness, males, which are hemizygous, are fully color-blind, while heterozygous females display a mosaic expression of color-blindness.
         - Transparency 56, page 160, figure 8-5.
         - For anhidrotic ectodermal dysplasia, males show an absence of teeth, sparse hair growth, and lack of sweat glands, whereas heterozygous females express patterns of tissue with and without sweat glands.
         - In both examples, the occurrences are caused by random inactivation of one or the other X chromosome in heterozygous females.

D. There is a single region on the human X chromosome that is the major control unit, and it is called the **X-inactivation center (XIC)**.
   1. The expression of this region results in the inactivation of an X chromosome.

2. The critical locus of the XIC region is thought to be the gene ***XIST* (*X-inactivation specific transcript*)**.
   a. There are comparable regions within the mouse X chromosome known as the *Xic* region and the *Xist* gene.
   b. In 1996, a research group led by Graeme Penny offered the most convincing data yet that the expression of the *Xist* is the critical event in X inactivation.
      - This group introduced a targeted deletion into a cell with two X chromosomes.
      - This targeted deletion was able to inactivate the gene responsible for X-inactivation, which eliminated inactivation of the chromosome bearing this alteration.

IV. Chromosome Composition and Sex Determination in Drosophila

A. In 1916, Calvin Bridges reported that the Y chromosome is not involved in sex determination in *Drosophila*, and instead he proposed that the X chromosome together with the autosomes play a critical role in sex determination.
B. There are two phases in which Bridges work can be classified: a study of offspring resulting from nondisjunction of the X chromosomes during meiosis in females, and the work with progeny of triploid females.
   1. **Nondisjunction** is the failure of paired chromosomes to segregate or separate during meiosis.
      a. The result of nondisjunction is two abnormal gametes, one with an extra chromosome ($n+1$), and the other with one less chromosome ($n-1$).
      b. When these gametes are fertilized, they produce aneuploid zygotes, ($2n+1$) and ($2n-1$).
         - The XXY flies were normal females, while the XO flies were sterile males.
         - The Y chromosome in the XXY females did not cause maleness, nor did the absence of the Y chromosome in the XO males cause femaleness.
         - Bridges concluded that the Y chromosome in *Drosophila* lacks male-determining factors, but contains genetic information essential to male fertility.

2. By studying the progeny of triploid females (3*n*), Bridges was able to determine the mode of sex determination in *Drosophila*.
   a. Triploid females arise from diploid eggs fertilized by normal haploid sperm.
   b. Look at figure 8-6 in the text.
   c. These females express characteristics such as, heavy-set bodies, coarse bristles, and coarse eyes.
   d. A wide variety of chromosome compositions are distributed into the gametes of these females.
3. Bridges soon realized that it was the ratio of X chromosomes to the number of haploid sets of autosomes present, which is the critical factor in sex determination.
   a. Normal (2X:2A) and triploid (3X:3A) are both a ratio of 1:1 and produce fertile females.
      - When the ratio exceeds 1:1, as in (3X:2A) or 1.5, a weak and infertile female.
      - At one time, these females were called **super-females** because they contained an extra X.
      - These females are presently called **metafemales** because they have lowered viability.
   b. Males which are normal (XY:2A) and males that are sterile (XO:2A) have ratios of 1:2, or 0.5.
      - When the ratio is decreased to 1:3, or 0.33, (XY:3A), an infertile male is produced.
      - This male is called a **metamale**.
   c. During Bridges experiments, he came across some ratios of X:A that were 0.5 and 1.0.
      - These flies were generally larger than other flies; they exhibited many morphological abnormalities, and contained rudimentary bisexual gonads and genitalia.
      - Flies, which expressed this ratio within their genome, are called **intersexes**.

4. The results of these experiments allowed Bridges to conclude that the male-determining factors are not located on the Y chromosome, but instead, they are located on the autosomes.
   a. However, there are some female-determining factors located on the X chromosome.
   b. Therefore, male gametes, which contain the normal number of autosomes and one Y chromosome, result in male flies because of the absence of an X chromosome.
   c. The **genic balance theory** explains this mode of sex determination.
      - This theory states that the threshold for maleness is reached when the X:A ratio is 1:2.
      - Although, the presence of an additional X (XX:2A) alters this balance and results in female differentiation.
5. There are several mutant genes now known that are also involved in sex determination in *Drosophila*.
   a. One of these genes is a recessive autosomal gene called *transformer* (*tra*), discovered by Alfred H. Sturtevant.
      - When females are homozygous for this mutation, they are transformed into sterile male flies.
      - On the other hand, males are not affected even when they are homozygous for *tra*.
   b. Another gene involved in sex determination is found on the X chromosome and is called *Sex-lethal* (*Sxl*).
      - This gene serves as the "master switch" for the activation of at least four other regulatory genes.
      - The activation of this gene is essential for female development, and relies on a ratio of 1:1, sex chromosomes: autosomes.
      - When the ratio is less than 1.0, activation does not occur and male development takes place.

V. Aneuploidy

A. Aneuploidy again is when an organism has extra or missing chromosomes within their diploid set.
   1. Transparency 57, page 163, figure 8-7.
   2. This condition occurs through primary or secondary nondisjunction.

B. The loss of a single chromosome is $2n$-1 and is called monosomy.
   1. Monosomy for the autosomes is not tolerated well in animals.
      a. In *Drosophila*, **Haplo-IV** is a condition in which the fly is missing one of its chromosome number 4, and experiences slower development, a reduced body size, and impaired viability.
         - Monosomy for the larger chromosomes is thought to be lethal since no flies have ever been recovered exhibiting this condition.
         - The reason that many animals do not survive from monosomic conditions is because if the chromosome contains a lethal allele, there is no homologous chromosome to keep the lethal allele from expressing.
      b. This type of aneuploidy is tolerated more within the plant kingdom, such as in maize, tobacco, the evening primrose, and the Jimson weed.
         - They are however less viable than their diploid kin.
         - Haploid pollen grains are particularly sensitive to monosomic conditions.

C. There are no live humans with monosomy, but there are people with **partial monosomy**.
   1. Partial monosomy is when only part of one chromosome is lost.
   2. These cases are also referred to as **segmental deletions**.
   3. Jerome LeJeune described the first case of this type of monosomy in 1963.
      a. Transparency 58, page 163, figure 8-8.
      b. He described the condition known as **cri-du-chat (cry of the cat) syndrome**.
      c. This syndrome is associated with a large loss of the short arm of chromosome 5.
      d. The genetic constitution is designated as **46,5p-**, which designates that there are a normal number of chromosomes, but that part of the short arm, the petite arm, on chromosome number 5 is missing.

- Characteristics of this syndrome include gastrointestinal and cardiac complications, as well as mental retardation.
- The abnormal growth of the larynx and glottis gives the characteristic cry in which this syndrome is named.
- The incidence of this syndrome is 1 in every 50,000 live births.

D. The loss of two chromosomes is $2n$-2 and is called trisomy.
   1. The addition of an extra chromosome is somewhat more viable in both the plant and animal kingdoms when compared to monosomy.
   2. The effect is usually small when the extra chromosome is a small chromosome, but when a larger chromosome exists with an extra copy in humans or *Drosophila*, severe effects are usually seen, and they are often lethal.
   3. Triploidy is tolerated well within the plant kingdom.
      a. Transparency 59, page 164, figure 8-9.
      b. An example is the Jimson weed *Datura*, whose normal diploid number is 24.
      c. Each of the twelve possible trisomic conditions has been reported within this species.
      d. Each different trisomy possibility alters the phenotype of the plant's capsule.

E. In 1866, Langdon Down discovered the only human autosomal trisomy apparent in many individuals who live past one year of birth.
   1. The condition that Langdon Down reported resulted from trisomy of chromosome 21.
      a. This condition is now known as **Down syndrome**, or simply **trisomy 21**.
      b. Figure 8-10 in the text shows a boy with Down syndrome.
      c. This condition is found in approximately 3 in every 2000 live births.
   2. There are many characteristics, which are associated with Down syndrome.
      a. Many affected individuals display epicanthic folds in the corner of the eye and are usually very short.
      b. They may have round heads; protruding, furrowed tongue, which causes them to keep their mouths open; and short broad hands and fingers.
      c. The psychomotor, physical, and mental development is retarded, and the IQ is rarely above 70.

d. Affected individuals also have a shortened life expectancy, and are prone to heart malformations, respiratory disease, and show a high incidence of leukemia, 15 times higher than that of the normal population.

3. One way in which this condition might occur is through nondisjunction of chromosome 21 during meiosis.
   a. This results in gametes with $n + 1$ composition.
   b. Following fertilization with a normal gamete, the triploid zygote is produced.
   c. Although the additional chromosome can come from either the maternal or paternal gamete, the ovum is most often the source.
4. Before techniques were available to distinguish maternal and paternal chromosomes, the conclusion that the ovum was most often the source was supported by other evidence from studies of the age of mothers giving birth to Down babies.
   a. Figure 8-11 in the text shows the distribution of maternal age and the incidence of Down syndrome children.
   b. As the mother's age increases, the incidence of Down syndrome children increases dramatically.
      - One reason that this is thought to occur is because all primary oocytes are formed at birth.
      - Therefore, when ovulation begins, the egg is held in arrest one month longer than the previous one.
      - Women, who are at ages 40-50, have had their eggs in arrested in meiosis for a much longer time than women who are at ages 20-30.
   c. **Genetic counseling** is available for women who are pregnant in their later years, and it serves two purposes.
      - One is to inform the parents about the probability that their child will be affected and to educate them about Down syndrome.
      - Another is to possibly recommend a prenatal diagnosis such as **amniocentesis** or **chorionic villus sampling (CVS)**.
        - These techniques require a culture of fetal cells.

- A karyotype is then determined and analyzed to see if the child will be affected with Down syndrome.

d. Although Down syndrome is not inherited since it is caused by a random error, it can "run" in families if it is caused by a translocation of chromosome 21 (we will talk about this later in the chapter).

F. Individuals with monosomic and trisomic conditions definitely have a reduced viability.

1. There are only two other trisomy cases in humans that survive to term, and they are **Patau** and **Edwards syndromes, 47,13+** and **47,18+**.
   a. These both result in severe malformations and early fatalities.
   b. Figure 8-12 in the book shows a karyotype for a patient affected with Edwards syndrome.
2. There have been studies performed on spontaneously aborted fetuses.
   a. Reports have been made that at least 15 to 20 percent of all conceptions end in a spontaneous abortion.
   b. Approximately 30 percent of all spontaneous abortions are due to chromosome anomalies.
   c. Many of the spontaneous aborted fetuses were shown to demonstrate aneuploidy.
      - The most common form of aneuploidy is the 45,X condition.
      - If the fetus survives to term, the individual is afflicted with Turner syndrome.
   d. Studies performed by David H. Carr, recovered trisomies for every human chromosome, but monosomies were rarely found.

VI. Polyploid and Its Origins

A. Polyploid describes instances in which more than two multiples of the haploid genome are found.

B. A triploid has $3n$ chromosomes, a tetraploid has $4n$ chromosomes, and a pentaploid has $5n$ chromosomes and so on.

C. Although this condition rarely occurs in animal species, it is commonly found in lizards, amphibians, and fish, but is much more common in plant species.

D. Odd numbers of chromosome sets are not usually passed on from generation to generation because the gametes that these individuals produce are genetically unbalanced; therefore, this condition is not generally found in species that rely solely on sexual reproduction.

E. There are two ways that polyploidy can originate.
   1. **Autopolyploidy** results when there is an addition of one or more extra sets of chromosomes identical to the normal haploid complement of the same species.
   2. **Allopolyploidy** results when there is a combination of chromosome sets between different species.

F. In autopolyploidy, each additional set of chromosomes is identical to the parent species.
   1. Triploids are represented as *AAA* and tetraploids are represented as *AAAA*.
   2. There are several ways in which **autotriploids** can be produced.
      a. One way begins with the failure of all chromosomes to segregate during meiotic divisions, which may result in the production of a diploid gamete, which if fertilized, results in a triploid zygote.
      b. A very uncommon way for autotriploidy to occur is by having two sperm fertilize one ovum.
      c. Experiments are another way in which autotriploids are produced, a diploid can be crossed with a tetraploid and the resulting zygote is a triploid.
   3. Experiments can also be performed in order to create a tetraploid cell.
      a. Look at figure 8-13 in the text.
      b. One way is by applying cold or heat shock during mitosis of a somatic cell.
      c. **Colchicine** can also be added to somatic cells during mitosis; this interferes with the spindle formation and the replicated chromosomes cannot be separated.
   4. There are some general characteristics of autopolyploid plants.
      a. They are often larger than their diploid relatives.
      b. They often have increased fruit or flower size, which seems to be due to a larger cell size.

5. There are several triploid plants used for commercial value, these include several species of potatoes, Winesap apples, bananas, and seedless watermelons.
   a. The Winesap apples are larger than normal, which is good for commercial use.
   b. The commercial triploid bananas contain soft edible seeds, whereas diploid bananas contain hard seeds.
6. The commercial strawberry is an octaploid.

G. Allopolyploid is derived from the hybridization of two closely related species.
   1. Transparency 60, page 168, figure 8-14.
   2. If a haploid ovum from one species contains chromosome sets *AA* and is fertilized by a sperm from another species with chromosome sets *BB*, a hybrid is produced with *AB*.
      a. The hybrid produced may be sterile due to the fact that all or some of the *a* and *b* chromosomes cannot synapse.
      b. However, if the *AB* hybrid undergoes a natural or induced chromosomal doubling a fertile *AABB* tetraploid is produced, which is called an **allotetraploid**.
   3. The most common form of allotetraploidy in plants is *Gossypium*, the cultivated species of American cotton.
      a. This species consists of 26 chromosomes, 13 large and 13 much smaller.
      b. Old World cotton was discovered to only have 13 pairs of large chromosomes, and allopolyploidy was suspected.
      c. J.O. Beasley reconstructed the origin of the cultivated cotton.
         - First, he crossed the Old World strain with the American strain.
         - Then, he treated the resulting hybrid with colchicine to double the chromosome number.
         - A fertile **amphidiploid**, a term equivalent to allotetraploid, resulted with similar characteristics to the cultivated variety.
   4. Amphidiploids generally exhibit characteristics of both parental species.
      a. Many hybridization techniques have been used on the grasses wheat and rye.
      b. Geneticists have produced a variety of allopolyploid hybrids.

5. The technique somatic cell hybridization has been used to produce allopolyploid plants.
   a. Transparency 61, page 169, figure 8-16.
   b. Cells from developing leaves are isolated and treated to remove their cell walls, which results in **protoplasts**.
   c. These protoplasts can be fused with protoplasts from other plant species to create new hybrids.

VI. Variation in Chromosome Structure and Arrangement: An Overview

A. Chromosomes can undergo structural changes that delete, add, or rearrange substantial portions.
B. These structural changes include deletions, duplications, inversions, and translocations.
C. These changes are generally due to one or more breaks along the axis of a chromosome, which is then followed by either the loss or rearrangement of some genetic material.
D. The ends of chromosomes, the telomeres, do not readily fuse with "broken" ends of chromosomes, but the ends produced from a break are "sticky" and can rejoin with other broken ends.

VII. Deletions

A. A **deletion** is when a portion of a chromosome is lost, also called a **deficiency**.
   1. Deletions can encompass a small or large part of the chromosome.
   2. They may occur at the ends or in the middle of the chromosome called **terminal** and **intercalary deletions**, respectively.
B. Look at figure 8-17 in textbook.
C. During synapsis of the deleted chromosome and its normal homologue, the unpaired region of the normal chromosome must "buckle out," in a configuration called a **deficiency loop** or **compensation loop**.

VIII. Duplications

A. A **duplication** is when any part of the genetic material is present more than once.
B. As in a deletion, the pairing heterozygotes may produce a compensation loop.
C. Look at figure 8-18 in the textbook.

D. Duplications can arise from unequal crossing over between synapsed chromosomes during meiosis, or through a replication error prior to meiosis.

E. Ribosomal RNA, along with other molecules, must be present in a large amount in every cell in order to perform protein synthesis.

1. To ensure the abundance of RNA, there are many genes encoding rRNA in each cell.
2. DNA that codes for ribosomal RNA is called **rDNA**, and the phenomenon is called **gene redundancy**.
   a. For example, about 0.4% of the haploid genome in *Escherichia coli*, consists of rDNA, which is equivalent to 7 copies of rDNA.
   b. About 0.3% of the genome in *Drosophila melanogaster* consists of rDNA, which is equivalent to 130 copies.
3. In oocytes, the normal abundance of rDNA is often insufficient to provide enough rRNA and ribosomes.
   a. The oocyte stores a copious amount of nutrients for use by the developing embryo.
   b. The oocyte also has more ribosomes than any other cell.
   c. **Gene amplification** is a phenomenon used by some cells to ensure an adequate amount of rRNA is present.
4. The **nucleolar organizer region (NOR)** is the area on the chromosome in which the genes for rRNA are located.
   a. The NOR is associated with the nucleolus, the processing center for ribosome production.
   b. Each NOR in the frog *Xenopus* contains the equivalent of 400 redundant gene copies which code for rRNA, and even this is not an adequate amount.
5. In order to amplify the number of rRNA genes, rDNA is selectively replicated.
   a. Each new set of genes is then released from its template.
   b. These form small nucleoli around each NOR in the oocyte.
   c. As many as 1500 of these "micronucleoli" have been seen in a single oocyte.

d. If the number of micronucleoli is multiplied to the number of gene copies per NOR, the resulting number, over half a million, is the number of gene copies in *Xenopus*.

F. Duplications can also cause phenotypic variation in organisms.

1. The *Bar* eye in *Drosophila* is an example of a phenotypic variation caused by a duplication.
   a. Instead of the normal oval shaped eye, these individuals have narrow, slitlike eyes.
   b. This phenomenon is inherited in the same manner as a dominant X-linked mutation.
2. Transparency 62, page 172, figure 8-19.
3. In the 1920s, Alfred H. Sturtevant and Thomas H. Morgan discovered and investigated this mutation.
   a. Normal wild-type females (B^+/B^+) have about 800 facets in each eye.
   b. Heterozygous females (B/B^+) only average about 350 facets.
   c. Homozygous females (B/B) only had 70 facets in each eye.
   d. Females with even fewer facets were termed *double Bar* (B^D/B^+).
4. In the 1930s, Calvin Bridges and Herman J. Muller compared the polytene X chromosome banding of the wild-type fly with that of the *Bar* fly.
   a. They reported that in wild-type flies, there was one copy of region 16A on the X chromosome, whereas in the *Bar* flies, the region was duplicated, and in the *double Bar* flies, the region was tripled.
   b. They concluded that the *Bar* phenotype was in fact due to a duplication.

G. In 1970, Susumo Ohno published a paper suggesting that gene duplication was essential to the origin of new genes during evolution.

1. He suggested that there were unique genes within each genome that were essential for life in an organism.
   a. Since these unique genes were essential for life, it is obvious that they cannot mutate.
   b. However, if the unique gene duplicated, the new copy would be inherited to all subsequent generations.

- Since there is an extra copy, mutations can occur because the original gene is still intact and function, as it needs to.
- Over long periods of evolutionary time, the gene may change sufficiently so that its product assumes a divergent role in the cell.
- This "new" product could then be used as an "adaptive" measure to increase an organism's fitness!

2. The presence of **gene families**, groups of contiguous genes whose products perform the same function, supports this hypothesis.
   a. Members of a family show a significant amount of DNA sequence homology, and it can therefore be assumed that they arose from a common origin.
   b. Examples include T-cell receptors, and antigens encoded by the major histocompatability complex.

IX. Inversions

A. When a segment of chromosome is turned around 180° within the chromosome, it is called an **inversion**.
   1. An inversion does not involve loss of genetic material; instead it is the rearranging of the linear sequence of the genes.
   2. Transparency 63, page 173, figure 8-20.
   3. An inversion requires two breaks within the chromosome.
   4. The chromosome forms a loop prior to the breakage, so that the newly created "sticky ends" are brought close together and rejoined.

B. The actual segment that is inverted can be short or long and may or may not include the centromere.
   1. If the inversion includes the centromere, it is called a **pericentric inversion**.
   2. If the inversion does not include the centromere, it is called a **paracentric inversion**.

C. Sometimes, only one of the homologous chromosomes in a pair experiences an inversion.
   1. In this case, synapsis during meiosis is not possible.
   2. Organisms with one inverted chromosome and one noninverted homolog are called **inversion heterozygotes**.

3. The way pairing occurs between these two is that they form an **inversion loop**.

D. When the inversion loop forms, crossing over is possible.

1. Transparency 64, page 174, figure 8-21.
2. If no crossing over occurs, the homologs will separate and two normal and two inverted chromatids are distributed into gametes.
3. If crossing over does occur, abnormal chromatids are produced.
4. Figure 8-21 (a) illustrates an example of a paracentric inversion.
   a. If a single crossover event is completed, two parental and two recombinant chromatids are produced.
   b. If the centromere is involved, one recombinant chromatid is **dicentric** (two centromeres), while the other one is **acentric** (lacking a centromere).
      - The acentric chromatid will move randomly to either pole, or be lost during anaphase.
      - The dicentric chromatid will be pulled in two directions, forming **dicentric bridges**, during anaphase until it finally splits.
   c. If either of the two recombinant chromatids go into the gametes, then that gamete is deficient in genetic material, and the zygote develops abnormally, if it develops at all.
5. A similar chromosomal imbalance is produced when crossing over occurs between a pericentric inverted chromatid and its noninverted homolog.
   a. This example is illustrated in figure 8-21 (b).
   b. No acentric or dicentric chromatids are produced from this crossover event.
   c. The gametes that are produced give rise to inviable embryos following fertilization.
6. The inversion located in inversion heterozygotes has the effect of suppressing the recovery of crossover products when chromosome exchange occurs within the inverted region.

X. Translocations

A. The movement of a segment of chromosome to a new place in the genome is called a **translocation**.

B. Translocations can occur within a single chromosome or between nonhomologous chromosomes.

C. A **reciprocal translocation** is the exchange of segments between two nonhomologous chromosomes.

1. Transparency 65, page 175, figure 8-22.
2. Figure 8-22 (a) illustrates an example for a reciprocal translocation.
   a. The easiest way for this to occur is for the nonhomologous arms to come very close to each other in order to facilitate exchange between them.
   b. For this example, only two breaks are required, one on each chromosome.
   c. If the translocated segments are located within the center of the chromosome, a total of four breaks is required, two on each chromosome.

D. Translocations do not make genetic information be lost or gained; instead it is a rearrangement of the genetic material.

E. Homologs that are heterozygous for a reciprocal translocation, shown in figure 8-22 (b), undergo a crosslike configuration when pairing.

1. In the case of translocations, aberrant gametes are not necessarily the result of crossing over.
2. Focus on the homologous centromeres in figure 8-22 (b).
   a. The chromosome that contains centromere 1 will migrate randomly to either pole, and will migrate with the chromosome containing either centromere 3 *or* the chromosome containing centromere 4.
   b. The chromosome with centromere 2 will migrate to the other pole along with either the chromosome containing centromere 3 *or* the chromosome containing centromere 4.
   c. This results in four potential meiotic products.
      - The 1,4 combination contains chromosomes not involved in translocation, while the 2,3 combination contains translocated chromosomes, which contain a complete complement of genetic information and are balanced.
      - The other possible products 1,3 and 2,4 combinations contain chromosomes displaying both duplicated and deleted segments.

- When these are incorporated into gametes, the meiotic products are unbalanced.
- If fertilized, lethality often results.
- As few as 50% of the progeny of parents that are heterozygous for a reciprocal translocation may survive, this condition is called **semisterility**.
- In humans, this condition leads to monosomies and trisomies, which in turn lead to many different birth defects.

F. A **Robertsonian translocation** is a large submetacentric or metacentric chromosome.

1. This is a common type of translocation, and involves the breakage of extreme ends of the short arms of two nonhomologous acrocentric chromosomes.
2. The small segments are subsequently lost, and the larger segments fuse at their centromeric region.
3. It is this type of translocation that is responsible for inherited or familial Down syndrome.
   a. Transparency 66, page 176, figure 8-23.
   b. Studies have shown that familial Down syndrome can be caused by a **14/21 D/G translocation**.
   c. This is where one parent has the majority of the G-group of chromosome 21 translocated to the D-group of chromosome 14; this individual is normal even though he/she only has 45 chromosomes.
      - Following meiosis, ¼ of the gametes produced will have two copies of chromosome 21: a normal chromosome and a second copy of 21 translocated on chromosome 14.
      - When fertilized, the gamete will only have 46 chromosomes, but it will have three copies of chromosome 21.
      - Other potential surviving offspring will either have the standard diploid genome, or the balanced translocation (like the parent).

XI. Fragile Sites in Humans

A. An discovery occurred around 1970, where cells derived from certain individuals contained a specific area along one of the chromosomes that did not stain and gave the appearance of a gap.
   1. This "gap" was present in other individuals, but in appeared in different positions.
   2. These gaps came to be known as **fragile sites** because it was thought that the gaps represented areas where the chromosome had broken due to the handling of the cells.
   3. There soon became a strong association between one of the sites and a form of mental retardation.
   4. All these experiments have been performed *in vitro*, and it is not known how the phenotype is expressed *in vivo*.

B. Although most fragile sites do not appear to be associated with any type of clinical syndrome, there are individuals bearing a folate-sensitive site on the X chromosome, which exhibit the **fragile X syndrome** (or **Martin-Bell syndrome**).
   1. Look at figure 8-24 in the text.
   2. This is the most common form of inherited mental retardation.
   3. Fragile X syndrome affects about 1 in 1250 males, and 1 in 2500 females.
   4. This is a dominant trait; therefore, females carrying only one affected X chromosome can be mentally retarded.
   5. The trait is not fully expressed, only about 30% of fragile X females are mentally retarded, whereas 80% of fragile X males are mentally retarded.
   6. Males also have characteristic protruding chins, enlarged ears, and increased testicular size.
   7. There is a gene that spans the fragile site, and may be responsible for this syndrome.
      a. The gene is known as *FMR-1*, and is a sequence of three nucleotides repeated over and over.
      b. This phenomenon is called **trinucleotide repeats**, and has been recognized in other human disorders including Huntington disease.

c. The number of repeats varies immensely, but correlates directly with the expression of fragile X syndrome.
   - Normal individuals have up to 50 repeats, whereas those with 50-200 repeats are considered carriers.
   - Individuals with over 200 repeats express the syndrome.

C. Fragile X syndrome illustrates the phenomenon known as **genetic anticipation**, where expression becomes more severe in successive generations.
   1. The number of trinucleotide repeats increases as the affected X chromosome passes through each generation.
   2. The correlation between the number of repeats and the severity of the disorder is very strong, and it is not limited to fragile X syndrome.

D. In 1996, Carlo Croce, Kay Huebner, and colleagues reported a gene on chromosomes 3, the *FHIT* (standing for *f*ragile *hi*stidine *t*riad) gene, which was altered in cells taken from tumors recovered from individuals with lung cancer.
   1. 80% of the tumors or cell lines derived from them had an abnormal *FHIT* gene.
   2. This gene is part of the area of the autosome *FRA3B*, also thought to be associated with cancer of the colon, esophagus, and stomach.
   3. It is thought that this gene may be more fragile in some than in others, leading to lung cancer following environmental carcinogens, such as cigarette smoke.
   4. The discovery of this gene, and others like it, aid in the potential for **genetic screening**.

Lecture Outline

Chapter Eleven: Organization of DNA in Chromosomes and Genes

I. Viral and Bacterial Chromosomes

A. Less complicated

1. Single nucleic acid molecule
2. Less genetic information

B. DNA or RNA for viruses

1. Single- or double-stranded
2. Circular or linear form
   a. **ϕX174 bacteriophage**
   b. **Polyoma virus**
   c. **Bacteriophage λ**
   d. **T-even series of bacteriophages**
3. Figure 11-1 in the text
   a. Package DNA in small volume
   b. Phage λ
4. Table 11.1

C. Bacterial chromosomes

1. Double-stranded DNA
   a. **Nucleoid**
   b. *E. coli*
2. **DNA-binding proteins**
   a. **H** and **HU**
   b. Small and abundant in cell
   c. **Histones**
   d. Functional after packaging

II. Mitochondrial and Chloroplast DNA

A. Own genetic information

1. Transmission of mutations
2. **Uniparental mode of inheritance**

B. **Mitochondrial DNA (mtDNA)**

1. Circular duplex
2. Free of chromosomal proteins
3. Figure 11-4 for mtDNA
4. The size of mtDNA
   a. Table 11.2
   b. In vertebrates
   c. Plants
5. Characteristics
   a. Few or no gene repetitions
   b. Dependent upon nuclear DNA
   c. Function of products

C. **Chloroplast DNA (cpDNA)**

1. Own set of genetic information
   a. Dependent on nuclear DNA
   b. Larger
2. Figure 11-5 for chloroplast DNA
   a. Circular and double-stranded
   b. Semiconservative
   c. Free of associated proteins
3. Number of copies
   a. In *Chlamydomonas*
   b. 195 kb
   c. Higher plants

D. **Endosymbiont theory**

1. Incorporation with primitive eukaryotic cells
2. Lost ability to function independently

III. Organization of DNA in Chromatin

A. Organization in eukaryotes

1. Large amount of DNA per chromosome
   a. *E. coli*
   b. Humans

c. Nucleus is 5 μm in diameter

2. Different cells assume specific functions
   a. All cells have complete genetic complement
   b. Highly ordered regulatory system
3. Organized cell cycle
   a. **Chromatin**
   b. Recognizable during prophase
   c. **Folded-fiber model**
4. Associated proteins
   a. **Histones**
   b. **Nonhistones**

B. Extensive coiling and folding
   1. Most essential structural role
      a. Positively charged amino acids
      b. Electrostatic bonding
      c. Table 11.3
   2. X-ray diffraction studies
      a. Repeating structural units
      b. If histones are removed
   3. Model for chromatin structure
      a. The digestion of chromatin
         1. Not a random process
         2. Fragment sizes
         3. Repeating unit
      b. Linear arrays of spherical bodies
         1. Look at figure 11-6 in the text
         2. Ada and Donald Olins
         3. **V-bodies** or **nucleosomes**
      c. Two types of tetramers
         1. $(H2A)_2$-$(H2B)_2$ and $(H3)_2$–$(H4)_2$
         2. Repeating units

3. Octamer

d. Extended nuclease digestion time

1. **Nucleosome core particle**

2. Number of base pairs

3. Lost DNA

4. **Linker DNA**

e. John T. Finch, Aaron Klug, and others

1. Transparency 67, page 239, figure 11-7

2. 146-base-pair DNA core

3. Ellipsoidal shape

4. Packaging of chromatin

a. 110 Angstroms in diameter

b. Long strings of repeating nucleosomes

c. **Solenoid**

C. **Unineme model of DNA**

1. Condensation of chromosomes

2. **Heterochromatin** and **euchromatin**

3. Characteristics of heterochromatin

a. Genetically inactive

b. Later replication

c. Assumptions made from this discovery

d. Maintaining of the chromosome's structural integrity

e. Areas around centromeres

4. Effecting the expression of genes

a. Translocation of heterochromatin

b. **Position effect**

D. **Chromosome banding techniques**

1. Mary Lou Pardue and Joe Gall

a. Giemsa stain

b. Centromeric regions took stain

c. Stains heterochromatic areas

d. **C-banding**

2. Swedish researchers, lead by Tobjorn Caspersson
   a. Fluorescent dyes
   b. Fluorochrome quinacrine mustard
   c. The 23 chromosomes of humans
   d. **Q-bands**
3. **G-bands**
   a. Trypsin
   b. Giemsa stain
   c. Figure 9-11
   d. **R-band**

IV. Organization of the Eukaryotic Genome

A. **Genome**

B. Multicellular eukaryotes
   1. Cell differentiation and tissue organization
   2. Coordinated development and function

C. ***C* value** is
   1. Figure 11-10
   2. Trends
      a. More DNA
      b. Evolutionary progress
   3. Increasing *C* values
      a. Increases are very dramatic
      b. Degree of complexity
      c. Amphibians and flowering plants
   4. ***C*-value paradox**

D. Repetitive DNA
   1. Chromosome distribution
      a. Estimates of infidelity
      b. DNA sequences of the centromeric regions
      c. **CEN**

2. John Carbon and Louis Clarke
   a. Similar in organization
   b. Three different regions
      1. Transparency 68, page 242, figure 11-11
      2. Regions I and III
      3. Region II
3. Analysis of these regions
   a. Mutations in regions I and II
   b. Mutations in region III
   c. Conclusion
4. Extensive amount of DNA associated with centromere
   a. **"Satellite" DNA**
   b. Common in most multicellular eukaryotic organisms
      1. *Drosophila*
      2. Humans; **alphoid family**
      3. Role
         - Not transcribed
         - Some sequence variation
         - Specific number of repeats
5. **Telomere**
   a. Stability
   b. Do not fuse
6. Two types
   a. **Telomeric DNA sequences**
      1. Short tandem repeats
      2. Stability and integrity
      3. *Tetrahymena*
      4. Humans
      5. Analysis of telomeric DNA
   b. **Telomere-associated sequences**
      1. Location

2. Function is not known

7. **Telomerase**

a. When telomerase is absent

b. Essential for survival of single-celled eukaryotes

c. Humans

d. Cell aging

e. Cancer cells

E. **Moderately repetitive DNA**

1. Interspersed sequences

2. **Short interspersed elements, SINEs**

a. ***Alu* family**

1. 200-300 base pairs

2. Ten percent of human genome

b. Function

1. Sometimes transcribed

2. Role of resulting RNA

3. Reverse transcriptase

3. **Long interspersed elements (LINEs)**

a. **LI**

1. 6400 base pairs

2. 5' end

b. Role within genome

4. Clustered

a. Functional genes

b. Humans

5. **Variable-number tandem repeats (VNTRs)**

a. 15 to 100 base pairs

b. Number of copies

c. **DNA fingerprinting**

d. Location

6. 30% of human genome

a. 60% unaccounted for

b. Varies between different organisms

1. *Drosophila*

2. Humans

V. Eukaryotic Gene Structure

A. Transparency 69, page 244, figure 11-12

B. Not represented in "mature" mRNAs

1. **Intervening sequences**, or **introns**

2. **Exons**

3. **Heterogeneous nuclear RNA (hnRNA)**

4. Intron regions are excised

C. 5' region upstream from coding sequence

1. **Promoter, TATA box**

2. **CCAAT box**

3. **Enhancer region**

D. Distribution of related genes

1. **Multigene families**

2. Usually located on single chromosome

E. **Alpha-** and **beta-globin gene families**

1. Alpha family

2. Beta family

3. Homology

4. Globin polypeptides

5. Members turned on and off

F. Alpha family characteristics

1. ζ gene

2. $\alpha 2$, and $\alpha 1$ genes

3. **Psuedogenes**

a. Similar in sequence

b. Not transcribed

c. First and second psuedogenes

d. Designation of psuedogenes

4. Three functional genes

a. Intergenic regions

b. Two introns

c. Polypeptide chains

d. Divergent

G. Beta-globin family characteristics

1. Order of expression during development

2. Five functional genes

3. TATA box and CCAAT box

4. Coding sequences, 5%

a. Remaining 95%

b. Introns

VI. Genome Analysis

A. Human genome project

1. $200 million dollars

2. Year 2003

Lecture Content

Chapter Eleven: Organization of DNA in Chromosomes and Genes

I. Viral and Bacterial Chromosomes

A. The chromosomes of viruses and bacteria are much less complicated than eukaryotic chromosomes.
   1. The chromosomes usually consist of a single nucleic acid molecule.
   2. There is also much less genetic information, since there is only one chromosome as compared to the many chromosomes found in eukaryotic organisms.

B. The nucleic acid molecule within viruses can either be DNA or RNA.
   1. This molecule can also either be single- or double-stranded.
   2. The nucleic acid molecule can exist in a circular form or a linear form.
      a. The **ϕX174 bacteriophage** consists of a single-stranded DNA molecule.
      b. The **polyoma virus** forms ring-shaped molecules within the protein coat of the mature virus and within the host cell.
      c. The **bacteriophage λ** possesses a linear double-stranded DNA molecule prior to infection, which forms a loop upon infection of the host cell.
      d. Other viruses such as the **T-even series of bacteriophages** have linear double-stranded chromosomes of DNA, which do not form circles, even inside of host cells.
   3. In figure 11-1 in the text, a picture of a mature bacteriophage lambda is shown with its double-stranded DNA molecule.
      a. Bacteria, viruses, and eukaryotic cells all share the ability to package a long strand of DNA within a small volume.
      b. For example, phage λ contains DNA that is approximately 17 μm long, which must fit into the phage head, a relatively small place.
   4. A table comparing the length of chromosomes and the size of several viruses' heads is illustrated in table 11.1.
      a. Rarely does the available space in the head of a virus exceed chromosome volume by more than a factor of 2.
      b. Once the genetic information is packaged in the head, it remains inert until it is released into a host cell.

C. Bacterial chromosomes are also simple when compared to those of eukaryotic cells.
   1. Bacterial chromosomes always consist of double-stranded DNA.
      a. They are usually packaged in a structure called the **nucleoid**.
      b. Figure 11-3 shows the chromosome of an *E. coli*, which is a large circular chromosome measuring approximately 1200 μm in length.
   2. The DNA of bacteria is associated with many **DNA-binding proteins**.
      a. Two of these proteins known are called **H** and **HU**.
      b. These proteins are small and abundant in the cell, and they contain a high percentage of positively charged amino acids, which bond ionically to the negatively charged phosphate groups on DNA.
      c. These proteins are similar in structure to **histones**, proteins associated with eukaryotic DNA.
      d. Unlike viral DNA, bacterial DNA is functional even after packaging, and replicates and transcribes readily.

II. Mitochondrial and Chloroplast DNA

A. The discovery of mutations in yeast, other fungi, and plants that altered the function of mitochondria and chloroplast, suggested that these organelles contain their own genetic information.
   1. The transmission of these mutations did not always follow the patterns of biparental inheritance.
   2. Instead, a **uniparental mode of inheritance** was observed.
   3. Since the origin of both mitochondria and chloroplasts is usually uniparental, many suggested that these organelles did in fact carry their own genetic information.

B. There has been extensive work done on **mitochondrial DNA (mtDNA)** and its related gene functions.
   1. The mtDNA, in most eukaryotes, is a circular duplex that replicates semiconservatively.
   2. This DNA is also free of the chromosomal proteins which are characteristic of eukaryotic DNA.
   3. Figure 11-4 shows an electron micrograph of mtDNA.

4. The size of mtDNA varies from organism to organism.
   a. Table 11.2 gives examples of organisms and the size of their mtDNA.
   b. In vertebrates, there are about 5 to 10 molecules per organelle.
   c. There is a greater amount of DNA present in plant mitochondria as well.
5. There are some characteristics of mtDNA known.
   a. It appears that there are few or no gene repetitions within this DNA.
   b. Replication is dependent upon enzymes encoded by the nuclear DNA.
   c. The products, which are encoded by mtDNA, are essential in transcription and translation, including ribosomal RNAs, transfer RNAs, and many products needed for cellular respiration.

C. There have also been extensive studies on **chloroplast DNA (cpDNA)**.
   1. Chloroplasts also contain their own set of genetic information, which is distinct from that found within the nucleus of the cell.
      a. Like mitochondrial DNA, the components needed for transcription and translation are derived from both nuclear and organellar genetic information.
      b. Chloroplast DNA is much larger than mtDNA, and it is very similar to DNA found within prokaryotic cells.
   2. Figure 11-5 shows an electron micrograph of chloroplast DNA derived from maize.
      a. The DNA of chloroplasts has been found to be circular and double-stranded.
      b. The cpDNA replicates semiconservatively.
      c. The cpDNA is also free of associated proteins, which are characteristic of eukaryotic DNA.
   3. The number of copies of cpDNA also varies from organism to organism.
      a. In *Chlamydomonas*, there are approximately 75 copies of the cpDNA molecule per organelle.
      b. Each of these copies contains 195 kb.
      c. In higher plants, there are multiple copies of the DNA molecule in each organelle, but the length is considerably smaller than that in the *Chlamydomonas*.

D. Lynn Margulis and others came up with the **endosymbiont theory** to explain the observation of mtDNA and cpDNA.
   1. This theory states that mitochondria and chloroplasts have originated as distinct bacteralike particles, which became incorporated with primitive eukaryotic cells.
   2. The symbiotic relationship caused the particles to lose their ability to function independently, while the eukaryotic cells became dependent on them.
   3. This theory explains why these two organelles have their own genetic information system, but not all questions are answered by this theory.

III. Organization of DNA in Chromatin

A. The organization of genetic information is much more intricate in eukaryotes than in bacteria or viruses.
   1. The complexity is due to the large amount of DNA per chromosome, as well as the presence of protein associated with DNA in the eukaryotes.
      a. For example, *E. coli* contains chromosomes that are 1200 μm in length, while human chromosomes range from 14,000 to 73,000 μm in length.
      b. If the DNA in a single human nucleus is stretched out, it will extend almost 2 meters!
      c. This genetic material, along with the associated proteins, is contained in a nucleus that is generally only 5 μm in diameter.
   2. Different cells assume specific functions based upon highly specific biochemical activity.
      a. All cells carry a complete genetic complement, but different cells activate different sets of genes.
      b. A highly ordered regulatory system governing the readout of the information must be present; this system must be imposed on or somehow related to the molecular structure of the genetic material.
   3. Eukaryotic cells exhibit a highly organized cell cycle (already discussed).
      a. During interphase of this cycle, the genetic material and the associated proteins are uncoiled and dispersed throughout the nucleus in a condition referred to as **chromatin**.

b. The chromatin condenses greatly when mitosis begins, and it becomes recognizable as chromosomes during prophase.

c. The condensation represents a contraction in length of some 10,000 times for each of the chromatin fibers; this is the basis for the **folded-fiber model**.

4. The associated proteins, mentioned before, are divided into two categories.

a. The positively charged **histones**.

b. The less positively charged **nonhistones**.

B. DNA and protein undergo extensive coiling and folding as they are condensed within the nucleus of the cell.

1. Of the associated proteins, histones exhibit the most essential structural role.

a. Histones contain large amounts of positively charged amino acids lysine and arginine.

b. These amino acids bond electrostatically to the negatively charged phosphate groups present on the nucleotides.

c. Table 11.3 shows the five main types of histones.

2. X-ray diffraction studies have shown that chromatin produces regularly spaced diffraction rings.

a. This suggests that there are repeating structural units occur along the chromatin axis.

b. If the histones are removed, the regularity of this diffraction pattern is disrupted.

3. It was not until the mid-1970s that a model was created for the chromatin structure; the following are the observations of this model.

a. The digestion of chromatin by certain endonucleases yields DNA fragments of approximately 200 base pairs.

- This demonstrated that enzymatic digestion is not a random process.
- If it were random, the fragment sizes produced would be of varying lengths.
- Therefore, chromatin must contain some type of repeating unit, each protected from enzymatic cleavage, except where two units are joined.

b. Chromatin fibers are composed of linear arrays of spherical bodies.

- Look at figure 11-6 in the text.
- This was discovered by Ada and Donald Olins, who reported that these particles occur regularly along the axis of a chromatin strand, and appear like beads on a string.
- These particles are now referred to as **v-bodies** or **nucleosomes**.

c. Studies showed that histones H2A, H2B, H3, and H4 occurred as two types of tetramers.
   - The tetramers are $(H2A)_2$-$(H2B)_2$ and $(H3)_2$–$(H4)_2$.
   - This suggested that each repeating nucleosome unit consisted of one of each tetramer.
   - The octamer interacts with about 200 base pairs of DNA.

d. Studies showed that when the nuclease digestion time was extended, DNA was removed from both the entering and exiting strands.
   - This created a **nucleosome core particle**, which consists of 146 base pairs.
   - The number of base pairs is fairly constant in other organisms studied.
   - The DNA that is lost due to this prolonged digestion is responsible for linking nucleosomes together.
   - This **linker DNA** is associated with histone H1.

e. John T. Finch, Aaron Klug, and others created a detailed model of the nucleosome.
   - Transparency 67, page 239, figure 11-7.
   - The 146-base-pair DNA core exits as a secondary helix surrounding the octamer of histones.
   - The coiled DNA does not complete two full turns, and the entire nucleosome is of ellipsoidal shape, measuring about 110 Angstroms in its greatest dimension.

4. The extensive investigations have lead to a basis for predicting how the packaging of chromatin occurs within the nucleus.
   a. The chromatin fiber is about 110 Angstroms in diameter at its largest site.
   b. The chromatin fibers consist of long strings of repeating nucleosomes.

c. The nucleosome string is then further folded into a thicker fiber, called a **solenoid**, which is approximately 300 angstroms in diameter.

C. The **unineme model of DNA** is based on the fact that DNA is a continuous fiber.

1. Experiments in the early part of this century, revealed that some of the chromosome remains condensed during interphase, but most do not.
2. In 1928, the terms **heterochromatin** and **euchromatin** were used to describe the parts of chromosomes that remain tightly coiled and condensed and those that are uncoiled.
3. There are many characteristics that distinguish heterochromatin from euchromatin.
   a. Heterochromatin areas are genetically inactive because they either lack genes or contain genes that are repressed.
   b. Heterochromatin also replicates later in the S phase of the cell cycle.
   c. The discovery of heterochromatin provided the first clues that not all parts of the eukaryotic chromosomes encode proteins.
   d. Instead, some regions are thought to aid in the maintaining of the chromosome's structural integrity, as well as in other functions such as movement of the chromosome during cell division.
   e. The areas around the centromeres are composed of heterochromatin, as well as the telomeres.
      - In some cases, the whole chromosome is heterochromatic, like in the mammalian Y chromosome.
      - In some species, all chromosomes of one entire haploid set are heterochromatic.
4. Sometimes the position of the heterochromatin can effect the expression of genes.
   a. When certain heterochromatic areas are translocated to new sites, the genetically active area can become inert.
   b. This is known as the **position effect**.

D. **Chromosome banding techniques** are very useful in distinguishing chromosomes.

1. Mary Lou Pardue and Joe Gall devised one of the first chromosome banding techniques.
   a. They took chromosome preparations from mice, denatured them by adding heat, and then added Giemsa stain.
   b. They noticed that the centromeric regions of the chromosomes preferentially took up the stain.
   c. Apparently, this technique stains heterochromatic areas of chromosomes.
   d. Figure 11-8 shows the mouse karyotype, this staining pattern is referred to as **C-banding**.
2. Around the same time, a group of Swedish researchers, led by Tobjorn Caspersson devised a technique that produced an even greater staining differentiation of metaphase chromosomes.
   a. They used fluorescent dyes, which bind to nucleoprotein complexes and in turn creates unique banding patterns.
   b. The metaphase chromosomes are treated with fluorochrome quinacrine mustard and then viewed under a fluorescent microscope; the result is differential brightness.
   c. Each of the 23 chromosomes of humans can be distinguished this way.
   d. The bands that are produced by this technique are called **Q-bands**.
3. Another technique results in what are called **G-bands**.
   a. Digestion of the mitotic chromosomes with proteolytic enzyme trypsin is the first step.
   b. The cells are then subjected to the Giemsa stain.
   c. Figure 9-11 shows a karyotype with G-bands.
   d. A method that produces staining patterns exactly reverse of G-banding, is called an **R-band** pattern.

IV. Organization of the Eukaryotic Genome

A. The **genome** is the complete haploid set of chromosomes constituting the genetic material of an organism.

B. Prior to 1970, it was reasoned that multicellular eukaryotes had several unique requirements that distinguished them from phages and bacteria.

1. One of the requirements is cell differentiation, as well as tissue organization.
2. It was also thought that coordinated development and function depended on the regulation of genetic expression.

C. The ***C* value** is the amount of DNA contained in the haploid genome.
   1. Figure 11-10 shows the DNA content for the haploid genomes of many organisms.
   2. These values exhibit several trends.
      a. Eukaryotes have more DNA in their genomes and exhibit a wide variation among different groups as compared to viruses and prokaryotes.
      b. An increased amount of DNA has accompanied evolutionary progress.
   3. It is thought by some, that the increasing *C* values are the result of a greater need for increased amounts and varieties of gene products for more complex organisms.
      a. This is not the case however.
      b. Studies have shown that the increases are very dramatic; viruses and bacteria contain 10^4 to 10^6 nucleotide pairs in their single chromosomes, whereas eukaryotes contain 10^7 to 10^{11} nucleotide pairs in each set of chromosomes.
      c. It is also apparent that closely related organisms with the same degree of complexity in body form and tissue and organ types often vary tenfold or more in DNA content.
      d. Finally, amphibians and flowering plants have DNA content that varies as much as 100-fold within their taxonomic classes.
      e. There is no correlation between genome size and morphological complexity in these groups.
   4. These trends have been the basis for the ***C*-value paradox**, which states that excess DNA is present that does not seem to be essential to the development or evolutionary progression of eukaryotes.

D. Repetitive DNA, discussed in an earlier chapter, is a series of noncoding sequences.
   1. The separation of the chromatids during mitosis and meiosis is essential to the fidelity of chromosome distribution.
      a. The estimates of infidelity during meiosis are extremely low.

b. It has been assumed that the analysis of DNA sequences of the centromeric regions will provide insights to the remarkable features of this chromosomal region.

c. This DNA region is termed the **CEN**.

2. John Carbon and Louis Clarke first described a model system of the CEN regions of the yeast *Saccharomyces cerevisiae*.

a. They discovered that all the CENs were very similar in their organization.

b. The CEN region of the yeast they were studying consists of about 225 base pairs, which can be divided into three different regions.

- Transparency 68, page 242, figure 11-11.
- The first and third regions are relatively short, but highly condensed; consisting of only 8 to 26 base pairs.
- Region II, on the other hand, is larger, approximately 80-85 base pairs, and is extremely A = T rich.

3. Analysis of these regions has shown that regions I and II are less critical to centromere function than region III.

a. Mutations in regions I and II are generally tolerated.

b. However, mutations in region III usually disrupt the function of the centromere.

c. Therefore, it is thought that the DNA sequence in region III may be essential to binding of spindle fibers.

4. Multicellular eukaryotic organisms exhibit a much more extensive amount of DNA associated with the centromere than do yeast.

a. In mice, there are highly repetitive **"satellite" DNA** sequences that are localized in the centromere region.

b. These sequences are common in most multicellular eukaryotic organisms, but they vary considerably in size.

- *Drosophila* contain a 10-base-pair sequence (AATAACATAG) that is tandemly repeated many times in the centromeres of all four chromosomes.

- One of the most recognized satellite DNA sequences in humans is the **alphoid family**, which contain approximately 170 base pairs and are present in tandem arrays of up to 1 million base pairs.
- The role of this highly repetitive DNA in centromere function is unclear.
  - It is known that the sequences are not transcribed.
  - Also, there is some sequence variation between members of the alphoid family.
  - Each human chromosome has a specific number of repeats.

5. The **telomere** is the structure that is found at the ends of chromosomes.
   a. It functions to provide stability to the chromosome, by rendering the ends of the chromosome generally inert in interactions with the ends of other chromosomes.
   b. Telomere regions do not fuse with one another, nor do they fuse with broken ends of chromosomes.
6. There have been two types of telomere sequences discovered.
   a. The first type is simply **telomeric DNA sequences**.
      - This type consists of short tandem repeats.
      - This group is what contributes to the stability and integrity of the chromosome.
      - In the ciliate *Tetrahymena*, over 50 tandem repeats of GGGGTT occur.
      - In humans, the sequence GGGATT is repeated numerous times.
      - The analysis of telomeric DNA sequences has shown that they are highly conserved over long periods of evolutionary time.
   b. The second type is **telomere-associated sequences**.
      - They are also repetitive and found adjacent to as well as within the telomere.
      - These sequences vary from organism to organism, and their function is not known.
7. Replication of the telomere requires a unique RNA-containing enzyme called **telomerase**.

a. When telomerase is absent, the ends of the chromosomes become shorter with each replication.
b. Telomerase is essential for the survival of single-celled eukaryotes.
c. In humans, telomerase is essential in the germline cells, but is inactive in most somatic cells.
d. The shortening of chromosomes is considered a natural process of cell aging, and serves as an internal clock.
e. In cancer cells, cells which have become immortalized, the transition to malignancy appears to require the activation of telomerase so that the normal senescence is overcome.

E. **Moderately repetitive DNA** consists of either interspersed or tandemly repeated sequences.
1. The interspersed sequences can either be short or long.
2. The **short interspersed elements**, called **SINEs** are less than 500 base pairs long, but are present as many as a million times.
a. The ***Alu* family** is the best-characterized human SINE.
- Members of this family consist of 200-300 base pairs, and are present 70,000 to 900,000 times throughout the genome, both between and within genes.
- This family encompasses almost ten percent of the entire human genome.

b. The function of these *Alu* sequences is not known.
- It is however known that they are sometimes transcribed.
- The role of the resulting RNA is not yet defined.
- These sequences are thought to have arisen from an RNA element whose DNA complement was dispersed throughout the genome as a result of the activity of reverse transcriptase.

3. The second category representative of moderately repetitive DNA is known as **Long interspersed elements (LINEs)**.
a. The most prominent example of LINEs in humans is designated **L1**.
- Members of this family are around 6400 base pairs and are present 3000 to 40,000 times within the genome.

- Their 5' end has been shown to be highly variable.

b. Their role within the genome has not yet been identified.

4. Moderately repetitive DNA can also be clustered rather than interspersed throughout the genome.
   a. This category includes functional genes that are present in many copies.
   b. Humans have many copies of the genes encoding rRNA, which are clustered on the p arms of chromosomes 13, 14, 15, 21, and 22.
5. A second type of tandem repeat is called **variable-number tandem repeats (VNTRs)**.
   a. These may be up to 15 to 100 base pairs long.
   b. The number of copies varies in each individual.
   c. These regions in humans are used for the forensic technique, **DNA fingerprinting**.
   d. VNTRs may be found within or between genes.
6. All together, the various forms of moderately repetitive DNA composes up to 30% of the entire human genome.
   a. That leaves over 60% unaccounted for.
   b. Even though the proportion of the genome accounted for by repetitive DNA varies between different organisms, one fact seems to be shared, only a small part of the genome codes for proteins.
      - In *Drosophila*, only 5 to 10 percent of the genome is occupied by genes coding for proteins.
      - In humans, genes encoding proteins occupy only 1 to 2 percent of the genome.

V. Eukaryotic Gene Structure

A. Transparency 69, page 244, figure 11-12.

B. It has been found that many of the internal base sequences of genes are not represented in the "mature" mRNAs.
   1. These sequences are referred to as **intervening sequences**, or just **introns**.
   2. The remaining areas, which are ultimately translated into the amino acid sequence of the protein, are called **exons**.

3. The entire gene is transcribed into one large precursor mRNA called **heterogeneous nuclear RNA (hnRNA)**.
4. The intron regions are then excised, and the exon regions are then spliced back together before translation occurs.

C. It is now clear that the 5' region upstream from the coding sequence is critical for efficient transcription.
1. The **promoter** is the closest to the coding sequence, and consists of a **TATA box**.
   a. The promoter is where the RNA polymerase binds before initiation of transcription.
   b. The TATA box is named because its base sequence has been conserved in most eukaryotic genes.
2. Further upstream is the **CCAAT box**, which is involved in the regulation of transcription.
3. In many cases, an **enhancer region** is present.
   a. The enhancer region modulates transcription.
   b. These may be found within coding sequences, or as part of the 3' downstream region.
4. There are upstream (5') and downstream (3') noncoding sequences present, which are part of the primary transcript of the gene, but they are trimmed off as the mRNA matures.

D. The distribution of related genes is another aspect of the organization of the eukaryotic genome.
1. **Multigene families** consist of members that share DNA sequence homology, and produce functionally related gene products.
2. They are often, but not always, located along a single chromosome together.

E. Two of the most extensive and best-characterized groups of multigene families are the human **alpha-** and **beta-globin gene families**.
1. The alpha family consists of five genes, and is located on the short arm of chromosome 16.

2. The beta family consists of six genes, and is located on the short arm of chromosome 11.
3. Homology is apparent between members of both families, although not to the extent that members within the same family exhibit.
4. Both families code for globin polypeptides, which combine into a single tetrameric molecule that interacts with a heme group to reversibly bind to oxygen.
5. Within each group, members are turned on and off during different stages of development, in the order in which they occur along the chromosomes.

F. The alpha family spans more than 30,000 nucleotide pairs and contains five genes: the zeta gene (ζ), two nonfunctional genes, and two copies of the alpha (α) gene.
   1. The ζ gene is only functional during the embryonic stage of development.
   2. The $\alpha 2$ gene is expressed during the fetal stage, whereas the $\alpha 1$ gene is expressed during the adult stage.
   3. The two nonfunctional genes are called **pseudogenes**, and are found in both families.
      a. These genes are similar in sequence to the other genes in their family.
      b. They contain significant substitutions, deletions, and duplications that prevent them from being transcribed.
      c. The first pseudogene shows greater similarity to the zeta gene, while the second pseudogene shows greater similarity to the alpha gene.
      d. The pseudogenes are designated with the symbol ψ followed by the symbol of the gene they most resemble, for example, $\psi\alpha 1$ indicates a pseudogene of the first alpha gene.
   4. There are three functional genes that occupy the chromosomal housing of the entire alpha family.
      a. The region consists almost entirely of intergenic regions.
      b. There are two introns, precisely at the same position, within the functional genes of this family.
      c. Both genes lead to the production of a polypeptide chain that is 141 amino acids long.

d. The intron sequences, although approximately the same sizes, are very divergent.

G. The beta-globin family in humans contains six genes, one of which is nonfunctional, and covers a span of 60-kb.

1. As in the alpha family, the order of expression during development parallels the sequence of genes along the chromosome.
2. The five functional genes encode products, which are 146 amino acids long, and contain two similarly sized introns at the same positions.
3. Both a TATA box and a CCAAT region have been observed within the flanking regions for this family.
4. Coding sequences are found only in about 5% of the 60-kb region.
   a. The remaining 95% include introns, flanking regions, and spacer DNA found between genes.
   b. Introns actually only consist of about 11% of this 95%, the remainder serves no known function.

VI. Genome Analysis

A. In 1990, an international agreement was made, and the search for the sequences of the human genome began.

1. The United States government allowed $200 million dollars in funding for this project.
2. The completion date is thought to be in the year 2003.

Lecture Outline

Chapter Twelve: Storage and Expression of Genetic Information

I. The Genetic Code: An Overview

A. Storage of genetic information

B. Characteristics of the genetic code

1. Linear form
2. **Triplet codon**
3. **Unambiguous**
4. **Degenerate**
5. **Ordered**
6. **Initiation** and **termination** codons
7. **Commaless**
8. Nonoverlapping
9. Almost **universal**

II. Early Thinking About the Code

A. DNA directly encodes synthesis of protein

1. Four nucleotides
2. mRNA

B. Sydney Brenner

1. Two at a time
2. Four at a time
3. Triplet code

C. Observations

1. Messenger RNA as an intermediate between DNA and protein
2. Nature of the code
   a. Phage T4
   b. **Frameshift** mutations
      1. Transparency 70, page 252, figure 12-1
      2. Shift of the reading frame
      3. One or two nucleotides involved
      4. Three nucleotides involved

5. Supported idea of a degenerate code

III. Deciphering the Code: Initial Studies

A. First specific coding sequences
   1. Two experimental tools
   2. **Cell-free protein-synthesizing system** and **polynucleotide phosphorylase**
   3. mRNAs served as templates
   4. Amino acids incorporated into polypeptide chains
      a. Must contain essential elements
      b. Radioactive amino acid
      c. Addition of mRNA

B. Synthetic mRNA
   1. Enzyme to degrade RNA
   2. Formation of RNA
   3. Does not require DNA template
      a. Random assembly
      b. Concentration of four ribonucleoside

C. **RNA homoploymers**
   1. Amino acids associated with newly formed proteins
   2. Different labeled single amino acids
   3. Table 12.1
      a. Incorporation of ^{14}C-phenylalanine
      b. Poly U directs
         - AAA and CCC
         - Poly G
         - Composition of triplets

D. **RNA heteropolymers**
   1. Transparency 71, page 254, figure 12-3
   2. Ribonucleoside diphosphates added
   3. Correlation and assignments
   4. Example: 1A:5C
      a. Insertion of a ribonucleotide

b. 1/6 possibility for A, and 5/6 possibility for C

c. Calculate the frequency

1. AAA

2. AAC, ACA, and CAA

3. CCC

5. Probable base compositions

6. Sequence of the triplets

E. **Triple binding assay**

1. Ribosomes that would bind and attract charged tRNA

2. Transparency 72, page 255, figure 12-4

a. UUU

b. **Anticodon**

3. Determination of tRNA-amino acid

4. Nitrocellulose filter

a. Smaller components pass through

b. Specific codon assignment made

5. Importance

a. Table 12.2

b. Degenerate and unambiguous code

F. Gobind Khorana

1. Short replicated sequences of nucleotides

2. Figure 12.5

3. Filled in the gaps

IV. The Coding Dictionary

A. Transparency 73, page 257, figure 12-6

1. Assignments for triplets

2. 61 triplet codon assignments

B. Code is degenerate

1. Two, three, or four triplets

2. Six triplets

3. Single triplets

C. **Wobble hypothesis**
   1. Pair of more than one triplet
   2. Substitutions at third base position
   3. 30 different tRNAs needed

D. **Ordered code**
   1. U or C
   2. A or G
   3. Mutation in second position
   4. Chemically similar amino acid

E. The initiation and termination processes
   1. ***N*-formylmethionine (fmet)**
      a. AUG
      b. **Initiator codon**
      c. Present internally
   2. In eukaryotes
   3. UAA, UAG, and UGA
      a. Termination signals
      b. **Nonsense mutation**

V. Confirmation of Code Studies: Phage MS2

A. Analysis with bacteriophage **MS2**

B. Walter Fiers and coworkers
   1. Infects *E. coli*
   2. 3500 ribonucleotides
   3. Three genes
   4. Sequence for gene encoding the protein coat
      a. 129 amino acids
      b. **Colinear relationship**
   5. Last two genes deciphered

VI. Universality of the Code?

A. Assumed that the code was universal

B. Deviation from principle of universality of genetic language

1. Mitochondria
2. Several exceptions
3. Change in coding capacity

VII. Expression of Genetic Information: An overview

A. Nature of **genetic expression**

1. **Information flow**
2. Production of proteins

B. Genetic information, transferred to RNA

1. **Transcription**
2. **Translation**
   a. Polypeptide chains synthesized
   b. **Transfer RNA (tRNA)**
   c. **Ribosomes**

VIII. Transcription: RNA Synthesis

A. Four main observations

1. DNA occurs in nucleus, protein synthesis occurs in cytoplasm
2. RNA synthesized in nucleus
3. Migration out of nucleus
4. Amount of RNA

B. Initial experiments

C. Model for gene regulation in bacteria

D. **RNA polymerase**

1. Enzyme capable of directing synthesis
2. Similar to DNA polymerase
3. The overall reaction

$$n(\text{NTP}) \xrightarrow[\text{enzyme}]{\text{DNA}} (\text{NMP})_{n+1} + \text{PP}_i$$

   a. The nucleoside triphosphates (NTPs)
   b. Catalyzes the polymerization of nucleoside monophosphates (NMPs)
   c. 5' – 3' phosphodiester bonds
   d. PP_i

4. Addition of each ribonucleotide:

$$(NMP)n + NTP \xrightarrow[\text{enzyme}]{\text{DNA}} (NMP)_{n+1} + PP_i$$

a. Addition of one ribonucleotide (NMP)

b. Nucleoside triphosphate (NTP)

5. RNA polymerase of *E. coli*

a. α_2, β, β', and σ subunits

b. β and β' **polypeptides**

c. **σ subunit**

6. Three different types in eukaryotes

E. Single-stranded RNA molecule

1. Transparency 74, page 261, figure 12-7

2. Complementary to *one* of the two DNA strands

a. **Template strand**

b. **Partner strand**

3. **Promoters**

a. Location

b. Enzyme "explores" DNA

c. Complex forms on promoter

d. Binds to 60 nucleotides

4. Essential in process of transcription

a. Governs efficiency of initiation

b. Strong and weak promoters

c. Mutations within promoter

5. **Consensus sequence**

F. Insertion of first 5'-ribonucleotide

1. No primer is required

2. Phosphodiester bonds

a. **Chain elongation**

b. DNA/RNA

3. σ subunit dissociates

4. Termination sequence

a. tRNA folds back on itself

b. RNA dissociates from DNA template

c. **Termination factor rho, (ρ)**

5. Core enzyme dissociates

6. Substitution within RNA strand produced

a. T substituted with U

b. Example

IX. Transcription in Eukaryotes

A. Major differences

1. Performed in nucleus of cell

2. More extensive interactions

3. "Processing," occurs

4. Modifications occur to internal sequence of RNA transcripts

a. **Pre-mRNAs**

b. **Heterogeneous nuclear RNA (hnRNA)**

c. **Heterogeneous ribonucleoprotein particles (hnRNPs)**

1. 25% of hnRNA converted to mRNA

2. **Split genes** and **splicing**

B. Polymerases and promoters

1. Three different forms of polymerases

2. ***Cis*-acting elements**

a. **TATA box**

1. Location

2. A and T residues

3. Function

a. **CCAAT box**

1. Location

2. Loss of CCAAT box

3. CCAATT is frequent sequence

b. **Enhancer**

- Location
- Function

- Essential for initiation

3. **Trans-acting factors**
   a. **Transcription factors**
   b. Essential
   c. **TFIIA, TFIIB**
   d. **TATA-binding proteins (TBPs)**
   e. **TFIID**
      1. Ten subunits
      2. Binding to TFIID
      3. Pre-initiation complex

C. Internal base sequences
   1. Transparency 75, page 264, figure 12-8
   2. James Darnell and coworkers
      a. **Heterogeneous nuclear ribonucleoproteins**
      b. Similar sequences to mRNA
      c. mRNA must be processed in nucleus
   3. **Posttranscriptional modification**
      a. **7-methylguanosine (7mG) cap**
      b. Protective measure
      c. Complex
   4. Another modification
      a. hnRNAs and mRNAs
      b. **Poly-A sequences**
      c. AAUAAA sequence
      d. Polyadenylation
      e. Almost all eukaryotic organisms
   5. AAUAAA sequence
      a. Mutation in the AAUAAA sequence
      b. RNA transcripts degrade

D. Susan Berget, Philip Sharp, and others
   1. **Intervening sequences**

a. **Introns**

b. **Exons**

c. **Splicing**

2. Two main approaches

a. Molecular hybridization.

1. **Heteroduplexes**

2. Figure 12-9

3. Introns

b. Comparison of nucleotide sequences

3. **Beta-globin gene**

a. Figure 12-10

b. Mouse gene

c. Rabbit gene

d. Similar introns

4. Bert O'Malley and Pierre Chambon

a. **Ovalbumin gene**

b. "Silent" DNA

E. Different groups of introns

1. One group (type I)

a. Must be excised

b. Intron itself contains enzymatic activity necessary

c. Thomas Cech and his colleagues

d. **Riboenzymes**

e. Two chemical reactions

1. Transparency 76, page 266, figure 12-11

2. First reaction

3. Second reaction

f. Mitochondria and chloroplasts

1. Group II introns

2. Autocatalytic reactions

F. Nuclear-derived transcripts

1. Larger introns
2. Complex mechanism for removal
   a. Sequence of introns is similar
   b. GU dinucleotide at 5'-end, AG dinucleotide at 3'-end
   c. **Spliceosome**
      1. Very large
      2. **Small nuclear RNAs (snRNAs)**
      3. **Small nuclear ribonucleoproteins (snRNPs,** or **snurps)**
      4. U1, U2…
3. The U1 snRNA
4. Two reactions associated splicing
   a. Transparency 77, page 266, figure 12-12
   b. The first reaction
   c. **Branch point**
   d. Formation of an intermediate
   e. The second reaction
5. **Alternate splicing**, results in **isoforms**

G. **RNA editing**

1. Nucleotide sequence is changed
2. Two main types
   a. **Substitution**.
   b. **Insertion/deletion**.
   c. *Physarum polycephalum*

X. Translation: Components Necessary for Protein Synthesis

A. Polymerization of amino acids
   1. Associated with ribosomes
   2. Transfer RNA (tRNA)
   3. Adapter role

B. The ribosome
   1. 10,000 ribosomes
   2. Structure

a. Two subunits

b. **Ribosomal RNA (rRNA)** and **ribosomal proteins**

3. Figure 12-13 in the text

a. Sedimentation behaviors

b. **Monosome**

1. Prokaryotes = 70*S*, eukaryotes = 80*S*

2. Not additive

c. Larger subunit

d. Smaller subunit

4. Degree of redundancy of genes

a. *E. coli*

b. Eukaryotes

1. *Drosophila*

2. Mammals

3. Clusters

- **Tandem repeats**.
- **Spacer DNA** sequences
- Humans

c. 5S rRNA component in eukaryotes

1. Different in *E. coli*

2. Gene cluster

C. Transfer RNA

1. Small size and stability

a. 75 to 90 nucleotides

b. Almost identical structure

c. Large precursor molecules

2. Sequence of $tRNA^{ala}$ isolated from yeast

a. Modified nucleotides

1. **Inosinic acid** (base is hypoxanthine)

2. *Unusual, rare*, or *odd bases*

3. Enzymatically modified bases

b. **Cloverleaf model**

1. Transparency 78, page 269, figure 12-14
2. Secondary structure
3. Paired stems and unpaired loops

c. Loops

d. Anticodon of $tRNA^{ala}$ molecule

1. CGI
2. **Anticodon loop** was established

3. Constant features

a. **pCpCpA-5'**

b. **pG**

c. Lengths were similar

d. All contain anticodon

4. X-ray crystallography

a. Alexander Rich and his coworkers in the United States, as well as J. Roberts B. Clark, Aaron Klug, and their colleagues in England

b. Individual nucleotides discernible

c. Three-dimensional model

1. Figure 12-15 in text
2. Reversed L-shape
3. Structures

D. Linkage to respective amino acids

1. **Charging; Aminoacyl tRNA synthetases**
2. Transparency 79, page 270, figure 12-16

a. Form an **aminoacyladenylic acid**

1. Covalent linkage
2. Associated with enzyme

b. Transferring of amino acid

1. Covalently bonded
2. Participate directly
3. **Isoaccepting tRNAs**

4. Crucial to fidelity

X. Translation: The Process

A. Transparency 80, pages 271-272, figure 12-17

B. Initiation

1. Ribosomes
2. Three **initiation factors (IFs)**
3. **Formylmethionine** (prokaryotes)
4. Bind to small subunit
   a. Binds to mRNA
   b. Sequence of up to six ribonucleotides
   c. **Shine-Dalgarno** sequence
5. Another initiation protein
   a. "Sets" reading frame.
   b. **Initiation complex**
   c. Hydrolyzing of GTP molecule

C. Elongation

1. Binding sites formed
   a. **P site**, or **peptidyl site**
   b. **A site**, or **aminoacyl site**
2. Initiator tRNA binds
3. Positioned at A site
4. **Peptidyl transferase**
5. The covalent bond broken
6. **Elongation**
7. Uncharged tRNA released
   a. **E site,** or **exit site**
   b. Occupied for brief time
8. **mRNA-tRNA-aa_2- aa_1** shifts
   a. **Elongation factors (EFs)**
   b. Requires use of energy
9. Accept another charged tRNA

10. Steps occur over and over
    a. Chain emerges
    b. A **tunnel** exists
11. Efficiency
    a. Error rate is 10^{-4}
    b. *E. coli*

D. Termination.
1. UAG, UAA, or UGA.
    a. Do not code
    b. **Release factors**
    c. Cleaves chain
2. Termination codons found internally

E. Another initiation complex
1. **Polyribosomes,** or just **polysomes**
2. Figure 12-18 in text
3. Efficient use of components

XI. Translation in Eukaryotes

A. Notable differences for eukaryotes
1. mRNAs live much longer
2. Two aspects
    a. Addition of 7-methyguanosine residue "cap"
        1. Absent in prokaryotes
        2. Most contain **5'-ACCAUGG... the Kozak sequence**
    b. Second factor
        1. Formylmethionine is not required
        2. AUG is essential

B. Similar protein factors
1. Many factors are homologous
2. May be more complex

Lecture Content

Chapter Twelve: Storage and Expression of Genetic Information

I. The Genetic Code: An Overview

A. DNA provides the chemical basis for storage of genetic information.

B. There are many characteristics of the genetic code.

1. The code is written in linear form, which uses the ribonucleotide bases that compose the letters in the mRNA molecules.
2. Each of the code words contains three letters, called a **triplet codon**.
3. The code is **unambiguous**, which means that each triplet codes for only one amino acid.
4. The code is **degenerate**, meaning that more than one triplet may code for a specific amino acid.
5. The code is **ordered**, which means that degenerate codons, specifying one amino acid, are grouped together, usually varying only by the third base.
6. There are "stop" and "start" codons, which are used to **initiate** and to **terminate** translation.
7. The code is said to be **commaless**, without internal punctuation; therefore, when translation begins, each three ribonucleotides are read one after the other.
8. The code is nonoverlapping, which means once translation begins, any single ribonucleotide at a specific location within the mRNA is part of only one triplet.
9. Finally the code is almost **universal**, which means that almost all viruses, prokaryotes, and eukaryotes use a single coding dictionary.

II. Early Thinking About the Code

A. It was once thought that DNA might encode the synthesis of protein directly.

1. The central question was how only four nucleotides could specify 20 amino acids.
2. When mRNA was discovered, it became clear that even though genetic information is stored in the DNA, the code that is translated into proteins resides in RNA.

B. Sidney Brenner, in the early 1960s, argued that the code was a triplet codon.

1. If it consisted of four nucleotides taken two at a time (4^2), it would only provide 16 unique code words.
2. If the code consisted of four nucleotides taken four at a time (4^4), then there would 256 words specified.
3. A triplet code (4^3) would specify for 64 words, more than is needed, but obviously the least complex.

C. By 1961, many significant observations were made concerning the genetic code.
   1. Francois Jacob and Jacques Monod suggested the existence of messenger RNA (mRNA) as an intermediate between DNA and protein.
   2. Francis Crick and his colleagues were some of the earliest scientists to provide information about the nature of the code.
      a. They studied phage T4 in their experiments.
      b. They recognized **frameshift** mutations within the phage.
         - Transparency 70, page 252, figure 12-1.
         - Frameshift mutations result from the addition or deletion of one or more nucleotides in the gene, as well as the mRNA transcribed by the gene, resulting in a shift of the reading frame.
         - Crick and his colleagues discovered that if one or two nucleotides were involved, then a mutation occurred.
         - On the other hand, if three nucleotides were involved, the reading frame was reestablished.
         - This would not occur is the code was anything besides a triplet, and that codons encode for amino acids, which supported the idea of a degenerate code.

III. Deciphering the Code: Initial Studies

A. Marshall Nirenberg and J. Heinrich Matthaei characterized the first specific coding sequences in 1961.
   1. They used two experimental tools within their studies.
   2. The two systems were **cell-free protein-synthesizing system** and an enzyme, **polynucleotide phosphorylase**, which produced synthetic mRNAs.

3. The mRNAs served as templates for polypeptide synthesis in the cell-free system.
4. In the cell-free system (*in vitro*), amino acids are incorporated into polypeptide chains.
   a. The *in vitro* mixture must contain essential elements, such as ribosomes, tRNAs, amino acids, and other molecules.
   b. At least one of the amino acids must be radioactive, so that protein synthesis can be traced.
   c. The last step is to add mRNA, which serves as the template to be translated.

B. The enzyme polynucleotide phosphorylase allowed for the production of synthetic mRNA, since mRNA had not yet been isolated.
   1. The enzyme was discovered by Marianne Grunberg-Manago and Severo Ochoa to function metabolically in bacteria to degrade RNA.
   2. However, in high concentrations in a cell-free system, this enzyme can be "forced" in the opposite direction, resulting in the formation of RNA.
   3. This enzyme does not require a DNA template, like RNA polymerase does.
      a. Therefore, the ribonucleotides are assembled at random.
      b. The way in which they are assembled is dependent on the concentration of the four ribonucleoside diphosphates that are added into the system.

C. In Nirenberg and Matthaei's first experiments, they synthesized **RNA homoploymers**, which consisted of either UUUUUU.... GGGGGG.... AAAAAA...., or CCCCCC.
   1. Then, they were able to determine which, if any, amino acids were associated with the newly formed proteins.
   2. Different experiments, but in the same manner, were then conducted with different labeled single amino acids.
   3. Table 12.1 illustrates the results of one of their initial experiments.
      a. This experiment was associated with the incorporation of ^{14}C-phenylalanine into protein.
      b. From their data, they concluded that the poly U directs only the incorporation of phenylalanine into the homopolymer polyphenylalanine.

- In this same way, they quickly found that AAA codes for lysine and that CCC codes for proline.
- Poly G did not code for anything, possibly because it folds back on itself.
- This method yields only the composition of the triplets, not their sequence.

D. Nirenberg and Matthaei, as well as Ochoa and others began using **RNA heteropolymers**, in their experiments.

1. Transparency 71, page 254, figure 12-3.
2. For this method, two or more different ribonucleoside diphosphates are added in combination in order to form the message.
3. Following the addition of mRNA into the cell-free system, a correlation can be made and assignments predicted, from the percentage of any particular amino acid present in the new protein formed.
4. Suppose that we add A and C in a ratio of 1A:5C.
   a. The insertion of a ribonucleotide at any position along the RNA molecule during the synthesis, is determined by the resulting ration of A:C.
   b. There is a 1/6 possibility for an A, and a 5/6 possibility for a C to occupy this position.
   c. It is then possible to calculate the frequency of any given triplet appearing in the message.
      - For AAA, the frequency would be $(1/6)^3$, or about 0.4%.
      - AAC, ACA, and CAA have identical frequencies; $(1/6)^2(5/6)$ or about 2.3% chance for each of these three different sequences.
      - CCC is represented by $(5/6)^3$, or 57.9% of the triplets.
5. It is possible to propose the probable base compositions when examining the frequencies.
   a. Proline appears 69% of the time, and because 69 is close to 57.9, we suspect that proline is encoded by CCC, and by one triplet 2C:1A.
   b. Histidine appears 14%, and is probably coded by one 2C:1A and 1C:2A.
6. The determination of the composition of triplet code words for all 20 amino acids was complete, but there still was no experimental method for determining the sequence of the triplets.

E. In 1964, Nirenberg and Philip Leder developed the **triple binding assay**, which led to the assignments of triplets.
   1. This method included ribosomes that would bind to and attract the correct charged tRNA corresponding to the triplet code.
   2. Transparency 72, page 255, figure 12-4.
      a. If an RNA triplet of UUU was present, ribosomes would form a complex similar to the one that would actually occur *in vivo*.
      b. The mRNA codon would attract the complementary sequence called the **anticodon.**
   3. Next, the tRNA-amino acid complex bound to the RNA-ribosome complex needed to be determined.
      a. To determine this, the amino acid that was to be tested was made radioactive, producing a charged tRNA.
      b. Since code compositions were known, it was easier to decide which amino acids should be tested for each specific triplet.
   4. Then, the charged tRNA, the RNA triplet, and the ribosomes were incubated together on a nitrocellulose filter.
      a. The filter pores allowed the smaller components, the triple and charged tRNA, to pass through.
      b. If radioactivity was determined to remain on the filter, it was because the charged tRNA had been bound to the triplet associated with the ribosome, and a specific codon assignment could be made.
   5. This binding technique was a major factor in deciphering the genetic code.
      a. Table 12.2 in the text gives amino acid assignments to specific trinucleotides derived from the triplet binding assay.
      b. This technique also aided in the conclusion that the genetic code is both degenerate and unambiguous.

F. Gobind Khorana was able to chemically synthesize long repeating RNA sequences, which could be used in the cell-free protein synthesizing system.

1. He created short sequences of nucleotides, and then replicated them many times allowing them to become joined by way of enzymes to form long polynucleotides.
2. Figure 12.5 illustrates the steps for this method of sequence determination.
3. Khorana not only reaffirmed triplets that were previously deciphered, but he also filled in the gaps left from other approaches.

IV. The Coding Dictionary

A. Transparency 73, page 257, figure 12-6.
   1. These are the assignments for the triplets.
   2. The dictionary contains 61 triplet codon assignments, the remaining three triplets code for termination signals.

B. The coding dictionary shows that the code is degenerate.
   1. Two, three, or four triplets specify almost all of the amino acids.
   2. Serine, arginine, and leucine are each encoded by six different triplets.
   3. Only tryptophan and methionin are encoded by single triplets.

C. In 1966, Crick postulated the **wobble hypothesis**, which states that the third member is less critical than the first two members of the code are.
   1. This allows the anticodon of a single tRNA species to pair with more than one triplet in mRNA.
   2. The degeneracy of the code allows for substitutions at the third base position without changing the amino acid.
   3. A minimum of 30 different tRNAs are needed to accommodate the 61 triplet code when the wobble rule is applied.

D. Chemically similar amino acids often share one or two "middle" bases in the different codons that code for them, this is referred to as an **ordered code**.
   1. For example, U or C are often present in the second position of triplets that specify for hydrophobic amino acids.
   2. Charged amino acids are often specified by triplet codons with A or G in the second position.
   3. When a codon experiences a mutation in the second position, the amino acid coded for is usually changed.

4. However, since the change is often to a chemically similar amino acid, the protein function may not be noticeably altered.

E. The initiation and termination processes of protein synthesis are very specific.
   1. In bacteria, the initial amino acid inserted to begin protein synthesis is ***N*-formylmethionine (fmet)**, a modified form of methionine.
      a. There is only one codon which codes for methionin, AUG.
      b. This codon is referred to as the **initiator codon**.
      c. AUG can be present in the internal part of the mRNA, and code for methionine, but it is very rare.
   2. In eukaryotes, methionine is also the initial codon, but it is not formylated.
   3. There are three other codons that do not code for any other amino acid, UAA, UAG, and UGA.
      a. These codons serve as termination signals.
      b. A **nonsense mutation**, is a mutation that forms one of these signals in the internal part of the gene, consequently causing premature termination.

V. Confirmation of Code Studies: Phage MS2

A. Individual principles of the genetic code have been confirmed by analysis of the RNA-containing bacteriophage **MS2**.

B. Walter Fiers and his coworkers are credited with the work of MS2.
   1. The MS2 bacteriophage infects *E. coli*.
   2. It contains 3500 ribonucleotides, which make up only three genes.
   3. The genes encode for a protein coat, an RNA-directed replicase, and a maturation protein.
   4. In 1970, the amino acid sequence for the gene encoding the protein coat was completed, and in 1972, the nucleotide sequence was completed.
      a. The coat protein consists of 129 amino acids and the gene contains 387 nucleotides.
      b. Each of the triplets corresponds in linear sequence to the correct codon in the RNA, proving evidence for the **colinear relationship** between the nucleotide sequence and amino acid sequence.

c. The codon for the first amino acid is preceded by AUG, the initiator codon; and the last codon for an amino acid is succeeded by two termination codons, UAA and UAG.

5. By 1976, the sequences for the last two genes and their protein products had been deciphered.

VI. Universality of the Code?

A. Between the years of 1960 and 1978, it was assumed that the code was universal, applying to all organisms including bacteria, viruses, and eukaryotes.

1. This was thought to be true because of the similar mechanisms of transcription and translation.
2. Studies had shown that eukaryotic genes could be transferred into bacterial cells and become transcribed and translated.

B. However, in 1979, many reports surfaced claiming that DNA derived from yeast and human mitochondria deviated from the principle of universality of the genetic language.

1. Mitochondria contain unique DNA, and transcription and translation occur within this organelle itself.
2. Several exceptions to the coding dictionary were discovered, when fragments of mtDNA were cloned, then sequenced and compared to the mitochondrial proteins.
   a. Table 12.4 in the text shows some of the exceptions to the universal code.
   b. Most surprising was that UGA, which normally causes termination in the mRNA, specified for the insertion of tryptophan during translation of mRNA originating in yeast and human mitochondria.
3. The change in coding capacity involves the shift of the third, or wobble position.
   a. For example, AUA codes for isoleucine in cytoplasm and methionine in the mitochondria.
   b. It has been suggested that these changes in the recognition of the cope may represent an evolutionary trend towards reducing the number of tRNAs needed in mitochondria, but it has not been proven as of yet.

VII. Expression of Genetic Information: An overview

A. Geneticists were very interested in the nature of **genetic expression**, even while the code was being deciphered.
   1. Scientists were exploring the concept of **information flow**.
   2. They wanted to find out how DNA led to the production of proteins.

B. It was known that DNA stored the genetic information, and that it was transferred to RNA during the initial stages of gene expression.
   1. **Transcription** is the process by which RNA molecules are synthesized on a DNA template.
   2. **Translation** is the process by which the RNA sequence, written in a genetic code, is translated.
      a. During the process of translation, polypeptide chains are synthesized.
      b. There are a series of **transfer RNA (tRNA)** molecules that are needed in order for this synthesis to occur.
      c. The process is also dependent on **ribosomes**.

VIII. Transcription: RNA Synthesis

A. There are four main observations that suggest RNA is involved as an intermediate molecule in the process of information flow between DNA and protein.
   1. Since DNA occurs, for the most part, in the nucleus of the eukaryotic cell, and protein synthesis occurs on ribosomes located in the cytoplasm of the cell, it is obvious that DNA does not participate directly in protein synthesis.
   2. RNA is synthesized in the nucleus of the cell, the location of DNA, and it is chemically similar to DNA.
   3. RNA, following synthesis, migrates out of the nucleus and into the cytoplasm, the area in which protein synthesis occurs.
   4. Finally, the amount of RNA is proportional to the amount of protein in a cell.

B. As with most genetically new ideas, initial experiments, concerning the association of RNA with protein synthesis, utilized bacteria and their phages.
   1. It was established that during initial infection, RNA synthesis precedes phage protein synthesis.
   2. It was also shown that the RNA is complementary to the phage DNA.

C. Francois Jacob and Jacques Monod in 1961, with the knowledge of data from the above experiments, formulated a model for gene regulation in bacteria, which included messenger RNA (mRNA) being made from a DNA template and directing the synthesis of specific proteins.

D. By 1959, many investigators independently discovered **RNA polymerase**.

1. RNA polymerase is an enzyme that is capable of directing synthesis.
2. Its properties are very similar to those of DNA polymerase.
3. The overall reaction of synthesis of RNA from a DNA template can be expressed as:

$$n(\text{NTP}) \xrightarrow[\text{enzyme}]{\text{DNA}} (\text{NMP})_{n+1} + \text{PP}_i$$

   a. The nucleoside triphosphates (NTPs) serve as substrates for the enzyme.
   b. The enzyme catalyzes the polymerization of nucleoside monophosphates (NMPs), nucleotides, into a polynucleotide chain $(\text{NMP})_n$.
   c. The nucleotides are linked by 5' – 3' phosphodiester bonds.
   d. The PP_i are inorganic phosphates, which constitute the energy that is created from the breaking of the triphosphate precursor.

4. A second equation which facilitates the addition of each ribonucleotide as the process of transcription can be expressed as:

$$(\text{NMP})n + \text{NTP} \xrightarrow[\text{enzyme}]{\text{DNA}} (\text{NMP})_{n-1} + \text{PP}_i$$

   a. Each step of transcription involves the addition of one ribonucleotide (NMP) to the growing polypeptide chain $(\text{NMP})_{n-1}$.
   b. The precursor for this step is the nucleoside triphosphate (NTP).

5. There have been extensive studies on the RNA polymerase of *E. coli*.
   a. These studies have shown that the RNA polymerase consists of the following subunits, α_2, β, β', and σ.
   b. The β and β' **polypeptides** provide the catalytic basis and active site for transcription.
   c. The σ **subunit** plays a regulatory function involving the initiation of RNA transcription.

6. Although there is only one RNA polymerase in *E. coli*, there are three different types in eukaryotes, which are summarized in table 12.5 in the text.

E. The final result of transcription is the synthesis of a single-stranded RNA molecule.
   1. Transparency 74, page 261, figure 12-7.
   2. The RNA molecule produced is complementary to a region along *one* of the two DNA strands.
      a. The strand of DNA that is transcribed is called the **template strand.**
      b. The strand that is the complement is called the **partner strand**.
   3. The initial site of binding in bacteria is achieved by the recognition by the σ subunit of the holoenzyme of specific DNA sequences called **promoters**.
      a. The promoters are located in the 5' upstream region from the initial point of transcription.
      b. The enzyme "explores" the length of the DNA, until the promoter is found.
      c. At that time, a complex forms on the promoter and the helix is unwound locally, to allow the DNA template to be accessible to the action of the enzyme.
      d. The polymerase binds to about 60 nucleotides, 40 of them are upstream from the point of initiation.
   4. The promoter sequence is essential in the process of transcription.
      a. It governs the efficiency of initiation of transcription by directing RNA polymerase to the proper starting point.
      b. Both strong and weak promoters exist.
      c. Mutations within the promoter sequence reduce the initiation of gene expression severely.
   5. A **consensus sequence** is one that is present in similar areas of genes in diverse organisms.

F. Following the recognition of the promoter, RNA polymerase catalyzes the insertion of the first 5'-ribonucleotide for the synthesis of RNA.
   1. Unlike DNA synthesis, no primer is required.
   2. Subsequent ribonucleotides are inserted and linked together by phosphodiester bonds.
      a. This process is called **chain elongation**, which proceeds in a 5' –3' direction.

b. The result is a temporary DNA/RNA duplex whose chains run antiparallel to one another.

3. Following the addition of the first few ribonucleotides, the σ subunit dissociates from the holoenzyme, and elongation proceeds under the direction of the core enzyme.
4. Eventually, the enzyme encounters a specific DNA sequence that when translated, serves as a termination sequence.
   a. When this occurs, the tRNA folds back on itself forming a hairpin loop, which interacts with the RNA polymerase.
   b. This interaction causes the enzyme to pause, which in turn causes the RNA to dissociate from the DNA template.
   c. In many cases, there is a **termination factor rho, (ρ)** present; not much is known about this termination factor.
5. Once the transcribed RNA molecule is released, the core enzyme dissociates.
6. While the DNA template is being transcribed, there is a substitution made within the RNA strand produced.
   a. The T in DNA is substituted with a U in RNA.
   b. Therefore, a DNA sequence ATCGATTC produces an RNA sequence of UAGCUAAC.

IX. Transcription in Eukaryotes

A. Although transcription is similar in eukaryotes and prokaryotes, there are some major differences.
   1. Eukaryotes carry out transcription within the nucleus of the cell under the direction of three RNA polymerases; therefore, the RNA transcript is not free to associate with ribosomes, until transcription is complete.
   2. There is a more extensive interaction between upstream DNA sequences and protein factors involved in stimulating transcription.
   3. The maturation of eukaryotic mRNA involves many complex stages referred to as "processing," such as the addition of a 5' cap and a 3" tail to most transcripts.
   4. Extensive modifications occur to the internal sequence of the RNA transcripts in eukaryotes.

a. The initial transcripts, called **pre-mRNAs**, are much larger than the mature mRNA.

b. They form a group known as **heterogeneous nuclear RNA (hnRNA)** these vary in size.

c. When they are complexed with proteins, they are called **heterogeneous ribonucleoprotein particles (hnRNPs)**.

- Only about 25% of the hnRNA molecules are converted to mRNA.
- The converted ones have had much of their internal sequence excised, leaving the rest to be spliced back together, giving rise to the concept of **split genes** and **splicing** in eukaryotes.

B. Polymerases and promoters are much more complex in eukaryotes, as compared to prokaryotes.

1. RNA polymerases exist in three different forms in eukaryotes.

a. Each of these forms is larger and more intricate than the prokaryote counterpart of the enzyme.

b. The eukaryote RNA polymerases consist of two large subunits and 10 to 15 smaller subunits.

c. RNA polymerase II is the most understood of the three polymerases.

2. There are at least three ***cis*-acting elements** of the eukaryotic gene that function in the efficient initiation of transcription by polymerase II.

a. The first of these elements is called the Goldberg-Hogness or **TATA box**.

- This is found about 25 nucleotides upstream from the start point of transcription.
- It consists solely of A and T residues.
- The TATA box is thought to be nonspecific and responsible for fixing the site of initiation by facilitating the denauturation of the helix.

b. The second element is found farther upstream from the TATA box, and is called the **CCAAT box**.

- Positions anywhere from 5 to 500 nucleotides upstream appear to modulate transcription in different genes.
- The loss of these sequences appear to reduce transcription drastically.

- The name comes from the fact that CCAATT is a frequent sequence in these regions.

c. The final element is called an **enhancer**.

- The location of these enhancers can vary from upstream, downstream, or even within the gene.
- They apparently have the effect of modulating transcription from a distance.
- Although they do not participate in transcription directly, they are essential for efficient initiation of transcription.

3. There are also **trans-acting factors**, which serve the role of facilitating template binding and initiation of transcription.
   a. These proteins are referred to as **transcription factors**.
   b. They are essential because the RNA polymerase II cannot bind directly to a eukaryotic promoter.
   c. The transcription factors in humans, the ones that are best characterized are known as **TFIIA, TFIIB,** and so on.
   d. The ones that bind directly to the TATA box are termed **TATA-binding proteins (TBPs)**.
   e. For example, there is one such TBP that is part of **TFIID**, which is responsible for initial binding to the TATA box.
      - The TFIID consists of ten subunits.
      - Once the binding has initially begun, there are at least seven other transcription factors that bind sequentially to TFIID.
      - This forms an extensive pre-initiation complex that is then bound by RNA polymerase II.

C. It has been shown that many of the internal base sequences of a gene may never appear in the mature mRNA in eukaryotes.
   1. Transparency 75, page 264, figure 12-8.
   2. By 1970, James Darnell and coworkers working with hnRNA accumulated evidence to show that the eukaryotic mRNA initially transcribed is much larger than the mRNA that is actually transcribed.

a. Heterogeneous RNA, complexed with a large variety of proteins, creates **heterogeneous nuclear ribonucleoproteins**, which are large, but vary in size, are only found in the nucleus.
b. hnRNA was determined to contain similar sequences to the mRNA located in the cytoplasm.
c. From this observation, it was suggested that the initial mRNA must first be processed in the nucleus before it could initiate in translation.

3. The initial **posttranscriptional modification** in eukaryotic RNA transcripts involves the 5'-end of the molecule.
   a. A **7-methylguanosine (7mG) cap** is added to the 5' end of the molecule.
   b. It is thought that the cap might be a protective measure against nuclease attack.
   c. The cap is complex and distinguished by a 5'-5' bonding between the cap and the initial ribonucleotide of the RNA.
4. Another modification involves the 3'-end of the molecule.
   a. Both hnRNAs and mRNAs have been shown to contain a stretch of as many as 250 adenylic acid residues.
   b. These **poly-A sequences** are added following the addition of the 5'-7mG cap.
   c. First, the 3'-end is cleaved at a point about 10 to 35 ribonucleotides from a highly conserved AAUAAA sequence.
   d. Next, polyadenylation occurs by sequential addition of adenylic acid residues.
   e. This modification has been seen in almost all eukaryotic organisms studied.
5. The AAUAAA sequence was determined to be extremely important for this modification.
   a. Cells bearing a mutation in the AAUAAA sequence cannot add the poly A sequence to the 3'-end.
   b. Consequently, the RNA transcripts readily degrade.

D. In 1977, Susan Berget, Philip Sharp, and others confirmed that genes of certain animal viruses contain internal nucleotide sequences that are not expressed in the amino acid sequences of the proteins they encode.
   1. The nucleotide sequences have been called **intervening sequences**.

a. The DNA sequences that are not present in the mature RNA are called **introns**.
b. The DNA sequences that are left and are expressed are called **exons**.
c. The process by which introns are removed and exons are placed together, is called **splicing**.

2. There are two main approaches used to test for introns and exons in eukaryotes.
   a. One such approach is by using molecular hybridization.
      - When hybridization occurs, **heteroduplexes** are formed.
      - Figure 12-9 is a picture of hybrid molecule taken by an electron microscope.
      - Introns that are present in the DNA, but absent in the mRNA, must loop out, and not become paired.
   b. The second approach involves the comparison of nucleotide sequences of DNA with those of mRNA and amino acids.
3. One of the first genes that was shown to contain introns was the **beta-globin gene** found in mice and rabbits, by Philip Leder and Richard Flavell.
   a. Figure 12-10 illustrates the sequences of nucleotides present in three organisms.
   b. The mouse gene contains an intron that is 550 nucleotides long.
   c. The rabbit gene contains an intron that is 580 base pairs.
   d. Similar introns have been found within this gene in all mammals examined.
4. Bert O'Malley and Pierre Chambon have done extensive studies on chickens.
   a. A more extensive set of introns has been located in the **ovalbumin gene** of chickens.
   b. This gene contains seven introns, making most of the DNA in the gene "silent."

E. It appears that there are different mechanisms for splicing genes in different RNAs as well as in RNAs produced by mitochondria and chloroplasts caused by different groups of introns.
   1. One group (type I) is located in the primary transcript of rRNAs derived from the ciliate protozoan *Tetrahymena*.

a. This group contains introns that must be excised in order for mature rRNA to be formed.
b. The intron itself contains the enzymatic activity necessary for the excision process.
c. Thomas Cech and his colleagues made the discovery of the self-excision process in 1982.
d. RNAs that are capable of splicing themselves are often called **ribozymes**.
e. There are two chemical reactions that take place in order for this to occur.
    - Transparency 76, page 266, figure 12-11.
    - First, there is an interaction involving guanosine.
        - The guanosine acts as a cofactor in the reaction and the primary transcript.
        - The 3'-OH end group of the guanosine is transferred to the nucleotide adjacent to the 5'-end of the intron.
    - The second reaction involves the interaction between the 3'-OH group, on the left-hand of the exon, and the phosphate group on the right-hand exon; resulting in the introns being sliced out and the exon regions being ligated.
f. Self-excision also seems to govern the removal of introns present in the primary mRNA and the tRNA produced from the mitochondria and chloroplasts.
    - These are referred to as the group II introns.
    - The splicing involves the autocatalytic reactions, which lead to the excision of introns, like in the group I introns, but guanosine is not involved.

F. Nuclear-derived transcripts representing mRNAs contain yet another group of introns.
1. Introns located here can be much larger than others, and they are more plentiful.
2. Their removal also consists of a much more complex mechanism.
    a. First, the nucleotide sequence around the different introns is often similar.

b. They usually begin with a GU dinucleotide at the 5'-end, and terminate with an AG dinucleotide at the 3'-end.

c. Consensus sequences, such as these, often attract specific molecules that forma complex called a **spliceosome**, which is essential to splicing.

- The spliceosome is very large, consisting of 40*S* in yeast and 60*S* in mammals.
- **Small nuclear RNAs (snRNAs)**, which are usually 100 to 200 nucleotides long, are one group of components of these complexes.
- When the snRNAs are associated with proteins in the spliceosome, they are called **small nuclear ribonucleoproteins (snRNPs,** or **snurps)**.
- These are designated U1, U2... because they are rich in uridine.

3. The U1 snRNA contains a homologous nucleotide sequence for the 5'-end of the intron.

a. The base pairing, which results from this homology, promotes binding that is the initial formation of the spliceosome.

b. The addition of other snurps occurs, and splicing commences.

4. Similar to group I, this group also has two reactions associated with the splicing of the introns.

a. Transparency 77, page 266, figure 12-12.

b. The first reaction involves the 2'-OH group from an adenine (A) present within the intron and the phosphate group at the 3'-end of the exon.

c. The **branch point** of the spliceosome is represented by the A residue position.

d. Following the formation of an intermediate, the second reaction begins, which involves the –OH group, already added to the 3'-endof the exon.

e. The intron has then been removed and the exons are spliced together.

5. **Alternate splicing** is a process in which the same gene is spiced in more than one way, yielding a group of mRNAs that upon translation, result in a series of related proteins called **isoforms**.

G. In the late 1980s, another form of modification was discovered called **RNA editing**.

1. In RNA editing, the nucleotide sequence of a pre-mRNA is actually changed prior to translation, resulting in a mRNA exhibiting a different sequence than what was encoded in the exons of the DNA.
2. There are two main types of RNA editing.
   a. One type is called **substitution**.
      - This is where the identities of individual nucleotides are altered.
      - Substitution is highly prevalent in mitochondrial and chloroplast RNAs transcribed in plants.
      - Substitution occurs without any form of template information, and the phenomenon remains a mystery still.
   b. The second type of RNA editing is called **insertion/deletion**.
      - This is where nucleotides are added or subtracted from the total number of bases.
      - *Trypanosoma*, a parasite that causes African sleeping sickness, use insertion/deletion editing in mitochondrial RNAs.
      - Insertion/deletion methods in Trypanosoma are directed by **gRNA (guide RNA)** templates, which are also transcribed from the mitochondrial genome.
      - They base pair with the pre-edited mRNAs to direct the editing mechanism to make the correct changes.
   c. *Physarum polycephalum*, a slime mold, uses both substitution and insertion/deletion methods for editing its mitochondrial RNAs.

X. Translation: Components Necessary for Protein Synthesis

A. Translation is the biological polymerization of amino acids in to polypeptide chains.
   1. Translation only occurs in association with ribosomes.
   2. Transfer RNA (tRNA) are molecules that serve as adapters between specific triplet codons in mRNA and the corresponding amino acids.
   3. An adapter role was first postulated by Crick in 1957.

B. The ribosome has been studied immensely because of its critical role in protein synthesis.

1. A single bacterial cell can contain 10,000 ribosomes, while an eukaryotic cell can contain many times more than that.
2. Ribosomes have been viewed under the electron microscope, so that its structure could be determined.
   a. The ribosome consists of two subunits, one large and one small.
   b. Each subunit of every ribosome is associated with one or more molecules of **ribosomal RNA (rRNA)** and an array of **ribosomal proteins**.
3. Figure 12-13 in the text summarizes the differences between ribosomes in prokaryotes and eukaryotes.
   a. The sedimentation behaviors in sucrose gradients are the most easily used data to form characteristics about ribosomes.
   b. The two subunits associated together make up the **monosome**.
      - In prokaryotes, the monosome is a 70*S* particle, whereas in eukaryotes the monosome is an 80*S* particle.
      - Sedimentation coefficients are not additive, that is the 70*S* monosome consists of a 50*S* and a 30*S* subunit, and the 80*S* monosome consists of a 60*S* and a 40*S* subunit.
   c. The larger subunit in the prokaryotes consists of a 23*S* RNA molecule, a 5*S* rRNA molecule, and 32 ribosomal proteins, whereas the eukaryotic equivalent a 28S rRNA molecule consists of a 5.8*S* and 5*S* rRNA molecule and about 50 proteins.
   d. In the smaller subunits of the prokaryotes, there is a16S rRNA component and 21 proteins, whereas in the eukaryotes, there is an 18*S* rRNA component and approximately 33 proteins.
4. The degree of redundancy of the genes coding for the rRNA components has been studied using molecular hybridization.
   a. In *E. coli*, there are seven copies of a single sequence, which encodes for all three components of the rRNA; the initial transcript produces a 30*S* RNA molecule that is enzymatically cut into these smaller pieces.
   b. In eukaryotes, there are many more copies of the sequence encoding the 28*S* and 18*S* components are present.

- *Drosophila* contain approximately 120 copies which are each transcribed into a molecule of about 34*S*, which is then processed into the 28*S*, 18*S*, and 5.8*S* rRNA species.
- In mammals, the original transcript is 45*S*.
- The rRNA genes are part of a moderately repetitive DNA fraction, which is present in clusters at various chromosomal sites.
  - Each of these clusters consists of **tandem repeats**.
  - The tandem repeats are separated by **spacer DNA** sequences, which are noncoding.
  - For human beings, these clusters have been localized on the short arm ends of chromosomes 13, 14, 15, 21, and 22.

c. The unique 5S rRNA component in eukaryotes is not part of the larger transcript, instead, the genes encoding for it are located separately.
   - This is different than in *E. coli*.
   - A gene cluster encoding for this has been reported on the human chromosome number 1.

C. Transfer RNA is the best-characterized RNA molecule.

1. The fact that is the best characterized RNA molecule is due to it small size and stability in the cell.
   a. It is composed of only 75 to 90 nucleotides.
   b. tRNA also displays an almost identical structure in bacteria and eukaryotes.
   c. tRNAs are transcribed as large precursor molecules, which are later cleaved into mature 4S tRNA molecules.
2. Robert Holley and his coworkers reported the complete sequence of $tRNA^{ala}$ isolated from yeast, in 1965.
   a. They discovered a number of nucleotides unique to tRNA, each of which are modifications of one of the four nitrogenous bases expected in RNA.
      - These modified bases include **inosinic acid**, which contains purine hypoxanthine, ribothymidylic acid, and pseudouridine, among others.
      - These modified bases, which are sometimes referred to as *unusual*, *rare*, or *odd bases* are created posttranscriptionally.

- The unmodified base (A, U, C, or G) is produced during transcription, and then enzymatically modified.

b. Holley's analysis led him to create the two-dimensional **cloverleaf model** of transfer RNA.
   - Transparency 78, page 269, figure 12-14.
   - It had already been determined that tRNA exhibits secondary structure due to base pairing.
   - Holley's model consists of a series of paired stems and unpaired loops.
   - The loops consistently contained modified bases, which do not generally form base pairs.

c. Because Holley knew that GCU, GCC, and GCA coded for alanine, he looked for the anticodon of his $tRNA^{ala}$ molecule.
   - Holley determined it to be in the form of CGI, located at the top loop of the model.
   - Thus, the **anticodon loop** was established.

3. Numerous constant features were observed, following the examinations of other tRNA species.
   a. One feature was that the 3'-end of all tRNAs contain the sequence ...**pCpCpA-5'**, and it is to the terminal adenine residue, that the amino acid is covalently bonded to during charging.
   b. Another feature was that all tRNAs contain...**pG**, at the 5' terminus.
   c. Also, the lengths of the various stems and loops were similar.
   d. Finally, all tRNAs contain an anticodon complementary to the known amino acid for which it is specific, and is present at the same position of the cloverleaf as well.
4. X-ray crystallography was used to examine the three-dimensional structure of tRNA.
   a. By 1974, Alexander Rich and his coworkers in the United States, as well as J. Roberts B. Clark, Aaron Klug, and their colleagues in England had successfully crystallized tRNA.

b. The resolution they had was at 3 angstroms, and the patterns made by individual nucleotides were discernible.

c. Following the results of these experiments, a complete three-dimensional model became available.

- Look at figure 12-15 in the text for an illustration of the three-dimensional model.
- The model shows that tRNA exhibits a reversed L-shape.
- At one end of the L, the anticodon loop and stem is apparent, while at the other end, is the presence of the 3'-acceptor region, which is where the amino acid is bound.

D. The transfer RNAs must be linked to their respective amino acid, before translation can continue.

1. The process by which this occurs is called **charging**.

a. The activation of this process is done by the direction of enzymes called **aminoacyl tRNA synthetases**.

b. It is commonly thought that since there are 20 amino acids, there must be 20 different tRNA molecules.

c. Because charging is so specific, there must also be a unique synthetase enzyme for each reaction.

d. After careful research, it is now suggested that there are at least 32 different tRNAs and 20synthetases.

2. Transparency 79, page 270, figure 12-16.

a. The first step of the charging process occurs when the amino acid is converted to an activated form, which reacts with ATP to form an **aminoacyl adenylic acid**.

- A covalent linkage between the 5'-phosphate group and the carboxyl end of the amino acid is formed.
- This remains associated with the enzyme, forming an activated complex, which in turn reacts with a certain tRNA molecule.

b. In the second step, the amino acid is transferred to the tRNA.

- The amino acid binds covalently to the adenine residue at the 3'-end.

- This charged tRNA can participate directly in protein synthesis.
- The aminoacyl tRNA synthetases are highly specific, and recognize only one amino acid and only a subset of the tRNAs called **isoaccepting tRNAs**.
- This is crucial to the fidelity of translation.

X. Translation: The Process

A. Transparency 80, pages 271-272, figure 12-17.

B. Initiation is the first part of translation, and it is diagramed in steps 1-3.

1. Most ribosomes are dissociated into their separate subunits, when they are not involved in translation.
2. In *E. coli*, initiation involves the small subunit, a mRNA molecule, a specific initiator tRNA, GTP, Mg^{+2}, and at least three **initiation factors (IFs)**, which are not part of the ribosome, however they are essential for initiation to begin.
3. The initiation code of mRNA, AUG, calls for the modified amino acid **formylmethionine**, in prokaryotes.
4. Several initiation proteins are then bound the small subunit of the ribosome.
   a. This complex then binds to mRNA.
   b. In bacteria, this binding involves a sequence, which can be up to six ribonucleotides (AGGAGG), which is located upstream from the AUG codon.
   c. This sequence consisting of only purines, is called the **Shine-Dalgarno** sequence, and it base pairs with a region of the 16S rRNA of the small ribosome subunit.
5. Next, another initiation protein facilitates the binding of charged formylmethionyl-tRNA to the small subunit of the ribosome.
   a. This "sets" the reading frame.
   b. This also represents the **initiation complex**, which then combines with the large ribosomal subunit.
   c. This process requires the hydrolyzing of a GTP molecule, and finally the initiation factors are released.

C. Elongation is the second part of translation, and it is represented in steps 4-9.

1. Binding sites for the two charged tRNA molecules are formed once both subunits of the ribosome are assembled with mRNA.
   a. One of the sites is called the **P site**, or **peptidyl site**.
   b. The other site is called the **A site**, or **aminoacyl site**.
2. Assuming that the AUG triplet is in the P site, the initiator tRNA binds to the corresponding site of the small subunit.
3. The charged tRNA, which is dictated by the second triplet in the sequence, becomes positioned at the A site.
4. **Peptidyl transferase**, which is part of the large subunit of the ribosome, then catalyzes the formation of the peptide bond, which links the two amino acids together.
5. The covalent bond between the amino acid and the tRNA occupying the P site is then hydrolyzed, or broken, resulting in a dipeptied which is attached to the tRNA at the A site, as its product.
6. The step in which the growing polypeptide chain increases in length by one amino acid is termed **elongation**.
7. The uncharged tRNA attached to the P site must be released from the large subunit, in order for elongation to continue.
   a. It is thought that the uncharged tRNA moves transiently through a third site, which is called the **E site,** or **exit site**.
   b. The E site is only occupied for a brief amount of time throughout the whole process of translation.
8. Next, the entire **mRNA-tRNA-aa_2- aa_1** shifts over to the P site.
   a. This event requires several protein **elongation factors (EFs)**.
   b. It also requires the use of energy produced from the hydrolysis of GTP.
9. The third triplet of the mRNA is now in the position to accept another specifically charged tRNA into the A site.
10. The steps of elongation occur over and over.
    a. Once a reasonably sized polypeptide chain is assembled, 30 amino acids, the chain begins to emerge from the base of the large subunit.

b. A **tunnel** exists within the subunit, in which the elongated polypeptide chain works its way out of the ribosome.

11. The efficiency of this process is extremely high.
    a. The error rate that is observed is only 10^{-4}!
    b. In *E. coli*, elongation occurs at a rate of about 15 amino acids per second.
    c. This is the same as the rate of a tape moving through a tape player!

D. The final action observed is termination.
    1. The termination of the growing polypeptide chain is signaled by one or more of three codons: UAG, UAA, or UGA.
        a. These codons do not code for any amino acid, nor do they summon a tRNA into the A site.
        b. The termination codon signals the action of GTP-dependent **release factors**.
        c. These release factors will cleave the chain at the terminal tRNA, and it is then released from the ribosome, which dissociates into its subunits.
    2. If one of the termination codons is found internally on the gene, caused by a mutation, then the same result will occur, and the polypeptide chain will terminate prematurely.

E. As the mRNA passes through the ribosome, it is free to associate with another small subunit to form yet another initiation complex.
    1. This process, which can be repeated several times with a single mRNA, results in the production of **polyribosomes,** or just **polysomes**.
    2. These can then be isolated and analyzed, look at figure 12-18 in the text.
    3. Polysome complexes represent efficient use of the components available during a unit of time in protein synthesis.

XI. Translation in Eukaryotes

A. The model that was just presented was initially derived from bacteria; therefore, there are so other notable differences between that model and the model for eukaryotes.
    1. Eukaryotic mRNAs are able to live much longer than their prokaryotic counterparts; they can live for hours rather than just minutes.

2. There are two aspects that involve the initiation of translation that are different as well.
   a. One is the addition of the 7-methyguanosine residue "cap" on the 5'-end.
      - The presence of this cap is absent in prokaryotes, but is essential for translation to proceed smoothly in eukaryotes.
      - Also, most eukaryotic mRNAs contain a short recognition sequence that surrounds the initiating AUG codon, **5'-ACCAUGG... the Kozak sequence**, which seems to function in the same way that the Shine-Dalgarno sequence does in prokaryotic mRNA.
   b. The second factor involves the insertion of the first amino acid.
      - In eukaryotes, the amino acid formylmethionine is not required to be first, as it is in prokaryotes.
      - However, AUG is essential in the formation of the translational complex in both prokaryotes and eukaryotes.

B. Finally, the protein factors similar to those in prokaryotes guide initiation, elongation, and termination of translation in eukaryotes.
   1. Many of the eukaryotic factors are homologous to their counterparts in prokaryotes.
   2. There may be a greater number of factors required during each of these steps, and they may be more complex than they are in the prokaryotes.

Lecture Outline

Chapter Thirteen: Regulation of Gene Expression

I. Genetic Regulation in Prokaryotes: An Overview

A. Turn genes on and off

B. *E. coli*

C. Basal level of gene products

1. **Inducible enzymes**
2. **Constitutive enzymes**

D. Inhibition of genetic expression

1. End products of biosynthetic pathways
2. Amino acids
   a. Present in the environment
   b. Suppression factor
   c. **Repressible** system

E. **Negative** or **positive** control

1. **Repressor** protein
2. **Active regulator** protein

II. Lactose Metabolism in E. coli: An Inducible System

A. Single regulatory site

1. ***Cis*-acting element**
2. Location
3. Interactions at regulatory site
   a. ***Trans*-acting elements**
   b. Which genes will be expressed

B. Lactose metabolism in *E. coli*

1. Presence of lactose
2. **Inducible**
3. Contents of cluster
   a. Transparency 81, page 283, figure 13-1
   b. **Polycistronic mRNA**
   c. **Operons**

d. Rapid response

C. **Structural genes**

1. **β-galactosidase**
2. **β-galactoside permease**
3. **Transacetylase**
4. Mutants of *lacZ⁻ or lac Y⁻*

D. Constitutive mutants

1. Regulatory scheme for lactose metabolism
2. *lacI⁻*
3. *lacO^c*

E. **Operon model**

1. Transparency 82, page 284, figure 13-3
2. Unit
   a. *Z*, *Y*, and *A* structural genes
   b. **Repressor molecule**
      1. **Allosteric**
      2. Reversible interactions
3. Repressor interaction
   a. RNA polymerase
   b. Allosteric conformational change
   c. Operator DNA
   d. Repressor-operator interaction absent
   e. **Negative control**

F. Genetic methods

1. Major assumptions
   a. *lacI* gene
   b. *lacO* region
   c. *lacO* location
2. Bacteria
   a. Extra copy of one or even a few genes
   b. **F factor**

c. *lacI*$^+$ added to *lacI*$^-$ cell

d. Adding a *lacO*$^+$ region to *lacO*^{C} cell

3. Table 13.1

4. Mutations in *lacI* gene

a. Cannot interact with the inducer

b. Structural genes permanently repressed

c. Additional *lacI*$^+$ gene

5. *lacI*^{S}

G. Isolation and characterization of repressor molecule

1. Water Gilbert and Benno Muller-Hill

a. Contents of repressor

b. Experiments

2. Mitchell Lewis, Ponzy Lu, and colleagues

a. Four monomers

b. Each tetramer

c. Distortion of conformation of DNA

d. Illustrated in figure 13-4

3. Three-dimensional conformational changes

H. *lac* repressor bound to inducer

1. **Promoter region**

a. Location of promoter

b. **Catabolic-activating protein (CAP)**

2. **CAP-binding site**

a. Inducible cellular conditions

b. **Catabolite repression**

c. Lactose present

3. Transparency 83, page 287, figure 13-5

a. Regulation of the *lac* operon

b. CAP exerts positive control

4. Efficient utilization of energy

III. Tryptophan Metabolism in E. coli: A Repressible Gene System

A. **Enzyme repression**

1. Wild-type *E. coli*
   a. **Tryptophan synthase**
   b. **Repressed**
   c. Economical to cell
2. Five contiguous genes
3. Model for gene regulation
   a. Inactive repressor
   b. Can bind to tryptophan
   c. Activation of repressor
   d. Negative control
   e. **Corepressor**

B. Two categories of constitutive mutations

1. *trpR*$^-$
   a. Repressor protein
   b. Inhibition by *trpR*$^-$
   c. Repressor is absent
   d. *trpR*$^-$ gene is present
2. Another constitutive mutant
   a. Location
   b. Addition of a wild-type gene in mutant cells
3. Figure 13-6

C. "Strong" and "weak" repression

1. Binding between protein repressor and operator DNA
2. **Attenuation**
   a. Charles Yanofsky
   b. Termination of mRNA
   c. **Attenuator**
   d. Attenuation overcome
3. Folding of RNA

a. "Hairpin loop"

b. Premature termination

IV. Genetic Regulation in Phage Lambda: Lysogeny or Lysis?

A. Genetic regulation at transcriptional level

1. Lambda DNA

2. Two different cycles

a. **Lysogenic pathway**

1. **λ repressor protein**

2. ***c*I repressor**

b. **Lytic pathway**

1. *c*I gene repressed

2. **Cro** protein

3. *c*I repressor

a. 236 amino acids

b. Controls the rate of λ mRNA

4. Cro protein

a. When repressor is produced

b. O_L and O_R

c. Early genes repressed

d. During binding

5. No repressor bound

a. N protein

b. Cro protein

c. Mutations

d. Dimers

V. Genetic Regulation in Eukaryotes: An Overview

A. Similar regulatory systems

B. Eukaryotic cells

1. Genetic information

a. Many chromosomes

b. Transcription

c. Transcripts of eukaryotic genes

d. Cellular interactions and external signals

2. Transparency 84, page 289, figure 13-8

a. Eukaryotic gene expression

b. Transcriptional level

c. Two main components of transcriptional control

V. Regulatory Elements and Eukaryotic Genes

A. The internal structure of eukaryotic genes

1. **Promoters** and **enhancers**

2. Location

a. *Cis* regulators

b. *Trans* regulators

B. **Promoters**

1. Necessary to initiate transcription

2. Binding protein factors

a. Promoters that recognize RNA polymerase II

b. 100 base pairs upstream

c. TATA box

3. Other components

a. **CAAT box**

b. A third element

C. **Enhancers**

1. More complex

2. Distinguished from promoters

a. Position is not fixed

b. Can be inverted

c. If enhancer is moved

3. Control of enhancers

a. Immunoglobulin heavy-chain genes

b. Human β-globin gene

c. **Upstream activator sequences (UAS)**

VI. Transcription Factors and Gene Regulation

A. **Transcription factors**
   1. Two functional domains
      a. **DNA-binding domain**
      b. **Trans-activating domain**
   2. Protein responsible for binding function

B. Galactose metabolism
   1. Expression of genes
      a. Absence of galactose
      b. Presence of galactose
      c. Mutation (*GAL4)*
   2. **UAS_Gs**
   3. Product from *GAL4* gene
      a. Transparency 84, page 291, figure 13-11
      b. DNA-binding domain
      c. Gene activation
   4. Interaction activates transcription
   5. **TFIID**
      a. Complex of several proteins
      b. **TATA-binding protein (TBP)**, and **TBP-associated factors**

C. Domains of eukaryotic transcription factors
   1. **DNA-binding domains**
      a. Three major types
      b. New groups will be established
   2. **Helix-turn-helix**
      a. HTH motifs
      b. Present in many prokaryotic DNA-binding proteins
      c. Geometric conformation
      d. Protein binds to DNA
      e. Always part of a larger DNA-binding domain
   3. Eukaryotic genes

a. **Homeobox**

b. **Homeodomain** sequence

c. Conserved sequence

4. **Zinc fingers**

a. Transcription factor TFIIIA

b. Discovered in other places

c. Different types of zinc fingers

5. Clusters of two cysteine and two histidine residues

a. Amino acids fold into loops (zinc fingers)

b. Content of the loops

c. Bind to specific DNA sequences

d. Major groove of DNA

e. Number of fingers

6. **Leucine zipper**

a. First seen in rat liver

b. Forms a helix

c. Leucine residues "zip" together

7. Transcription factors

a. 30 to 100 amino acids

b. Interact with other transcription factors

8. Generalizations of transcriptional regulation

a. Primary levels of regulation

b. Positive regulation

c. Binding of one or more factors

d. Promoter and enhancer sequences recognized and bound

D. Assembly of transcriptional factors

1. TFIID complex

a. 20 base pairs of DNA involved

b. Conformational changes

2. Eukaryotic polymerases

a. Transparency 85, page 294, figure 13-16

b. Minimal basal level

c. Above the basal level

d. Factors bound to enhancers

VII. Genomic Alterations and Gene Expression: DNA Methylation

A. Chromatin conformation alteration

1. Addition or deletion of methyl groups

a. DNA is modified after replication

b. **DNA methylation**

c. Five percent of cytosine residues are methylated

2. Ability of methylation to alter gene expression

a. Methylation of DNA in operator region

b. 5' position on cytosine

c. Protruding methyl group

B. CG doublets

C. Gene is expressed

D. Observations

1. Inverse relationship

2. Tissue-specific and heritable

E. Not a general phenomenon

VIII. Gene Regulation by Steroid Hormones

A. **Steroid hormones**.

B. Major sex hormones

C. Transparency 86, page 296, figure 13-18

1. General scheme for hormone action

2. Hormone enters cell

3. **Hormone receptor protein**

4. Translocated into nucleus

D. Three functional domains

1. N-terminus domain, central domain, and C-terminus domain

2. **Hormone responsive elements (HREs)**

a. Short consensus sequences

b. Several hundred bases upstream from start site

c. Many copies

d. Promoter or enhancer sequences

3. HRE not sufficient to cause activation on its own

a. Facilitate interaction of other transcription factors

b. Other binding sites available

IX. Postranscriptional Regulation of Gene Expression

A. **Post-transcriptional modes of regulation**

B. In eukaryotes

C. Noncoding introns removed, exons spliced together

Lecture Content

Chapter Thirteen: Regulation of Gene Expression

I. Genetic Regulation in Prokaryotes: An Overview

A. Evolution has allowed for highly efficient mechanisms that turn genes on and off, depending on the cell's metabolic needs in a particular environment.

B. *E. coli* has more than 4000 polypeptide chains encoded within the genome.
   1. There is a vast range of gene products produced in *E. coli*.
   2. Some of the proteins may be present as only 5 to 10 molecules in each cell, however others may be present is as many as 100,000 copies per cell, such as ribosomal proteins.

C. There is generally a basal level of gene products that exists, but the level can be altered in response to the chemical signals from the environment.
   1. **Inducible enzymes** are produced in response to environment signals.
   2. **Constitutive enzymes** are produced continuously regardless of the chemical makeup of the environment.

D. There are also cases where a specific molecule can cause inhibition of genetic expression.
   1. These products are usually the end products of biosynthetic pathways.
   2. Amino acids are examples because they can be present in the environment, or they can be produced by bacteria.
      a. If a certain amino acid is present in the environment, then it is inefficient for the organism to produce the necessary factors to make that amino acid.
      b. In this case, the amino acid is the factor that suppresses the transcription of mRNA for the appropriate biosynthetic enzyme.
      c. This is an example of a **repressible** system of gene regulation.

E. Gene regulation can be under **negative** or **positive** control, whether it is inducible or repressible.
   1. Gene expression occurs unless it is shut off by a **repressor** protein in negative control.
   2. In positive control systems, gene expression occurs only when there is an **active regulator** protein present.

II. Lactose Metabolism in E. coli: An Inducible System

A. Genes tend to be organized in clusters, which are controlled by a single regulatory site in prokaryotes.
   1. The site is linked to the gene cluster it controls, and is known as a ***cis*-acting element**.
   2. These elements are usually located upstream from the gene cluster in which they regulate.
   3. Interactions at the regulatory site include the binding of molecules that control transcription in the gene cluster.
      a. These molecules are called ***trans*-acting elements**.
      b. The actions performed at the regulatory site determine which genes will be expressed and which will not.
      c. The binding of these *trans*-acting elements to a *cis*-acting element can have either a positive reaction (turning on of the genes), or a negative reaction (shutting off of the gene).

B. In 1946, the work performed by Jacques Monod and contributions from Joshua Lederberg, Francois Jacob, and Andre Lwoff, provided insights into the way that genes responsible for lactose metabolism in *E. coli* are regulated.
   1. In the presence of lactose, these enzymes increase in concentration from 5 to 10 molecules to thousands per cell very rapidly.
   2. These enzymes are said to be **inducible**, with the lactose serving as the **inducer**.
   3. The lactose cluster contains a regulatory gene, a control site, and three adjacent structural genes that encode the enzymes involved in the metabolism of lactose.
      a. Transparency 81, page 283, figure 13-1.
      b. The three structural genes are transcribed into a single **polycistronic mRNA.**
      c. The clustered structural genes transcribed as a single mRNA and their adjacent control regions are termed **operons**.
      d. The genes in the *lac* operon function to provide a rapid response to the presence or absence of lactose in the environment.

C. **Structural genes** encode for the primary structure of enzymes.

1. The *lac Z* gene codes for **β-galactosidase**, which converts the disaccharide lactose into the monosaccharides glucose and galactose, which is essential for the conversion of lactose.
2. The *lac Y* gene encodes **β-galactoside permease**, which is a membrane-bound protein that assists in the transport of lactose into the bacterial cell.
3. The third gene in the cluster is the *lac A* gene, which codes for **transacetylase**, an enzyme whose role is not yet completely clear.
4. Mutants of the *lacZ⁻ or lac Y⁻* are unable to utilize lactose as an energy source.

D. The **constitutive mutants** produce enzymes whether or not lactose is present.
   1. These mutations served as a basis for studies that defined the regulatory scheme for lactose metabolism.
   2. The constitutive mutant *lacI⁻* is present at a site that is adjacent to, but independent from the locus of the structural genes, and it possesses its own promoter and termination regions.
   3. Another class of constitutive mutants is the *lacO⁻* that is located adjacent to the structural genes, and represents the **operator region** of the *lac* operon.

E. In 1961, Jacob and Monod proposed the **operon model**, which is a scheme of negative control regulation.
   1. Transparency 82, page 284, figure 13-3.
   2. This is where a group a genes is regulated and expressed together as a unit.
      a. In their model, the operon consists of *Z, Y,* and *A* structural genes, as well as the operator region.
      b. They suggested that the *lacI* gene regulated the transcription of the structural gene by producing a **repressor molecule**.
         - The repressor was thought to be **allosteric**.
         - This means that the molecule interacts reversibly with another molecule, causing a conformational shape change, as well as a change in the chemical activity.
   3. Jacob and Monod also suggested that normally the repressor interacts with the DNA sequence of the operator region.

a. This in turn inhibits the action of RNA polymerase, repressing the transcription of the structural genes.
b. On the other hand, when lactose is present, the disaccharide binds to the repressor, which causes allosteric conformational change.
c. The change alters the binding site of the repressor, rendering it incapable of interacting with operator DNA.
d. When the repressor-operator interaction is absent, RNA polymerase transcribes the structural genes and enzymes necessary for lactose metabolism are produced.
e. This is considered **negative control** since transcription occurs only when the repressor fails to bind to the operator region.

F. Genetic methods can be used to test the predictions made by the operon model.
1. The following are the major assumptions:
a. The *lacI* gene produces diffusible cellular products.
b. The *lacO* region does not encode a gene product.
c. The *lacO* region must be adjacent to the *lac* structural genes in order to regulate transcription.
2. Bacteria are generally haploid organisms, but they can exist in partially diploid strains.
a. In this case, the entire host chromosome is present along with an extra copy of one or even a few genes of choice.
b. These genes exist as part of a plasmid called the **F factor**, designated as F'.
c. The Jacob-Monod operon model explains that if the $lacI^+$ gene is added to a host with the $lacI^-$ cell, inducibility should be restored because a normal repressor would be produced again.
d. Adding a $lacO^+$ region to a $lacO^c$ cell, should no effect since regulation depends on the $lacO^+$ region of the host DNA.
3. Table 13.1 lists the results from these experiments, were Z represents all three structural genes.
4. Another prediction is that mutations in the *lacI* gene should have the opposite effect of the $lacI^-$ mutants.

a. The molecules of the mutant repressor would be produced that cannot interact with the inducer, lactose.
b. This would result in the repressor remaining bound to the operator sequence, and the structural genes would be repressed permanently.
c. The presence of an additional *lacI*$^+$ gene would have little or no effect on repression.

5. A mutation, *lacI*^{S}, was discovered in which the operon was "superrepressed," and even the addition of a *lacI*$^-$ gene would not relieve repression.

G. The isolation and characterization of the repressor molecule allowed for the most direct proof for the role of repressors in operon regulation.

1. Water Gilbert and Benno Muller-Hill reported the isolation in 1966.
   a. The repressor consists of a tetramer of four polypeptides, with two binding sites, one for lactose, and the other for the DNA sequences of the operator region.
   b. Experiments resulting with the repressor binding to the DNA containing a *lacO*$^+$ gene, but not a *lacO*^{C} gene, confirmed the role of the repressor.
   c. The repressor binding activity could not be demonstrated among the proteins isolated from the *lacI*$^-$ cells, as predicted.
2. The crystal structure of the lac repressor as well as the structure of the repressor bound to IPTG and to the operator DNA was demonstrated by Mitchell Lewis, Ponzy Lu, and colleagues in 1996.
   a. The four monomers within the repressor tetramer are identical and consist of 360 amino acids.
   b. Each tetramer binds to two symmetrical operator DNA helices.
   c. Distortion of the conformation of DNA results from the binding by the repressor, causing the DNA to bend away from the repressor.
   d. This is illustrated in figure 13-4.
3. Three-dimensional conformational changes, which accompany the allosteric transitions during the interactions with the inducer molecules, have also been defined within these studies.

H. The *lac* operon is activated and RNA polymerase transcribes the structural genes, when the *lac* repressor is bound to the inducer.

1. The binding between the polymerase and the nucleotide sequence of the **promoter region** initiates this process.
   a. The promoter, within the *lac* operon, is located between the *I* gene and the operator region, (O^C).
   b. A protein called the **catabolic-activating protein (CAP)** must be present in order for the polymerase binding to be efficient.
2. The CAP and the **CAP-binding site** were discovered as a result of an investigation, which produced very interesting observations.
   a. When the cellular conditions are inducible (in the presence of lactose), transcription of the operon is inhibited if glucose is present.
   b. This repression, **catabolite repression**, reflects the greater simplicity with which glucose may be metabolized in comparison to lactose.
   c. Since the cells "prefer" glucose, they do not activate the *lac* operon when lactose is present.
3. Transparency 83, page 287, figure 13-5.
   a. This figure represents the regulation of the *lac* operon by CAP and the role of glucose in catabolite repression.
   b. When under inducible conditions, and the absence of glucose, CAP exerts positive control by binding to the CAP site.
      - This situation facilitates the binding of RNA polymerase to the promoter, resulting in transcription.
      - Therefore, the repressor must be bound by lactose, and CAP must be bound to the CAP-binding site for maximal transcription.
4. Efficient utilization of energy is observed with the regulation of the *lac* operon by catabolite repression.
   a. The presence of the glucose overrides the need for the metabolism of lactose.
   b. Other inducible operons exhibit catabolite repression involving CAP as well.

III. Tryptophan Metabolism in E. coli: A Repressible Gene System

A. In 1953, Monod and his coworkers discovered the phenomenon of **enzyme repression**.
   1. He discovered that wild-type *E. coli* are capable of producing enzymes that are required for the biosynthesis of amino acids and other important biomolecules.
      a. Monod focused his studies on tryptophan and the enzyme **tryptophan synthetase**.
      b. If tryptophan is present in a large quantity, the enzymes necessary for the synthesis of tryptophan are **repressed**.
      c. This repression is very economical to the cell, because the synthesis would be unnecessarily performed, utilizing the cell's energy.
   2. There are five contiguous genes on the *E. coli* chromosome, which are involved in tryptophan synthesis.
      a. The genes are part of the *trp* operon.
      b. All the genes are systematically repressed in the presence of tryptophan.
   3. As of a result of their investigations, Jacob and Monod proposed a model for gene regulation analogous to the *lac* system.
      a. They suggested that an inactive repressor is normally made that alone cannot bind to the operator region within the operon.
      b. The repressor, however, can bind to tryptophan, when present.
      c. This binding results in the activation of the repressor, allowing it to bind to the operon, and thus inhibit transcription.
      d. This repressible system is obviously under negative control.
      e. Tryptophan, because it participates in the repression, is referred to as a **corepressor** in this regulatory scheme.

B. Two distinct categories of constitutive mutations have been isolated, which provide support by genetic evidence for the model of a repressible operon.
   1. The first class, *trpR*$^-$ maps at a distance away from the structural genes.
      a. This encodes for a repressor protein.
      b. The *trpR*$^-$ mutant either inhibits the interaction of a mutant repressor with tryptophan, or inhibits repressor formation entirely.

c. When the repressor is absent, the operon is transcriptionally active.

d. When the *trpR*$^+$ gene is present, repression is restored and the operon is transcriptionally active.

2. Another constitutive mutant behaves similarly to the *lacO*^{c} mutation in the *lac* operon.

a. It is located directly adjacent to the structural genes within the *trp* operon.

b. The addition of a wild-type gene in mutant cells does not restore enzyme repression.

3. Figure 13-6 in the text illustrates the entire *trp* operon.

a. There are five contiguous genes that are transcribed into a polycistronic message that directs translation of the enzyme for the biosynthesis of tryptophan.

b. RNA polymerase binds to a promoter region, (*trpP*), while the repressor binds to the operator region (*trpO*).

c. Transcription is initiated within the overlapping *trpP*/*trpO* region, in the absence of binding.

d. The leader sequence contains a regulatory sequence called an **attenuator**.

C. Repression can be considered "strong" and "weak."

1. Molecular binding between a protein repressor and operator DNA is a dynamic process that is subject to chemical equilibria.

a. If there is a high degree of infinity between the two, then repression at equilibrium will be quite effective.

b. On the other hand, if there is a low affinity between the two, then repression at equilibrium will be weak.

2. **Attenuation** is the process where a second level of regulation occurs at the *trp* operon.

a. Attenuation was studied extensively by Charles Yanofsky, and in this context means to reduce in amount.

b. mRNA synthesis, if initiated, is terminated at a point about 140 nucleotides along the transcript.

c. This is illustrated in figure 13-6 in the text; the point is called the **attenuator**.

d. Attenuation is overcome and transcription proceeds when tryptophan is present.

3. Attenuation involves the folding of the RNA transcribed from the leader sequence.
   a. When tryptophan is abundant, the structure of the RNA mimics that found in at the end of mRNA molecules, forming a "hairpin loop," during attenuation.
   b. The configuration leads to the premature termination of the transcription, resulting in a decreased amount to mRNA produced.

IV. Genetic Regulation in Phage Lambda: Lysogeny or Lysis?

A. The understanding of genetic regulation at the transcriptional level has benefited from the studies with bacteriophage lambda.
   1. Lambda DNA contains approximately 45,000 base pairs, which is enough to encode 35 to 40 genes.
   2. Phage lambda can enter two different cycles following infection of *E. coli*.
      a. One is the **lysogenic pathway**, in which phage DNA is incorporated into the bacterial genome and is almost totally expressed.
         - The genes responsible for phage reproduction are turned off by the λ **repressor protein**.
         - The protein is produced by the virus's own genes, *c*I, and will be referred to as the ***c*I repressor**.
      b. The other is the **lytic pathway**, in which phage DNA is transcribed and viral reproduction ensues.
         - The *c*I gene is repressed by a second protein in this pathway.
         - This protein is called the **Cro** protein and is produced by the *cro* gene.
      c. Both the *c*I repressor and the Cro protein have been isolated and characterized.
      d. Figure 13-7 in the text illustrates the operator regions that bind the repressor.
   3. Mark Ptashne isolated and characterized the *c*I repressor in 1967.
      a. It is a protein that consists of 236 amino acids.
      b. It controls the rate at which classes of λ mRNA are made.
   4. The Cro protein consists of 66 amino acids.

a. When the repressor is produced, it recognizes two operator regions in λ DNA.
b. The regions that it recognizes are called O_L and O_R, which are present on either side of the *c*I gene.
c. Two sets of early genes are repressed, when the repressor is bound to this region, causing the remainder of the λ genes to be turned off (negative control).
d. During binding, the *c*I receptor stimulates transcription of the *c*I gene, enhancing *c*I transcription by a factor of ten (positive control).

5. If there is no repressor bound, transcription initiation at promoters, P_{RM} and P_R, proceeds, resulting in the production of two proteins, N and Cro.
   a. The N protein functions as an antiterminator, which allows for the complete transcription of the genes essential to reproduction and lysis.
   b. The Cro protein acts as a repressor of the *c*I gene transcription.
   c. Mutations in either the O_L or O_R prevent repressor binding and abolish the potential for lysogeny, whereas *cro*⁻ mutants abolish potential for lysis.
   d. Both Cro and *c*I repressors form dimers, which bind to the major groove of the DNA helix.

V. Genetic Regulation in Eukaryotes: An Overview

A. Presently, there are similar regulatory systems between bacterial operons and yeast and the nematode *C. elegans*.
B. Eukaryotic cells contain a much greater amount of DNA, which is associated with histones and other proteins to form chromatin.
   1. Genetic information is carried on many chromosomes in eukaryotes, as compared to one in bacteria.
      a. These chromosomes are also located in the nucleus.
      b. The process of transcription is spatially and temporally separated from the process of translation, and it involves three different classes of RNA polymerases.
      c. Transcripts of eukaryotic genes are processed, cleaved, and realigned before they are transported into the cytoplasm.

d. Finally, the differentiation and changes in gene expression are often influenced by cellular interactions and external signals, i.e. hormones.

2. Transparency 84, page 289, figure 13-8.
   a. Eukaryotic gene expression can occur at many levels, transcriptional level, processing of the pre-mRNA, transport to the cytoplasm, stability of the mRNA, selecting which mRNAs are translated, and posttranslational modification of the protein product.
   b. Most eukaryotic genes are regulated via the transcriptional level.
   c. There are two main components of transcriptional control of gene expression: short DNA sequences that serve as recognition sites, and regulatory proteins that bind to these sites.

V. Regulatory Elements and Eukaryotic Genes

A. The internal structure of eukaryotic genes includes regulatory sequences adjacent to genes that control transcription.
   1. There are two types of these sequences: **promoters** and **enhancers**.
   2. These elements can be on either side of the gene, or at a distance from the gene.
      a. Those that are located adjacent to the structural genes are called *cis* regulators.
      b. Those that are not adjacent to the genes are called *trans* regulators.

B. **Promoters** are nucleotide sequences that serve as the recognition point for RNA polymerase binding.
   1. This region is necessary to initiate transcription.
      a. They are located adjacent to the genes in which they regulate.
      b. They usually consist of several hundred nucleotides.
   2. There is a number of binding protein factors that are required to initiate transcription.
      a. The promoters that recognize RNA polymerase II consist of short modular DNA sequences.
      b. They are usually located within 100 base pairs upstream of the gene.
      c. Promoters consist of a TATA box.
         - The TATA box is located 25 to 30 base pairs upstream form the initial point of transcription.

- It consists of an 8-base-pair sequence composed only of T=A pairs.
- Mutations in the TATA box severely reduce transcription, and deletions often alter the initiation point of transcription.

3. Many promoters contain other components as well.
   a. One of these is called a **CAAT box**.
      - Its consensus sequence is CAAT or CCAAT, and frequently appears in the region of –70 to –80 bases from the start site.
      - Mutations on either side of the CAAT box have little or no effect on transcription, whereas mutations within the CAAT box reduce the rate of transcription dramatically.
   b. A third element is called the GC box, which consists of GGGCGG and is usually found, around the –110 position.

C. **Enhancers** are regions that interact with regulatory proteins and can increase the efficiency of transcription initiation or activate the promoter.
   1. Enhancers appear to be much more complex in structure and function in the eukaryotic cell when compared to the prokaryotic cell.
   2. Enhancers can be distinguished from promoters in many ways.
      a. The position of the enhancer is not necessarily fixed.
      b. It can be inverted without significant effects on its action.
      c. If the enhancer is moved, then the transcription of the adjacent gene is enhanced.
   3. Most of the genes within an eukaryotic organism are under the control of enhancers.
      a. An example of the enhancer located within the gene is the immunoglobulin heavy-chain genes.
      b. In the human β-globin gene, the enhancer is located downstream.
      c. Yeast contain **upstream activator sequences (UAS)**, which are similar to enhancers, but they can function upstream at variable distances, whereas they cannot function downstream.

VI. Transcription Factors and Gene Regulation

A. **Transcription factors** control when, where, and how genes are expressed.

1. These factors are modular structures with at least two functional domains.
   a. One is that it binds to DNA sequences that are present in promoters and enhancers called the **DNA-binding domain**.
   b. The other activates transcription via protein-protein interaction, and is called the **trans-activating domain**.
2. Each domain consists of a specific sequence of amino acids within the protein that is responsible for the binding function.

B. One of the first model systems that was used to study eukaryotic gene regulation involves the set of genes in yeast, that encode for enzymes that are essential for galactose metabolism.
   1. Expression of these genes is regulated by the presence or absence of galactose.
      a. In the absence of galactose, these genes are not transcribed.
      b. If galactose is in the medium, transcription of the genes commences immediately, and the mRNA concentration of these transcripts increases by a thousandfold.
      c. A mutation in one of the genes, *(GAL4)* prevents the activation of the genes in even in the presence of galactose, which indicates that transcription is genetically regulated.
   2. **UAS_Gs** (upstream activating sequence of galactose genes) are DNA sequences that control the transcription of structural genes for galactose metabolism.
      a. The GAL4 protein binds to the UAS_G regions, and activates transcription of the genes for galactose metabolism.
      b. This establishes that the *GAL4* gene encodes for a transcription factor.
   3. The product produced from the *GAL4* gene is a protein of 881 amino acids and has been studied immensely.
      a. Transparency 84, page 291, figure 13-11.
      b. This protein includes a DNA-binding domain that recognizes and binds to sequences in the UAS_Gs; and a *trans*-activating domain, which is essential for the activation of transcription.
      c. Gene activation, in addition to UAS_G binding, requires direct interaction between the activating domain of the transcription factor and other proteins.

4. There are several possibilities on how this interaction activates transcription.
   a. One is from the stabilization of the binding between promoter and the DNA and RNA polymerase.
   b. Another is increasing the rate of which the double-stranded DNA within the transcribed region is unwound.
   c. And a final possibility is that there are attractive and stabilizing of other factors, which bind to the promoter, or to the RNA polymerase.
5. **TFIID** is an attractive candidate for an activator target.
   a. This consists of several proteins, which form a complex, which binds to the TATA sequence of promoters, and it is essential for transcription.
   b. TFIID consists of the **TATA-binding protein (TBP)**, and ten other proteins that are called **TBP-associated factors**, or TAFs.

C. The domains of eukaryotic transcription factors take on several different forms.
1. The **DNA-binding domains** have three-dimensional structural patters called **motifs**.
   a. There are three major types of these structural motifs: **helix-turn-helix (HTH), zinc finger, and leucine zippers**.
   b. It is assumed that other new groups will be established when new factors are characterized.
2. The **helix-turn-helix** was the first DNA-binding domain to be discovered.
   a. HTH motifs have been identified in the *cro* repressor, the *lac* repressor, *trp* repressor, and in other proteins, within prokaryotes.
   b. The HTH motif is present in many prokaryotic DNA-binding proteins.
   c. It has a geometric conformation rather than a distinctive amino acid sequence.
   d. There are two adjacent helices separated by a "turn" of several amino acids, which enables the protein to bind to DNA.
   e. The HTH pattern, unlike sever other DNA-binding motifs, cannot fold or function alone; therefore, it is always part of a larger DNA-binding domain.
3. A large number of eukaryotic genes, known to regulate developmental processes, have the potential for forming helix-turn-helix geometry.
   a. A **homebox** is present almost universally in eukaryotic organisms.

b. This consists of 180 base pairs, which specify a 60-amino-acid **homeodomain** sequence that can form the helix-turn-helix structure.

c. Many of these genes contain a conserved sequence as well.

4. One of the major structural families of eukaryotic transcription factors are the **zinc fingers**.

a. These were discovered in *Xenopus* transcription factor TFIIIA originally.

b. However, these have also been discovered in proto-oncogenes, genes that regulate development in *Drosophila*, in proteins whose synthesis is induced by growth factors and differentiation signals, and in transcription factors.

c. There are several different types of zinc fingers which each consist of a distinctive structural pattern.

5. One of these zinc fingers contains clusters of two cysteine and two histidine residues at repeating intervals.

a. The amino acids fold into loops (zinc fingers) by the covalent bonds between zinc atoms and the interspersed cysteine and histidine residues.

b. Each of these fingers consists of 23 amino acids and contains a loop with of 12 to 14 amino acids between the Cys and the His residues, as well as a linker between the loops, which consists of 7 or 8 amino acids.

c. The amino acids located in loop bind to specific DNA sequences.

d. Zinc fingers bind in the major groove of DNA, wrap at least partly around the DNA, and makes contact with a set of DNA bases which may then contain hydrogen bonds.

e. The number of fingers within a zinc finger transcription factor varies from 2 to 13, as does the length of the DNA sequence.

6. The **leucine zipper** represents the third type of domain.

a. This was first seen as a stretch of 35 amino acids in a nuclear protein in rat liver, with four leucine residues spaced 7 amino acids apart.

b. The regions form a helix with leucine residues protruding from every other turn.

c. When two of these structures dimerize, the leucine residues "zip" together.

- This dimer contains two alpha-helical regions adjacent to the zipper, which bind to the phosphate residues and specific bases of DNA.
- This results in the dimer looking like a pair of scissors.

7. Transcription factors contain domains that activate transcription as well.
   a. These regions can occupy from 30 to 100 amino acids and are distinct from the DNA-binding domains.
   b. These stretches of amino acids also interact with other transcription factors, or directly with RNA polymerase.
8. Overall, the transcriptional regulation in eukaryotes is somewhat complex, but there are a number of generalizations that can be made.
   a. The structural organization of chromatin and alterations in chromatic structure allow for the binding of transcription factors, which are considered to be the primary levels of regulation.
   b. The regulation of transcription by protein factors is largely positive.
   c. The binding of one or more factors at promoter regions is a prerequisite to transcriptional activation of a locus.
   d. Finally, promoter and enhancer sequences are recognized and bound by transcription factors.

D. Studies suggest that there are a series of transcriptional factors that are assembled at the promoter in a specific order.
   1. The TFIID complex binds to the TATA promoter via its TATA-binding protein to initiate formation of the apparatus.
      a. There are approximately 20 base pairs of DNA that are involved in the binding of the TBP, then other subunits bind to the growing complex.
      b. Conformational changes that expedite the binding of other transcription factors and RNA polymerase occur due to the contact between TFIID and activator proteins.
   2. Eukaryotic polymerases bind to transcription factor proteins that are in turn bound to the DNA promoter region.
      a. Transparency 85, page 294, figure 13-16.

b. Transcription of the DNA is then downstream and may ensue at a minimal basal level.
c. The final stage involves the achievement of the induced state, which is where transcription is stimulated above the basal level.
d. Factors bound to enhancers at a distance from the site are thought to interact with the transcription complex, resulting in a looping out of the DNA, which separates the enhancer from the complex.

VII. Genomic Alterations and Gene Expression: DNA Methylation

A. The chromatin conformation alteration is one of several methods in which gene expression can be regulated.
   1. One way to achieve a change in chromatin, is by the addition or deletion of methyl groups to the bases of DNA.
      a. DNA of most eukaryotic organisms is modified after replication by the enzyme-mediated addition of methyl groups to bases and sugars.
      b. **DNA methylation** most often involves cytosine.
      c. Approximately five percent of cytosine residues are methylated in the genome of any eukaryotic organism.
   2. Studies on the *lac* operon in *E. coli* have illustrated the ability of methylation to alter gene expression.
      a. The methylation of DNA in the operator region can cause a significant change in the affinity of the repressor for the operator.
      b. The methylation occurs at the 5' position on the cytosine.
      c. This causes the methyl group to protrude into the major groove of the DNA, where it is able to alter the binding of proteins to the DNA.
B. Methylation occurs most often in the cytosine of CG doublets in DNA.
C. If a gene is expressed, it is assumed that there is no methylation, or that there is very little.
D. The role of methylation as a factor in the regulation of eukaryotic gene expression is based mainly on observations.
   1. An inverse relationship exists between the degree of expression and the degree of methylation.

a. If there is a high degree of methylation, then the expression is low; and vice versa.
b. In mammalian females the inactivated X chromosome is highly methylated, while being almost totally inactive.

2. Second, the methylation patterns are tissue-specific and are heritable for all cells within that tissue.
   a. Some of the strongest evidence comes from studies utilizing base analogs.
   b. The nucleotide 5'-azacytidine is incorporated into DNA in place of cytidine, but cannot be methylated.
   c. This causes undermethylation, resulting in changes in the pattern of gene expression, and possible stimulation for expression of alleles on inactivated X chromosomes.

E. Methylation is not a general phenomenon in eukaryotes; for instance, there is no methylation in *Drosophila*.

VIII. Gene Regulation by Steroid Hormones

A. **Steroid hormones** are used to regulate growth and development, as well as to maintain homeostasis.
B. The major sex hormones are all steroids, as is vitamin D, and the homeostatic adrenal hormones that regulate glucose metabolism and mineral utilization.
C. Transparency 86, page 296, figure 13-18.
   1. This is the general scheme for hormone action.
   2. The hormone enters the cell by passing through the plasma membrane.
   3. It then binds to a specific **hormone receptor protein**, which is located in the cytoplasm.
   4. The receptor-hormone complex is then translocated into the nucleus and activates transcription of one or more specific genes.
D. All receptors have three functional domains.
   1. A variable N-terminus domain, which is unique to each receptor; a short, highly conserved central domain that binds to DNA; and a C-terminus domain that binds to the hormone.

2. The DNA-binding central domain contains two zinc fingers, which bind to specific DNA sequences known as **hormone responsive elements (HREs)**.
   a. HREs are composed of short consensus sequences that are related but not always identical.
   b. They are often located several hundred bases upstream from the transcription start site.
   c. They may be present in many copies.
   d. They are often associated with promoter or enhancer sequences.
3. The HRE is necessary for activation of a specific gene, but it may not be sufficient to cause activation on its own.
   a. It may serve simply to facilitate the interaction of other transcription factors by altering the chromatin structure in the HRE and adjacent regions.
   b. It may make other binding sites available to bind to transcription factors and RNA polymerase II, resulting in the initiation of transcription.

IX. Postranscriptional Regulation of Gene Expression

A. **Post-transcriptional modes of regulation**, are possibilities for regulation following the process of transcription.
B. In eukaryotes for example, there is an addition of a 5'-cap and a poly-A tail at the 3'-end.
C. Noncoding introns are removed and the remaining exons are spliced together.

Lecture Outline

Chapter Fourteen: Proteins: The End Product of Genes

I. Garrod and Bateson: Inborn Errors of Metabolism

A. Role of proteins in genetic processes

1. **Alkaptonuria**

a. **Homogentisic acid**

1. Excreted through urine
2. Oxidation product is black
3. Darkening of the nose and ears
4. Arthritic condition

b. Garrod's experiment

2. Inherited disorders and alternative modes of metabolism
3. Bateson

B. **Phenylketonuria (PKU)**

1. Phenylalanine

a. Differ by a hydroxyl group

b. **Phenylalanine hydroxylase**

c. Normal blood level

2. Accumulation of phenylalanine

a. Less efficiently reabsorbed by kidney

b. Enter the cerebrospinal fluid

c. Mental retardation

3. Newborn screening

C. Thousands of abnormal physiological conditions caused by errors in metabolism

II. The One-Gene:One-Enzyme Hypothesis

A. Genes responsible for synthesis of enzymes

1. George Beadle
2. Boris Ephrussi
3. Joining of Beadle and Edward Tatum

B. *Neurospora crassa*

1. Characteristic of this organism

a. Mutations introduced and isolated with ease
b. Much already known about its biochemistry
c. Genetic blocks of reactions essential for the growth

2. Manufactures almost everything needed
   a. Transparency 87, page 306, figure 14-2
   b. Irradiated asexual spores with X-rays
   c. Sexual cycle
   d. Placed on a "complete" medium
   e. Then on a "minimal" medium
      1. Growth occurred
      2. No growth occurred
3. Thousands of spores
   a. Many failed to grow on minimal medium
   b. Mutant type
      - Strains placed on different mediums
      - Individual components
      - Supplement that restores growth
4. The first and second mutant strains
5. Over 80,000 spores
   a. Genetics and biochemical events
   b. Loss of the enzymatic activity
   c. Enzymatically controlled reactions
   d. **One-gene: one-enzyme hypothesis**

III. Genes and Enzymes: Analysis of Biochemical Pathways

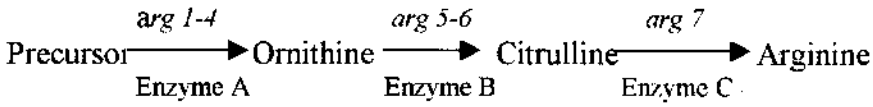
A. First metabolic pathway investigated
   1. Adrian Srb and Norman Horowitz
      a. Required arginine for growth
      b. Partial biochemical pathway
      c. Biochemical information
   2. **Citrulline** or **ornithine** added to medium
      a. Very similar compounds

b. *arg 1-4*

c. *arg 5-6*

d. *arg7*

e. Pathway for arginine



3. Reasoning

IV. One-Gene:One Protein/ One-Gene:One-Polypeptide

A. Two factors that modified the one-gene:one-enzyme hypothesis

1. Not all proteins are enzymes

a. Specification of proteins

b. **One-gene:one-protein**

2. **Polypeptide chains**

a. Quaternary structure

b. **One-gene:one-polypeptide chain**

3. Need for the modification

B. **Sickle-cell anemia**

1. Polymerization of hemoglobin

a. Figure 14-4 in the text

b. Attacks

c. Tissues and organs deprived of oxygen

d. Sickle-cell crisis

2. Anemic

a. Destruction of red blood cells

b. Compensatory mechanisms

c. Abnormal bone size and dilation of heart

3. Mendelian inheritance

a. Three different genotypes possible

b. Homozygous genotypes, Hb^A / Hb^A and Hb^S / Hb^S

c. Hb^A / Hb^S = **sickle-cell trait**

1. Less sickling

2. Carriers of disorder

4. Molecular basis of sickle-cell anemia
   a. Electrophoretic migration
   b. Rate of migration varies
   c. **HbA** and **HbS**
5. **Starch gel electrophoresis**
   a. Supporting medium
   b. Samples placed between cathode (-) and anode (+)
   c. All moved towards anode
   d. HbA moved farther than HbS
   e. Heterozygotes
6. Two possibilities
   a. **Hemoglobin**
      1. **Heme group**
      2. **Globin portion**
   b. α and β chains
   c. Alteration in net charge
7. Vernon Ingram
   a. Chemical change in the primary structure
   b. **Fingerprinting techniques**
8. Enzymatic digestion of protein
   a. Placed on paper and exposed to electric field
   b. Another migration in a second direction
   c. Results of spots, or "fingerprints"
   d. A single peptide fragment difference
   e. Due to single amino acid change
9. Significance
   a. Single gene, single polypeptide chain
   b. Mutation affects phenotype
   c. **Molecular disease**
10. Within the United States

C. Variety of hemoglobin molecules
   1. All are tetramers
      a. HbA
      b. 98% of hemoglobin
      c. **HbA_2**
         1. Alpha (α) chains
         2. **Delta (δ) chains**
   2. Embryonic and fetal development
      a. **Gower 1**
         1. **Zeta (ζ) chains**
         2. **Epsilon (ε) chains**
      b. Eight weeks of gestation
         1. Alpha chains
         2. **Gamma (γ) chains**.
   3. **HbF,** or **fetal hemoglobin**
   4. Figure 14.1

V. Colinearity

A. Transferring of nucleotide sequence
   1. **Colinear relationship**
   2. Direct correlation
B. **Tryptophan synthetase** in *E. coli*
   1. Many mutants
   2. Mapped mutations
   3. Colinear relationship detected
   4. Figure 14-6 in the text

VI. Protein Structure and Function

A. Polypeptides and proteins
   1. Polypeptides
      a. Assembly on the ribosome
      b. Higher order of structure
      c. Three-dimensional structure

d. Final conformation

2. Linear nonbranched polymers

a. Transparency 88, page 311, figure 14-7

b. Building blocks

c. Amino acids

1. **Carboxyl group**

2. **Amino group**

3. **R (radical) group**

4. **Hydrogen atom**

d. **Central carbon atom**

e. R group

f. Four classes

1. **Nonpolar (hydrophobic)**

2. **Polar (hydrophilic)**

3. **Negatively charged (acidic)**

4. **Positively charged (basic)**

3. Reactions of amino acids

a. Transparency 89, page 312, figure 14-8

b. **Peptide bond**

c. Dipeptide

d. Free amino group at **N-terminus** and free carboxyl group at **C-terminus**

B. Levels of **protein structure**

1. **Primary structure (I°)**

2. **Secondary structure (II°)**

a. Alpha helix

b. Rodlike

c. Spiral chain of amino acids

d. R groups extend outward

e. Another form for secondary structure

1. **β-pleated-sheet configuration**

2. Folding back on itself

3. Stabilized by hydrogen bonds

f. Mixture

1. Globular proteins

2. Rigid proteins

3. **Tertiary structure (III°)**

a. Specific structure

b. Three aspects for stabilization

c. Direct result of I° structure

d. Stabilizing factors

e. Most stable structure forms

f. **Myoglobin** shown in figure 14-10

4. **Quaternary level of organization (IV°)**

a. **Oligomeric**

b. **Promoter**

c. Promoters fit together

d. Hemoglobin

C. Final conformations

1. Spontaneous process

a. Properties of amino acid sequence

b. **Chaperones**.

2. Chaperones function

3. First discovered in the 1970s

a. **Heat-shock proteins**

b. Have been found in many organisms

c. Minimize denaturation of proteins

4. Present in cells that are not heat-shocked

a. Required

b. Bind to and stabilize polypeptide chains

5. Other chaperones

6. Another type of chaperone

D. **Posttranscriptional modification**

1. Examples
    a. N-terminus and C-terminus modified
    b. Amino acid residues modified
    c. Carbohydrate side chains attached
    d. Polypeptide chains are trimmed
    e. Signal sequences removed
    f. Metals incorporated
2. Essential in achieving final conformation

E. Proteins
1. Diverse roles
    a. Hemoglobin and myoglobin
    b. **Collagen** and **keratin**
    c. **Actin** and **myosin**
    d. **Immunoglobulin**
2. **Enzymes**
    a. Catalyzing biological reactions
    b. Biological catalysis
        1. **Energy of activation**
        2. Elevating the temperature
3. Catalytic properties and specificity
    a. **Active site**
    b. **Catabolic** or **anabolic**
    c. **Amphibolic**

Lecture Content

Chapter Fourteen: Proteins: The End Product of Genes

I. Garrod and Bateson: Inborn Errors of Metabolism

A. Sir Archibald Garrod and William Bateson made the first insights into the role of proteins in genetic processes.

1. Garrod studied albinism and cystinuria extensively, but what he is best known for is his work with the disorder **alkaptonuria**.
   a. This disorder is associated with the inability to metabolize the alkapton 2,5-dihydroxyphenlyacetic acid, known as **homogentisic acid**.
      - Homogentisic acid accumulates in the cells and tissues and is excreted through the urine.
      - The oxidation product of this molecule is black; it is therefore, easy to recognize in infant's diapers and urine samples.
      - These products accumulate in the cartilaginous areas, which causes darkening of the nose and ears.
      - It can lead to an arthritic condition because it can accumulate in the joints.
   b. Garrod studied this disorder by increasing the dietary protein or adding amino acids, phenylalanine or tyrosine to the diet.
      - Homogentisic acid increased in the urine of affected individuals, but not in that of unaffected individuals.
      - Garrod concluded that normal individuals break down, or catabolize, alkapton, whereas affected individuals cannot.
      - After further studies, Garrod discovered that alkaptonuria is inherited as a simple recessive trait.
2. Garrod suggested that hereditary information controlled the chemical reactions of the body and that inherited disorders, those that he studied, resulted from alternative modes of metabolism.
3. Bateson agreed with Garrod's findings, which went together with his belief that inherited conditions were caused by a lack of some critical substance.

B. **Phenylketonuria (PKU)** was first described in 1934, and is a disorder that may result in mental retardation and is inherited as an autosomal recessive disease.

1. Affected individuals are unable to convert the amino acid phenylalanine to the amino acid tyrosine.
   a. These two amino acids differ only by a hydroxyl group (-OH).
   b. The reaction is catalyzed by **phenylalanine hydroxylase**, which is inactive for affected individuals, and active at a 30% level in heterozygotes.
   c. The normal blood level of phenylalanine is about 1 mg/100 ml, whereas in phenlyketonurics, the level is as high 50 mg/100 ml.
2. When phenylalanine accumulates, it is sometimes converted into phenylpyruvic acid and other derivatives.
   a. These are less efficiently reabsorbed by the kidney and spill into the urine at a faster rate than phenylalanine.
   b. These derivatives, along with phenylalanine, enter the cerebrospinal fluid, which results in increased levels in the brain.
   c. The elevated levels, when present, during early developmental stages, are thought to cause mental retardation.
3. Newborn screening is performed routinely in hospitals.
   a. If a newborn is found to have this disorder, he/she is placed on a strict diet.
   b. The low-phenylalanine diet can reduce some of the by-products as phenylpyruvic acid, and abnormalities characterizing the disease can be diminished.
   c. Phenylketonuria occurs in approximately 1 in every 11,000 births.

C. It is now known that there are literally thousands of abnormal physiological conditions caused by errors in metabolism, which are the results of mutant genes.

II. The One-Gene:One-Enzyme Hypothesis

A. There were two separate investigations that led to the first convincing experimental evidence that genes are directly responsible for the synthesis of enzymes.
   1. George Beadle was the first to suggest this in 1933.
   2. The first investigation performed in collaboration with Boris Ephrussi involved *Drosophila* eye pigments.
   3. This encouraged the joining of Beadle and Edward Tatum to investigate nutritional mutations in the pink bread mold *Neurospora crassa*.

B. Beadle and Tatum began their work with *Neurospora crassa* in the 1940s.
   1. There were many reasons why they chose this organism to work with.
      a. One was that mutations could be introduced and isolated with ease.
      b. Another reason was that there was much already known about its biochemistry.
      c. Beadle and Tatum produced strains that had genetic blocks of reactions that were essential for the growth of the organism.
   2. *Neurospora* can manufacture almost everything needed for normal development.
      a. Transparency 87, page 306, figure 14-2.
      b. Beadle and Tatum irradiated asexual spores with X-rays, in order to increase the frequency of mutations.
      c. The organisms were allowed to progress through the sexual cycle, which results in haploid spores.
      d. These spores were placed on a "complete" medium, meaning everything needed for growth was present, and they were allowed to grow.
      e. These organisms were then transferred to a "minimal" medium and checked for production.
         - If growth occurred, the organism was able to produce the essential products for growth, and therefore did not contain a mutation.
         - If no growth occurred, the organism was unable to produce some essential products, and therefore did contain a mutation.
   3. There were thousands of these individual spores isolated and grown on a complete medium.
      a. Of these, Beadle and Tatum discovered that many cultures failed to grow on the minimal medium, meaning that a nutritional mutation had been induced.
      b. They then wanted to determine the mutant type.
         - The mutant strains were placed on different mediums containing different vitamins, amino acids, purines, or pyrimidines until a supplement that permitted growth was determined.
         - Once the general complement was determined, Beadle and Tatum tested to see what the individual components were.

- They suggested that the specific supplement that restores growth is the molecule that the mutant strain could not synthesize.

4. The first mutant strain produced by Beadle and Tatum required vitamin B-6 in the medium, and the second one required B-1 for growth.
5. Beadle and Tatum tested over 80,000 spores this way.
   a. Their results suggested to them that genetics and biochemical events have something in common.
   b. Each mutation was caused by a loss, which resulted in the loss of the enzymatic activity to facilitate the essential reaction needed for the particular nutritional requirement.
   c. Beadle and Tatum concluded also that a mutation could be found for nearly any enzymatically controlled reaction.
   d. Thus, they derived the **one-gene: one-enzyme hypothesis**, which means that one gene specifies one enzyme.

III. Genes and Enzymes: Analysis of Biochemical Pathways

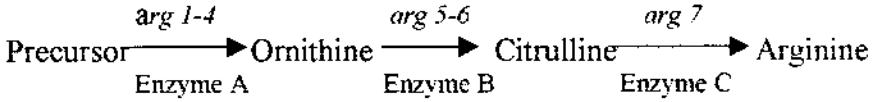
A. The synthesis of the amino acid arginine in *Neurospora* was one of the first metabolic pathways investigated in detail.
   1. Adrian Srb and Norman Horowitz studied several mutant strains of *Neurospora*.
      a. Each of the strains required arginine for growth; therefore they were *Arg*$^-$.
      b. From their studies, they were able to determine a partial biochemical pathway leading to the synthesis of this molecule.
      c. Their work illustrated how genetic analysis could be used to determine biochemical information.
   2. Srb and Horowitz tested the mutant strain's ability to reestablish growth when either **citrulline** or **ornithine** was added to the growth medium.
      a. These two compounds are very similar, chemically speaking, to arginine.
         - They reasoned that if the mutant strains could grow with either of these supplements, then it must be involved in the biosynthetic pathway of arginine.
         - They found that both molecules could be substituted in one or more strains.

b. Four of the mutant strains (*arg 1-4)* could grow if they were supplied with citrulline, ornithine, or arginine.

c. Two of the strains (*arg 5-6)* would grow only if they were supplied with citrulline or arginine.

d. One strain (*arg-7)* would only grow if arginine were supplied.

e. From these results, they deduced the following pathway for arginine.



3. The following, are the reasons that support this conclusion.

a. If mutants *arg 4-7* can grow regardless of which three molecules are supplied, the mutation must be a metabolic block that occurs prior to the involvement of ornithine, citrulline, or arginine.

- When any of these molecules are added, the block is bypassed.
- It can then be concluded that citrulline and ornithine are involved in the synthesis of arginine.

b. The mutants *arg 2* and *3*, grew when supplied with citrulline, but not when ornithine was the only supplement supplied.

- Thus, ornithine must occur prior to the block, and its presence will not overcome the block.
- Citrulline, however, does overcome the bock, and therefore must be involved beyond the point of blockage.
- The conclusion can then be made that the conversion of ornithine to citrulline represents the correct pathway.

c. It can finally be concluded that the mutant *arg 1* represents a mutation that prevents the conversion of citrulline to arginine.

- In this case, neither citrulline nor ornithine can overcome the metabolic block because they both participate in the pathway at an earlier time.
- From these results, a pathway can be formed for the synthesis of arginine.

d. The metabolic pathway is shown in figure 14-3 in the textbook.

IV. One-Gene:One Protein/ One-Gene:One-Polypeptide

A. There were two factors that soon modified the one-gene:one-enzyme hypothesis.

1. The first one was although all enzymes are proteins; not all proteins are enzymes.
   a. All proteins are specified by the information stored in genes.
   b. This lead to the revision of one-gene:one-enzyme, to **one-gene:one-protein**.
2. Secondly, proteins were shown to consist of a subunit structure of one or more **polypeptide chains**.
   a. This is the basis for quaternary structure of proteins.
   b. Each polypeptide sequence is encoded by a separate gene, a more modern statement is **one-gene:one-polypeptide chain**.
3. The need for the modification of the original hypothesis came with the studies performed on the hemoglobin structure of individuals with sickle-cell anemia.

B. **Sickle-cell anemia** is a disorder associated with the shape of the red blood cells.
   1. Individuals affected with this disorder contain erythrocytes, which under low oxygen conditions, become elongated and curved due to the polymerization of hemoglobin.
      a. Look at figure 14-4 in the text for a comparison of normal and sickle cell erythrocytes.
      b. Those afflicted with the disease, suffer attacks when the red blood cells aggregate on the venous side of the capillary systems, where oxygen tension is very low.
      c. A variety of tissues and organs may be deprived of oxygen as a result of this condition.
      d. This is called a sickle-cell crisis.
         - If this type of crisis goes untreated, it can be fatal.
         - If treatment is received, then the individual could avoid damage to tissues.
   2. Affected individuals are anemic as well.
      a. This is due to the fact that their red blood cells are destroyed more rapidly than normal.
      b. There are compensatory mechanisms for this, which include increased cell production by bone marrow and accentuated heart action.

c. These mechanisms lead to an abnormal bone size, as well as dilation of the heart.

3. The inheritance of this trait as a Mendelian inheritance, was first demonstrated by James Neel and E.A. Beet in 1949.
   a. Pedigree analysis revealed that there were three different genotypes possible, and that phenotypes were controlled by a single pair of alleles, Hb^A *and* Hb^S.
   b. Normal and affected individual were produced from the homozygous genotypes, Hb^A / Hb^A and Hb^S / Hb^S respectively.
   c. The heterozygote, Hb^A / Hb^S, exhibits **sickle-cell trait**, but not the disease.
      - This individual's red blood cells undergo much less sickling, since half of their hemoglobin is normal.
      - They are less affected than the homozygous individual, but they are carriers of the disorder.
4. Linus Pauling and his coworkers provided the first insight into the molecular basis of sickle-cell anemia in the same year.
   a. They demonstrated that isolated hemolglobins from diseased and normal individuals differed in their rates of electrophoretic migration.
   b. Charged molecules migrate in an electric field, and if the net charge of two molecules is different, the rate of migration will vary.
   c. Pauling and his colleagues concluded that there was difference between the two types of hemoglobin, which are now designated as **HbA** and **HbS**.
5. **Starch gel electrophoresis** of patterns of migration for hemoglobin derived from individuals with all three genotypes, is illustrated in figure 14-5 of the text.
   a. The gel is the supporting medium for the molecules in this experiment.
   b. Samples are placed at a point of origin between the cathode (-) and the anode (+), and an electrical field is applied.
   c. Since all molecules moved towards the anode, the conclusion can be made that the molecules contain a negative charge.
   d. HbA moved farther than HbS, revealing that HbA has a larger net negative charge than that of HbS.
   e. The heterozygotes revealed the presence of both HbA and HbS.

6. Pauling suggested two different possibilities associated with hemoglobin.
   a. It was known that **hemoglobin** consists of four nonproteinaceous iron-containing groups and a group that contains four polypeptide chains.
      - A **heme group** is the group that contains iron.
      - A **globin portion** is the name given to the group with four polypeptide chains.
   b. In the quaternary structure of adult human hemoglobin, there are two identical α chains, which contain 141 amino acids, and two identical β chains of 146 amino acids.
   c. The alteration in the net charge could be due to a chemical change in either the heme or the globin portion of the molecule.
7. Work done by Vernon Ingram between 1954 and 1957, answered Pauling's question.
   a. Ingram demonstrated that a chemical change occurred in the primary structure of the globin portion of the molecule.
   b. He used the **fingerprinting technique** to show that HbS differs in amino acid composition when compared to HbA.
8. The fingerprinting technique first involves the enzymatic digestion of the protein into peptide fragments.
   a. Then, the mixture is placed on paper while being exposed to an electric field, which force migration according to the net charge.
   b. The paper is turned at a right angle, and then is placed in a solvent, where chromatographic action forces another migration in a second direction.
   c. The result is a two-dimensional separation of the peptide fragments into a distinctive pattern of spots, or "fingerprints."
   d. This illustrated only a single peptide fragment difference between HbA and HbS.
   e. Further analysis proved that the peptide fragment difference was due to a single amino acid change: Valine was substituted for glutamic acid, at position six of the β chain.
9. This discovery has been very significant to modern day genetics.

a. It clearly established that a single gene provides the genetic information for a single polypeptide chain.
b. Also, that a mutation can affect the phenotype by directing only a single amino acid substitution.
c. As well as provided an explanation for the concept of **molecular disease**.

10. Sickle-cell anemia is found almost exclusively in blacks, within the United States.
    a. It affects one in every 625 black infants born here.
    b. There are approximately 50,000 to 75,000 individuals afflicted in the United States at this time.

C. There are a variety of hemoglobin molecules produced in humans.
1. All of these types are tetramers, which consist of seven distinct polypeptide chains in different combinations; each are encoded by a separate gene.
    a. HbA contains two alpha (α) and two beta (β) chains.
    b. HbA represents approximately 98% of hemoglobin found in individuals after the age of 6 months.
    c. The remaining 2% consist of **HbA_2**, a minor adult component.
        - This molecule consists of two alpha chains and two **delta (δ) chains**.
        - The delta chains are similar to the beta chains, in that they are made up of 146 amino acids; the difference is in the amino acid sequence.
2. During embryonic and fetal development, there is a different set of hemoglobins present.
    a. **Gower 1** is the earliest to develop.
        - This contains two **zeta (ζ) chains**, which are alphalike.
        - As well as two **epsilon (ε) chains**, which are betalike.
    b. This form is gradually replaced with different chains by eight weeks of gestation.
        - This form contains two alpha chains.
        - And two **gamma (γ) chains**.
            - The gamma chains are nearly identical, and are therefore designated as $^{G}\gamma$ and $^{A}\gamma$.

- They are both betalike, and differ by only one amino acid.
- Together, this molecule is called **HbF,** or **fetal hemoglobin**.

3. Figure 14.1 summarized the nomenclature and sequence of appearance for these five tetramers.
   a. The genes that encode for the seven different chains have been mapped.
   b. The genes that code for the α and γ are located on chromosome 6, and the genes that code for ε, $^{G}\gamma$, $^{A}\gamma$, δ, and β are located on chromosome 11.

V. Colinearity

A. It was then wondered how the nucleotide sequence of a gene could be transferred to the amino acid sequence of a polypeptide chain.
   1. It was suggested that a **colinear relationship** existed between the nucleotide sequence and the amino acid sequence.
   2. This means that the order of nucleotides in the DNA of a gene correlates directly with the order of amino acids in the corresponding polypeptide chain.

B. Charles Yanofsky performed studies that involved the A subunit of the enzyme **tryptophan synthetase** in *E. coli*.
   1. He isolated many mutants that had lost the activity of this enzyme.
   2. He was then able to map the mutations and establish the location of them with respect to one another within the gene.
   3. When two sets of data were compared, the colinear relationship could be detected.
   4. Figure 14-6 in the text illustrates his studies.

VI. Protein Structure and Function

A. Polypeptides and proteins are both composed of amino acids, but they differ in their state of assembly and function capacity.
   1. Polypeptides are precursors to proteins.
      a. The sequence of amino acids is termed a polypeptide chain during the assembly on the ribosome.
      b. Once it is released, the polypeptide folds up and assumes a higher order of structure.

c. This results in a three-dimensional structure, which is essential to the function of the molecule.

d. At the time when the final conformation is achieved, the molecule is appropriately called a protein.

2. Polypeptide chains of proteins consist of linear nonbranched polymers.

a. Transparency 88, page 311, figure 14-7.

b. The building blocks for the proteins, are the twenty amino acids.

c. Each amino acid consists of:

- A **carboxyl group**.
- An **amino group**.
- An **R (radical) group**, also known as a side chain.
- And a **hydrogen atom**.

d. The hydrogen atom is covalently bonded to the **central carbon atom**, referred to as the α carbon atom.

e. The R group is what provides the different chemical identities for each amino acid.

f. The twenty amino acids are broken into four classes:

- A **nonpolar (hydrophobic)** class.
- A **polar (hydrophilic)** class.
- A **negatively charged (acidic)** class.
- And a **positively charged (basic)** class.

3. Around the year 1900, Emil Fischer showed that the amino group of one amino acid could react with the amino group of another amino acid during a dehydration reaction.

a. Transparency 89, page 312, figure 14-8.

b. The dehydration reaction releases a molecule of H_2O, and results in a covalent bond known as a **peptide bond**.

c. A dipeptide is two amino acids linked together; a tripeptide is three amino acids linked together, and so on...

d. All polypeptide chain will always have a free amino group at one end (the **N-terminus**) and a free carboxyl group at the other end (the **C-terminus**).

B. There are four levels of **protein structure** recognized.

1. The **primary structure (I°)**, which is the sequence of amino acids in the linear backbone of the polypeptide.
   a. This sequence is specified by the DNA via a mRNA intermediate.
   b. The primary structure determines the specific characteristics of the higher orders of structure for the protein.
2. The **secondary structure (II°)** is a regular or repeating conformation in space assumed by the amino acids aligned closely to each other in the polypeptide chain.
   a. An alpha helix is one of type of the secondary structure.
   b. It is rodlike, and presents the greatest possible theoretical stability.
   c. The helix is composed of a spiral chain of amino acids that is stabilized through hydrogen bonds.
   d. The R groups extend outward from the helix in the secondary structure.
   e. In 1951, Pauling and Corey propose yet another form for secondary structure in proteins.
      - They called this structure the **β-pleated-sheet configuration**.
      - This occurs when a single polypeptide folds back on itself, or when several chains run next to each other.
      - The structure is stabilized by hydrogen bonds, which form between atoms present on adjacent chains.
   f. In fact, most proteins exhibit a mixture of the α-helical structure and the β-pleated-sheet structure.
      - Globular proteins contain a core of β-pleated-sheets, as well as many areas demonstrating α-helical structure.
      - The more rigid proteins rely on more on the β-pleated-sheet configuration for their structure.
3. The **tertiary structure (III°)** defines the three-dimensional conformation of the entire chain in space.
   a. This molecule twists, turns, and loops around itself to form a very specific structure characteristic of the corresponding protein.

b. There are three aspects of the tertiary structure that are important in determining the conformation and the stabilization of the molecule.
   - The unique amino acid cystine is formed by the covalent disulfide bonds between the closely aligned cysteine residues.
   - Located on the surface, are nearly all of the polar R groups, where they can interact with water.
   - Located on the inside of the molecule, are the hydrophobic R groups, where they can interact with each other and avoid interaction with water.

c. The III° structure is the direct result of the I° structure of the polypeptide.

d. The stabilizing factors are dependent upon the location of the amino acids relative to each other in the chain.

e. The most thermodynamically stable structure possible will form.

f. An example is the respiratory pigment **myoglobin** shown in figure 14-10.

4. The **quaternary level of organization (IV°)** represents the conformation of the associated polypeptide chains in relation to one another.
   a. This type of protein is called **oligomeric**.
   b. Each chain is called a **promoter**.
   c. The promoters fit together in a very specific complementary fashion.
   d. Hemoglobin and many enzymes, including DNA and RNA polymerase demonstrate IV° structure.

C. Many scientists soon began to wonder how polypeptide chains folded into their final conformation.
   1. It was first thought that the final conformation was a spontaneous process.
      a. This would be where the molecule achieved the maximum thermodynamic stability, based largely on the properties inherent in the amino acid sequence.
      b. However, there have been many studies that suggest that folding is dependent upon members of a family of still other ubiquitous proteins called **chaperones**.
   2. Chaperones function to facilitate the folding of other proteins.
      a. The exact mechanism is not known, but it is known that the bonds are not covalently linked.

b. The chaperones also do not become part of the final product.

3. Chaperones were first discovered in the 1970s.
   a. At that time, they were called **heat-shock proteins** because they were found to be induced by *Drosophila* following exposure to elevated temperatures.
   b. Since then, they have been found in many organisms including bacteria, animals, and plants, and are produced excessively in response to heat shock.
   c. It is thought that they might help to minimize the denaturation of proteins caused by heating.
4. Chaperones are also present in cells that are not heat-shocked.
   a. They seem to be required for normal assembly of proteins.
   b. They bind to and stabilize polypeptide chains in a conformation that is essential for subsequent folding.
5. Other chaperones are though to bind to proteins that must partially unwind in order to be transported across intracellular membranes.
6. Still another type of chaperone is known to function to maintain histone proteins in a conformation that is accessible to interact with DNA during assembly of nucleosomes.

D. It is important to note that **posttranscriptional modification** occurs in chaperones, similar to mRNA.
   1. There are several examples of posttranscriptional modification that have been identified.
      a. The N-terminus and C-terminus amino acids are usually removed or modified.
      b. The individual amino acid residues are sometimes modified.
      c. Sometimes, carbohydrate side chains are attached.
      d. Polypeptide chains are sometimes trimmed.
      e. Signal sequences are removed.
      f. Metals are often incorporated with the polypeptide chains.
   2. These modifications are essential in achieving the final conformation and status of proteins.

E. Proteins are the end products of genes, and they are the most abundant macromolecule found within a cell.

1. Different proteins play very diverse roles within the system.
   a. Hemoglobin and myoglobin are associated with the respiratory system and function to transport oxygen.
   b. **Collagen** and **keratin** are structural proteins that are associated with the skin, connective tissue, and hair of vertebrates.
   c. **Actin** and **myosin** are contractile proteins found in muscle tissue.
   d. **Immunoglobulin** functions in the immune system, and many more.
2. **Enzymes** are the largest group of proteins with a related function.
   a. They specialize in catalyzing biological reactions, by increasing the rate at which the reaction reaches equilibrium, without altering the end point of the reaction.
   b. Biological catalysis is a process in which the energy of activation is lowered.
      - The **energy of activation** is the increased kinetic energy state that molecules must usually reach before they can react with one another.
      - This state can be reached by elevating the temperature, but enzymes allow biological reactions to occur at a lower temperature.
3. A molecule's active site's chemical configuration determines the catalytic properties and specificity of an enzyme.
   a. The **active site** is associated with a crevice, a cleft, or a pit on the surface of the enzyme, which binds to reactants or substrates that facilitate their reaction.
   b. Each reaction is either **catabolic** or **anabolic**.
      - Catabolic is when larger molecules are degraded into smaller ones, and there is a release of chemical energy.
      - Anabolic is a synthetic phase of metabolism, which yields nucleic acids, proteins, lipids, and carbohydrates.
   c. A reaction that exhibits both catabolism and anabolism is said to be **amphibolic**.

Lecture Outline

Chapter Fifteen: DNA-Mutation, Repair, and Transposable Elements

I. Four Characteristics for Genetic Information

A. Four characteristics

B. Mutations

1. Genetic information
2. **Gene mutations**

II. Classification of Mutations

A. Classifications of mutations

1. **Spontaneous mutation**
   a. No specific agents
   b. Random sites
   c. Normal chemical processes
   d. DNA replication
2. **Induced mutation**
   a. Herman J. Muller
   b. Lewis H. Stadler

B. Somatic or gametic cells

1. Somatic cells (recessive)
   a. Dominant counterpart
   b. Dominant or X-linked
   c. Early developmental stages
   d. Cancer cells
2. Germ line
   a. Passed on to offspring
      1. **Dominant autosomal mutations**
      2. **X-linked recessive mutations**
   b. **Autosomal recessive mutations**

C. Classification by effect

1. **Morphological trait**
2. **Biochemical** or **nutritional mutations**

3. **Behavioral mutation**
4. **Regulatory mutation**
5. **Lethal mutations**
6. **Conditional mutation**
   a. May not be apparent
   b. **Temperature-sensitive mutation**

III. Detection of Mutation

A. Best study groups for detecting mutations
B. *Neurospora*
   1. Haploid during vegetative phase
   2. Wild-type *Neurospora*
      a. **Minimal medium**
      b. **Prototrophs**
   3. Induced mutant *Neurospora*
      a. **Complete medium**
      b. **Auxotrophs**
C. X-rays in *Drosophila*
   1. Rates of spontaneous and induced X-linked and autosomal mutations
   2. **Attached-X procedure**
      a. Attached-X females
      b. Cross between attached-X female and normal male
         1. Transparency 90, page 323, figure 15-1
         2. Triplo-X females
         3. Attached-X females
         4. YY males
         5. XY (normal) males
D. Plants
   1. Analysis of biochemical composition
      a. Determination of amino acid sequence
      b. ***Opaque*-2 mutant strain**
      c. Significance

2. Tissue culture of plant cells
    a. Resistant to herbicides or disease toxins
    b. Significance

E. Humans
    1. Detection techniques
        a. Pedigree
        b. Determine inheritance
    2. Transparency 91, page 324, figure 15-2
    3. Dominant mutations
        a. Present on X chromosome of father
        b. Autosomal
    4. X-linked recessive mutations
        a. Transparency 92, page 325, figure 15-3
        b. **Hemophilia**
    5. Autosomal recessive
    6. Analyze human cells cultured *in vitro*

IV. Spontaneous Mutation Rate

A. Relative rate of experimentally induced mutations

B. Points
    1. Low
    2. Varies between organisms
    3. Varies from gene to gene
        a. Viral and bacterial genes
        b. Humans, *Drosophila*, and maize

V. The Molecular Basis of Mutation

A. Change in the coded information
    1. Substitution of a single nucleotide
        a. Transparency 93, page 326, figure 15-4
        b. A change in only one letter
            1. "THE CAT SAW THE DOG," could be changed to "THE CAT SAW THE HOG."

2. *Missense*

c. **Base substitutions** or **point mutations**

d. Specific base mutations

- **Transition**
- **Transversion**

2. Insertion or deletion

a. Garbled message

b. **Frameshift mutations**

c. Premature termination

B. **Tautomeric forms**

1. Alternative chemical forms

2. **Tautomeric shifts**

3. Most stable tautomers

4. DNA replication phase

C. **Base analogs**

1. **5-bromouracil (5-BU)**

a. Figure 15-7 in text

b. Probability is increased

2. Other base analogs

3. **Reverse mutations**

D. Chemical mutagens

1. World War II

2. **Alkylating agents**

3. **Ethylmethane sulfonate (EMS)**

a. Transition mutations

b. **6-ethylguanine**

E. Other chemical mutagens

1. **Acridine dyes**

2. Model

a. Slippage and improper base pairing

b. Intercalation of the acridine dyes

F. Spontaneous loss of nitrogenous base
   1. Guanine or adenine
   2. **Apurinic sites (AP sites), Apyrimidinic (AP sites)**
   3. Altered genetic code
   4. **Deamination**
      a. Cytosine and adenine converted
      b. Alters the base-pairing specificities
      c. Nitrous acid

G. UV radiation mutagenic
   1. 1960
      a. Dimers
      b. Thymine residues
      c. Cytosine-cytosine and thymine-cytosine dimers
      d. Distort the DNA configuration
   2. Overcome inhibition of replication
      a. Bacteria
      b. Insertion relaxed
      c. Allows for survival

VI. Mutations in Humans: Case Studies

A. Two studies in humans

B. **ABO antigens**
   1. Antigenic determinants
   2. H substance
   3. Recombinant DNA technology
   4. Glycosyltransferase activity
      a. One consistent change
      b. Deletion of a single nucleotide

C. **Muscular dystrophy**
   1. Muscle degeneration
   2. Recessive X-linked
   3. Two forms

a. **Duchenne muscular dystrophy (DMD)**

b. **Becker muscular dystrophy (BMD)**

4. Over 2 million base pairs

5. BMD does not alter reading frame

6. J.T. Den Dunnen and associates

a. 128 substantial deletions or duplications

b. DMD mutations changed reading frame

c. High percentage of deleterious mutations

1. Nucleotide substitution.

2. Amino acid substitution

3. May not effect the functioning of the protein

7. Little or no effect on protein function

D. Molecular analysis of genes

1. **Fragile-X syndrome, myotonic dystrophy,** and **Huntington disease**

a. Unique trinucleotide DNA sequence

b. Significant increase in number of repeats

2. Correlation between number of repeats and onset

a. Greater number of repeats

b. **Genetic anticipation**.

3. *FMR-1*

a. 50 copies

b. 50 to 200

c. More than 200 copies

4. Myotonic dystrophy

a. Less than 35 copies

b. More than 35 copies

5. Huntington disease

a. 10 to 35 times more

b. **Spinobulbar muscular atrophy**

VII. Detection of Mutagenicity: The Ames Test

A. **Ames test**

B. *Salmonella typhimurium*

1. Four strains
   a. *his-*
   b. No DNA repair system
2. Innocuous
   a. *in vitro*
   b. Injected into a mouse
3. Carcinogens

IX. Repair of DNA

A. Counteract forms of DNA damage

B. Albert Kelner

1. **Photoreactivation repair** in *E. coli*
   a. Partially repaired
   b. Temperature sensitive
2. **Photoreactivation enzyme (PRE)**
   a. Cleave bonds between thymine dimers
   b. Photon of light
3. Have been preserved

C. Repair system not requiring light in *E. coli*

1. Several mutants
2. *uvr* (UV repair)
3. **Excision repair**
   a. Transparency 94, page 334, figure 15-11
   b. Three steps
      1. Distortion of the strand
      2. DNA polymerase I
      3. DNA ligase
4. *polA1* mutation
5. **AP endonuclease**
6. **DNA glycosylases**.

D. **Proof reading**

1. Recognizes error and "reverses" it
2. **Mismatch repair**
3. **DNA methylation**
   a. **Adenine methylase**
   b. Adenine residues
   c. Temporarily unmethylated

E. **Recombinational repair**

1. Damaged DNA escapes normal repair
   a. **SOS response**
   b. ***recA***
2. DNA polymerase
3. Over 20 different proteins
   a. LexA protein
   b. RecA

X. UV Radiation and Human Skin Cancer: Xeroderma Pigmentosum

A. **Xeroderma pigmentosum (XP)**

1. Skin tumors when exposed to sunlight
   a. Transparency 95, page 336, figure 15-12
   b. Rare disorder
   c. Thymine dimer production and XP
   d. Lack some repair mechanism
2. More than one mutant gene
3. **Unscheduled DNA synthesis**
   a. **Photoreactivation enzyme (PRE)**
   b. Betsy Sutherland
   c. Lower PRE activity
4. **Somatic Cell hybridization**
   a. Excision repair assessed following fusion
   b. **Complementation**
   c. Cells alone
   d. One copy of the normal gene

5. Seven complementary groups

B. High-energy radiation

1. **X-rays, gamma rays,** and **cosmic rays**

a. Higher energy status

b. Penetrate deep into tissue

c. **Ionizing radiation**

2. X-rays penetrate cells

3. **Target theory**

a. One or more sites

b. X-rays interact directly with DNA

4. Two other observations

a. Intensity of the dose

b. Portions of cell cycle more susceptible

XI. Knockout Genes and Transgenes

A. **Gene knockout**

1. **Knockout organism**

2. Cystic fibrosis and Duchenne muscular dystrophy

B. **Transgene**

1. **Transgenic organism**

2. Direct mutagenesis, or a foreign gene

3. Easier to perform

XII. Transposable Genetic Elements

A. **Transposable genetic elements, transposons**

B. Barbara McClintock

C. **Insertion sequences (ISs)**

1. Unique class of mutations

2. Heritable

3. Short DNA segments

4. **Inverted terminal repeats (ITRs)**

5. **Plasmids**

D. **Transposon (Tn) elements**

1. Transposons in bacteria
    a. Mobile
    b. Place to place within and between organisms
    c. Resistance to several antibiotics
2. **Bacteriophage mu**

XIII. Transposable Elements in Humans

A. ***Alu* family**

B. Found in all primates
    1. 200 – 300 base pair sequence
    2. 3% of the entire genome

Lecture Content

Chapter Fifteen: DNA-Mutation, Repair, and Transposable Elements

I. Four Characteristics for Genetic Information

A. The four characteristics are replication, storage, expression, and variation by mutation.

B. Mutations provide the basis for genetic studies.

1. A mutation is the failure to store genetic information correctly.
2. The term mutation includes both chromosomal changes and changes within a gene, **gene mutations**.

II. Classification of Mutations

A. There are various classifications of mutations known.

1. A **spontaneous mutation** is one that arises in nature.
   a. There are no specific agents, other than natural forces, which cause these mutations.
   b. They are generally assumed to be changes in the nucleotide sequence of genes that occur at random sites.
   c. Many of these can be linked to normal chemical processes that occur within the cell.
   d. They occur most often during enzymatic phases of DNA replication.
      - It is agreed that any natural force, which heightens chemical reactivity within a cell, will lead to more errors.
      - Once an error is present, it may be reflected in the amino acid of a specific protein, eventually leading to a functional alteration.
2. An **induced mutation** arises as a result of artificial factors.
   a. Herman J. Muller reported the first induced in mutation in 1927, caused by irradiation on *Drosophila*.
   b. Lewis H. Stadler reported the same finding in 1928.

B. Eukaryotes can experience mutations within somatic or gametic cells.

1. Autosomal recessive alleles within somatic cells are typically inert.
   a. That is, that they are usually masked by their dominant counterpart.

b. Somatic mutations generally exhibit a greater impact when they are dominant or X-linked.

c. Mutations are generally seen to be more influential during early developmental stages, whereas mutations in adults are usually masked by the thousands of cells performing normal function.

d. Cells that mutate and form cancer cells, on the other hand, have a very dramatic effect even in adult cells.

2. Mutations in the germ line occur in gametes or in gamete-forming tissue.

a. These mutations are of greater concern because they are passed on to offspring.

- **Dominant autosomal mutations** will be expressed phenotypically in the first generation.
- **X-linked recessive mutations** will arise in the gametes of a heterogametic female, and may be expressed in a hemizygous male offspring.

b. **Autosomal recessive mutations**, because of heterozygosity, may go unnoticed for many generations.

C. Many mutations are classified by the effect that is seen in the organism.

1. The most easily observed of all mutations are the ones that affect **morphological trait**.

a. These are obviously changes within the morphology of the organism.

b. Mendel's *Drosophila* expressed mutations that affected their morphological traits.

2. There are also **biochemical** or **nutritional mutations** observed in organisms.

a. An example would be when bacteria or fungi are unable to synthesize particular amino acids or vitamins.

b. In humans, sickle-cell anemia and hemophilia are examples of biochemical mutations.

c. These mutations can have a more general effect on the well being and survival of the affected individual.

3. A third type of mutation is a **behavioral mutation**.

a. Primary effects of behavioral mutations are often difficult to discern.

b. An example of a behavior mutation is the mating behavior for a fruit fly, which may be impaired if it cannot beat its wings.
   - This could be caused by a number of factors.
   - It may be that the fly's wing muscles are deformed, the nerves leading to them are impaired, or even the brain portion that initiates the beating of the wings is impaired.

4. Another type of mutation is a **regulatory mutation**.
   a. These may disrupt the normal regulatory processes and permanently activate or inactivate a gene.
   b. A regulatory gene may produce a product that controls the transcription of another gene.
5. **Lethal mutations** are yet another type of mutation.
   a. These mutations do not allow synthesis of a specific amino acid needed to grow and divide on a medium where the amino acid is absent.
   b. Examples of lethal mutations are Tay-Sachs disease and Huntington disease, which are lethal at different points of the human life cycle.
6. A final type of mutation is a **conditional mutation**.
   a. These mutations, although present in the genome, may not be apparent unless a specific type of environment is introduced.
   b. An example of a conditional mutation is a **temperature-sensitive mutation**.
      - There are certain "permissive" temperatures that allow the mutant gene to function normally, and "restrictive" temperatures that cause the gene to function incorrectly.
      - The shift from permissive to restrictive temperatures forces the mutation to become apparent and sometimes even lethal.

III. Detection of Mutation

A. Bacteria, fungi, and other haploid organisms make the best study groups for detecting mutations.
B. *Neurospora* is a pink mold that normally grows on bread, but can be cultured in lab.
   1. This organism is haploid during the vegetative phase of its life cycle.

2. Wild-type *Neurospora* grow on a **minimal medium**, which includes a few inorganic acids, salts, a nitrogen source, and the vitamin biotin.
   a. Wild-type microorganisms always require only minimal medium.
   b. Wild-type microorganisms are called **prototrophs**.
3. Induced mutant *Neurospora* can only grow on a **complete medium**, which includes a number of amino acids, vitamins, nucleic acid derivatives, and more.
   a. Induced mutant microorganisms require specific supplements on their medium to grow.
   b. These microorganisms are called **auxotrophs**.
      - Nutritional mutants are detected and isolated by their failure to grow on minimal mediums.
      - The mutant cells are then determined by growing the mutant organism in a series of test tubes, each containing a single compound.
      - The auxotrophic organism can be defined in this way, because if they grow, it is obvious that that specific compound is what the mutant is unable to synthesis.

C. Muller demonstrated the X-rays are mutagenic in *Drosophila.*
   1. Detection systems, created by Muller, can be used to estimate the rates of spontaneous and induced X-linked and autosomal mutations.
   2. The **attached-X procedure** assesses the rates of X-linked mutations.
      a. Attached-X females are ones that have two X chromosomes attached to a single centromere, along with one Y chromosome in addition to the normal diploid number of autosomes.
      b. When a cross is made between attached-X females and normal males, four types of progeny result.
         - Transparency 90, page 323, figure 15-1.
         - There are triplo-X females produced that die at birth.
         - There are viable attached-X females produced.
         - There are YY males, which also die.
         - And finally there are viable XY (normal) males.

D. There are many variations in plants, which are detected simply by observation; however, there are also biochemical techniques to detect this variation.
   1. One way to detect these mutations is through the analysis of biochemical composition.
      a. This technique involves the isolation of proteins, and the determination of their amino acid sequence.
      b. This has been preformed on maize endosperm, resulting in the detection of the ***opaque*-2 mutant strain**.
         - This mutant strain produces much more lysine than normal maize plants do.
         - It is because of this excess production that these plants are considered to have a higher nutritional value.
      c. This information has proved useful in combating malnutrition disease, which result from an inadequate amount of protein or amino acid in the diet.
   2. Another detection technique involves the tissue culture of plant cells in defined medium.
      a. This technique allows for the determination of strains that are resistant to herbicides or disease toxins.
      b. This information has been helpful in providing new herbicides that have not had the discovery of mutant strains.

E. It is obvious that humans do not make suitable experimental organisms.
   1. There are however detection techniques that can be used to explain mutation within families.
      a. A pedigree, tracing as far back as possible, must first be analyzed.
      b. It there is a trait that is inherited; it is possible to determine the inheritance pattern from a pedigree.
   2. Transparency 91, page 324, figure 15-2.
      a. This pedigree shows the occurrence of cataracts of the eye in a specific family.
      b. The parents in generation I were unaffected, but three of their offspring developed cataracts.

c. The female (proband) produced two children, of which the male child (generation III) was the only one to develop cataracts.
d. Of this male's six children (generation IV), four developed cataracts.
e. These observations are consistent, but do not prove, that this is an autosomal dominant mutation.
f. The unaffected daughter in generation IV argues against an X-linked inheritance; therefore, the conclusion is sound, provided the mutation is completely penetrant.

3. Dominant mutations are the simplest to detect.
   a. If a dominant mutation is present on the X chromosome of the father, the phenotypic trait is passed on to all daughters.
   b. If dominant mutations are autosomal, then about 50% of all offspring produced from an affected heterozygote will exhibit the trait.
4. X-linked recessive mutations are also possible to detect through pedigree analysis.
   a. Transparency 92, page 325, figure 15-3.
   b. The most famous case of X-linked recessive mutations is the mutation for **hemophilia** in the royal bloodline.
      - Hemophilia was detected in the descendents of Queen Victoria, who was obviously heterozygous (*Hh*) for the disorder.
      - Since hemophilia was not present in the bloodline before Queen Victoria, it can be assumed that she received the recessive allele through a mutation.
5. Autosomal recessive alleles are also possible to detect.
   a. These can be a little more difficult to detect because they are "hidden" in a heterozygous individual.
   b. A mating between an affected individual and a homozygous normal individual results in heterozygous carrier offspring.
   c. If the offspring mate with another carrier, approximately ¼ of their offspring will be affected by the trait.
6. It is also possible to analyze human cells that are cultured *in vitro*.

IV. Spontaneous Mutation Rate

A. Spontaneous mutation rates provide insights into evolution, as well as the baseline for measuring the relative rate of experimentally induced mutations.

B. There are many points that have been made concerning spontaneous mutation rates.

1. The rate is exceeding low for all organisms.
2. The rate varies considerable in different organisms.
3. Even within the same species, the spontaneous rate of mutation varies from gene to gene.
   a. Viral and bacterial genes undergo spontaneous mutations at a rate of about 1 in a 100 million cells.
   b. Humans, *Drosophila*, and maize demonstrate rates at a much higher magnitude.
      - Within these organisms, the rate averages between 1 in 1,000,000 and 1 in 100,000 gametes formed.
      - This variation might reflect the relative efficiency of enzyme systems whose function is to repair errors that are created during replication.

V. The Molecular Basis of Mutation

A. A mutation can be caused by any change in the coded information.

1. The least complex change is the substitution of a single nucleotide.
   a. Transparency 93, page 326, figure 15-4.
   b. A change in only one letter of the triplet code can result in an altered meaning of the sequence.
      - For example, if one letter is changed in the sentence, "THE CAT SAW THE DOG," it could be changed to "THE CAT SAW THE HOG."
      - Mutations such as these result in a *missense*.
   c. These are referred to as **base substitutions** or **point mutations**.
   d. There are two other more formal terms used to describe specific base mutations.
      - A **transition** is a mutation that results when a purine is replaced by another purine, or a pyrimidine is replaced by another pyrimidine.
      - A **transversion** is when a purine and a pyrimidine are interchanged.

2. Another type of change in the nucleotide sequence is an insertion or deletion of a single nucleotide along any part of the gene.
   a. When an insertion or deletion occurs, the remainder of the three-letter code words become garbled.
   b. These mutations are known as **frameshift mutations**.
   c. It is possible that a frameshift mutation could result in a codon such as UAA, UAG, or UGA, which are termination codons, which cause premature termination of the polypeptide synthesis.

B. In 1953, Watson and Crick recognized that purines and pyrimidines could exist in **tautomeric forms**.
   1. This means that the purines and pyrimidines can exist in alternative chemical forms, differing only by a single proton shift in the molecule.
   2. They suggested that **tautomeric shifts** could result in base-pair changes or mutations.
   3. The standard hydrogen bonds between the nitrogenous bases in DNA are the most stable tautomers.
      a. Hydrogen bonding between noncomplementary bases form the less frequent tautomers.
      b. These biologically important unstable tautomers involve keto-enol pairs for thymine and guanine, and amino-imino pairs for cytosine and adenine.
   4. Mutations can occur during the DNA replication phase, when a rare tautomer from the template strand pairs with a noncomplementary base.

C. **Base analogs** are molecules that may substitute for purines or pyrimidines during nucleic acid biosynthesis.
   1. A good example of a base analog is the halogenated derivative of uracil in the number-5 position of the pyrimidine ring **5-bromouracil (5-BU)**.
      a. Look at figure 15-7 in the text for an illustration of this analog.
      b. The probability that a tautomeric shift will occur is increased because of the bromine atom in place of the methyl group.
   2. There are other base analogs that are also mutagenic as well.
      a. One is the **2-amino purine (2-AP)**, which can serve as an analog of adenine.

b. This analog can base pair with thymine as well as with cytosine.

3. Base analogs may also as **reverse mutations**.

a. This is when the mutant form is reversed back into the wild-type form.

b. This occurs spontaneously, but at a very low rate.

D. One of the first groups of chemical mutagens discovered were the sulfur-containing mustard gases.

1. This discovery was made during the chemical warfare times of World War II.

2. Mustard gases are **alkylating agents**, which donate an alkyl group, like CH_3- or CH_3- CH_2- to amino or keto groups in nucleotides.

3. **Ethylmethane sulfonate (EMS)** alkylates the keto group in the number 6 position of guanine and the number 4 position of thymine.

a. The base-pairing affinities are then altered, which may result in transition mutations.

b. **6-ethylguanine**, for example, acts like a base analog of adenine, which causes it to pair with thymine.

E. There are other chemical mutagens that can cause frameshift mutations as well.

1. One such mutagen is **acridine dyes**, which is a group of aromatic molecules.

a. These are about the same dimension of the nitrogenous base pairs, and are known to intercalate between purines and pyrimidines.

b. This intercalation induces contortions in the DNA helix, consequently causing deletions and insertions.

2. There is one model that suggests that the gaps produced in DNA during replication, repair, or recombination result in the frameshift mutations.

a. It is during these events that there is a possibility of slippage and improper base pairing of one strand with another.

b. The intercalation of the acridine dyes can extend the existence of the slippage structures, resulting in a higher probability of mispaired configurations causing the addition or deletion of one or more bases from one of the strands.

F. The spontaneous loss of one of the nitrogenous bases in an intact double-helical DNA molecule is yet another type of mutation.

1. This type of event most frequently involves guanine or adenine.

2. **Apurinic sites (AP sites)** are created by the breaking of the glycosidic bond, which links the 1'-C of d-ribose and the 9-N of the purine ring together.
3. The genetic code is altered when a nitrogenous base is absent from a strand that is transcribed and translated.
   a. If replication occurs, the AP site is inadequate for use as a template, and the replication process might be stalled.
   b. If another nucleotide base is added, it could be the wrong one, resulting in yet another mutation.
4. **Deamination** is another type of lesion, which involves an amino group being converted to a keto group in cytosine or adenine.
   a. If deamination occurs, cytosine is converted into uracil, while adenine is converted into hypoxanthine.
   b. This alters the base-pairing specificities of these two molecules during replication.
   c. Nitrous acid is a known mutagen capable of inducing deamination.

G. In 1934, it was discovered that UV radiation in *Drosophila* eggs was mutagenic.
1. By 1960, there had been many studies concerning the *in vitro* effect of UV radiation on nucleic acids, which produced many conclusions.
   a. First, the major effect of UV radiation is on pyrimidines, where dimers form.
   b. This occurs particularly between two thymine residues.
   c. Cytosine-cytosine and thymine-cytosine dimers are also formed, but they are less prevalent.
   d. It is thought that these dimers distort the DNA configuration and inhibit normal replication, causing death.
2. Cells must overcome the inhibition of replication in order for the UV-induced lesion to be mutagenic instead of lethal.
   a. Bacteria have a system that when activated allows the "block" to be bypassed.
   b. The products of several genes allow the strict adherence of the insertion to be relaxed.
   c. This reduces the overall fidelity during replication, but it allows for the survival of otherwise lethal effects of UV radiation.

VI. Mutations in Humans: Case Studies

A. There are two studies of the actual gene sequence of various mutations have been investigated in humans.

B. The first investigation provides insight into the molecular basis of the **ABO antigens**.

1. The ABO system is based on a series of antigenic determinants that are found on erythrocytes and other cells.
2. There are three alleles that exist for this single gene, and the product of the gene is designed to modify the H substance.
   a. This modification involves glycosyltransferase activity, which converts the H substance to either the A or B antigen.
   b. The result is the product of the I^A or I^B alleles, or the failing to modify the H substance, which results in the I^O allele.
3. By using recombinant DNA technology, fourteen cases of varying ABO status have been examined.
   a. Four consistent nucleotide substitutions were found when DNAs of the I^A and I^B alleles were compared.
   b. It is assumed that the amino acid sequence of the gene product results from the substitutions and leads to the different modifications of the H substance.
4. Individuals, who are homozygous for the I^O allele, lack glycosyltransferase activity, which results in the failing of modification to the H substance.
   a. Analysis has shown that there is one consistent change associated with this allele, when compared to the other alleles.
   b. The deletion of a single nucleotide early in the coding sequence causes a frameshift mutation, which in turn causes a premature termination, resulting in a nonfunctioning gene product.

C. The second case studied is the X-linked disorder **muscular dystrophy**.

1. This disorder is characterized by muscle degeneration, or myopathy, which results in the death of the individual in early adulthood.
2. The condition is recessive X-linked; therefore, affected males do not reproduce, and it rarely affects females.

3. Two forms of this disorder exist.
   a. One form is **Duchenne muscular dystrophy (DMD)** and is most common and most severe.
   b. The allelic form is called **Becker muscular dystrophy (BMD)**.
4. The region containing the gene consists of over 2 million base pairs; unaffected individuals produce mRNAs containing approximately 14,000 bases, translated into the protein dystrophin, consisting of 3685 amino acids.
5. A hypothesis has been made that most mutations that cause BMD do not alter the reading frame, whereas most mutations causing DMD change the reading frame.
6. There was an extensive study performed J.T. Den Dunnen and associates, which consisted of 194 patients, 160 DMD and 34 BMD.
   a. They discovered that 128 of the mutations consisted of substantial deletions or duplications.
   b. 17 of the 115 deletions found, occurred in BMD, and of the 13 duplications found, 1 was in the BMD
   c. DMD mutations changed the reading frame of exon areas, whereas BMD mutations usually did not change reading frames.
   d. There was a high percentage of deleterious mutations that represented the deletion or duplication of nucleotides within the gene.
      - A nucleotide substitution may not change the amino acid, since the code is degenerate.
      - If an amino acid substitution does result, a change may not be present at a location within the protein that is critical to functioning.
      - Even if the alteration is at a location that is critical to functioning, it may not effect the functioning of the protein.
7. As a result, single-base substitutions may have little or no effect on protein function, or may simply reduce the efficiency of the protein.

D. During the years 1991 and 1993, molecular analysis of genes responsible for various human disorders provided remarkable observations.

1. The three separate genes responsible for **fragile-X syndrome**, and the autosomal disorders **myotonic dystrophy,** and **Huntington disease**, showed an important similarity.
   a. Each gene contains a unique trinucleotide DNA sequence that is repeated many times.
   b. Each mutation exhibits a significant increase in the number of times the sequence is repeated.
2. There was a correlation between the number of repeats and the onset of mutant gene expression found in many cases.
   a. The greater the number of repeats, the earlier the onset of the disorder.
   b. The number of repeats also increases with each subsequent generation; a phenomenon called **genetic anticipation**.
3. The gene responsible for fragile-X syndrome, *FMR-1*, has several hundred to several thousand copies of the sequence CGG.
   a. Individuals with up to 50 copies are normal.
   b. Individuals with 50 to 200 copies are considered "carriers."
   c. Individuals with more than 200 copies are affected with this disorder.
4. Myotonic dystrophy is the most common form of muscular dystrophy, and has a repeating sequence of GTG.
   a. An individual with fewer than 35 copies is considered normal.
   b. Individuals with more than 35 copies exhibit a range of symptoms, which correlate with the number of repeats.
5. Finally, the gene responsible for Huntington disease consists of a trinucleotide of CAG.
   a. Individuals that are affected with the disease contain copies 10 to 35 times the number in normal individuals.
   b. Another disorder **spinobulbar muscular atrophy** contains the same repeating sequence, but these individuals usually only contain 30 to 60 copies of the CAG sequence.

VII. Detection of Mutagenicity: The Ames Test

A. The **Ames test** measures the frequency of reverse mutations.

B. This test utilizes four strains of the bacteria *Salmonella typhimurium*, which are selected for sensitivity and specificity for mutagenesis.
   1. One strain is used in order to detect specific base-pair substitutions, while the other three are used to detect frameshift mutations.
      a. Each of the strains requires histidine to grow; therefore they are *his* -.
      b. The strains also bear mutations that have eliminated their DNA repair system, as well as their lipopolysaccharide barrier that coats and protects their surface.
   2. Most substances are innocuous when they are present in the human body until activated metabolically to a more chemically reactive product, which usually occurs in the liver.
      a. Because of this, the Ames test is performed *in vitro*, and the test compound is incubated in the presence of mammalian liver extract.
      b. Other times, the compounds are injected into a mouse, which is later sacrificed and the extracts are tested.
   3. A large number of known carcinogens were tested in the 1970s.
      a. Over 80% were shown to be strong mutagens.
      b. This is not surprising since transformation of cells to a malignant state undoubtedly occurs as a result of some alteration in DNA.

IX. Repair of DNA

A. Living systems have evolved a variety of elaborate systems that counteract many of the forms of DNA damage, which can ultimately lead to mutation.
B. Albert Kelner made the first relevant discovery of UV repair in bacteria in 1949.
   1. He observed a phenomenon called **photoreactivation repair** in *E. coli*.
      a. Kelner showed that UV-induced damage to *E. coli* DNA could be partially repaired if, following irradiation, the cells were exposed to a light in the blue range of the spectrum.
      b. This phenomenon was later shown to be temperature sensitive, which suggested that the light-induced mechanism involves an enzymatically controlled chemical reaction.
   2. Further studies revealed that the process is due to a protein called **photoreactivation enzyme (PRE)**.

a. The mode of action for this enzyme is to cleave the bonds between the thymine dimers, which reverse the damage created by the UV radiation.
b. It must absorb a photon of light to cleave the dimer.

3. The genes that are responsible for the PRE, have been preserved throughout evolution, and appear to be in other organisms as well.

C. In the 1960s, Paul Howard-Flanders and his co-workers suggested that there was a repair system that did not require light in *E. coli*.

1. They isolated several independent mutants demonstrating increased sensitivity to UV.
2. One group was called *uvr* (UV repair), which included *uvrA*, *uvrB*, and *uvrC* mutations.
3. These genes were shown to be involved in a process known as **excision repair**.
   a. Transparency 94, page 334, figure 15-11.
   b. There are three steps in this form of repair system.
      - Step one is the distortion of the strand, caused by the UV dimer, being recognized and clipped out by a nuclease that cleaves phosphodiester bonds.
      - Then, DNA polymerase I fills in the gap by inserting d-ribonucleotides complementary to those on the intact strand, and then adds bases to the clipped 3'-OH end of DNA.
      - Finally, DNA ligase seals the final "nick" that remains at the 3'-OH end of the last base inserted, which closes the gap.
4. *E. coli* with the *polA1* mutation, lack the functional polymerase I.
   a. Replication occurs normally, the cells are just unusually sensitive to UV light.
   b. The cells are unable to fill in the gap, which is created by the excision of the thymine dimers.
5. An enzyme called **AP endonuclease** makes a cut in the polynucleotide chain at the AP site, creating a distortion that is recognized by the excision-repair system, ultimately leading to the correction of the error, or the AP site.

6. **DNA glycosylases** are another group of enzymes, which are part of the repair system in DNA; these recognize uracil within DNA, cut it, leaving an AP site to be repaired.

D. The **proof reading** function is performed by DNA polymerase III.

1. The enzyme complex has the potential to recognize the error and "reverse" it.
   a. It performs this function by cutting out the incorrect nucleotide and replacing it.
   b. In bacteria, the proof reading system increases fidelity by two orders of magnitude.
2. Another mechanism utilized is called the **mismatch repair** system.
   a. Robin Holliday proposed this mechanism over 20 years ago.
   b. The steps that must be followed are that the alteration must be detected, the incorrect nucleotide must be removed, and the replacement with the correct nucleotide must occur.
   c. It was wondered how the repair mechanisms could distinguish between the correct template strand and the incorrect strand.
3. **DNA methylation** is the process that is used to distinguish the template from the newly synthesized strand.
   a. *E. coli* contain an enzyme **adenine methylase**, which recognizes DNA sequences as a substrate.
   b. A methyl group is added to each of the adenine residues, following recognition.
   c. At the end of another round of replication, the newly synthesized strands remain unmethylated temporarily.
      - The enzyme recognizes this mismatch and binds to the unmethylated strand.
      - *E. coli* gene products, MutH, L, S, and U are all involved in the discrimination step.
      - It is not yet known how organisms lacking the GATC methylation perform strand discrimination.

E. A final mode of repair is known as **recombinational repair**, and was first proposed by Miroslav Radman.
   1. This system is thought to respond when damaged DNA escapes normal repair, and the damage is significant enough to disrupt the process of replication.
      a. This system was initially referred to as the **SOS response**.
      b. These cells are dependent on a gene, ***recA***, which is involved in many of the recombinational phenomena of *E. coli*.
   2. DNA polymerase stalls the process of replication and skips the distorted part of the DNA strand.
      a. This creates a gap in one of the newly synthesized strands.
      b. The RecA protein then directs recombinational exchange, where the gap is filled with an insertion of a segment that was initially part of the intact homologous strand.
      c. A gap is then created on the "donor" strand, which is filled in with repair synthesis as replication proceeds.
   3. There have been over 20 different proteins described that are involved in this repair mode.
      a. The LexA protein produces a product, which serves to partially repress the transcription of the *recA* and *uvr* genes.
      b. When RecA binds to a single-stranded DNA molecule, the binding activates a second function of RecA, which has the ability to cleave the LexA repressor molecule, disrupting its normal capacity.

X. UV Radiation and Human Skin Cancer: Xeroderma Pigmentosum

A. **Xeroderma pigmentosum (XP)** is a rare autosomal recessive disorder, in humans, that predisposes individuals to epidermal pigment abnormalities.
   1. Affected individuals experience skin tumors when exposed to sunlight.
      a. Transparency 95, page 336, figure 15-12.
      b. This rare disorder is very serious, and can even be fatal.
      c. A causal relationship has been predicted between thymine dimer production and XP.

d. It was suspected that XP individuals were lacking in some repair mechanism, which made them more susceptible to UV-induced skin damage.

2. Studies have also suggested that XP may be due to more than just one mutant gene.
3. James Cleaver showed that cells from XP individuals were deficient in the **unscheduled DNA synthesis** produced by normal cells.
   a. In 1974, a **photoreactivation enzyme (PRE)** was determined to be present in human cells.
   b. Betsy Sutherland identified this enzyme in leukocytes and then in fibroblast cells.
   c. She also discovered the XP cells contain a lower PRE activity than other controlled cells.
4. **Somatic Cell hybridization** has established a link between xeroderma pigmentosum and inadequate repair of DNA.
   a. Once fusion has occurred between the two cells used in this technique, excision repair is assessed.
   b. When repair occurs within the heterokaryon, the two variants are said to demonstrate **complementation**.
   c. Neither of the cells alone will perform excision repair, suggesting that there is more than one gene involved in this disorder.
   d. The heterokaryon is able to fuse because each cell has at least one copy of the normal gene.
5. There have been seven complementary groups assigned to this disorder, suggesting that there are at least seven different genes involved.

B. There are different forms of high-energy radiation.
   1. **X-rays, gamma rays,** and **cosmic rays** all have shorter wavelengths than UV.
      a. This results in a higher energy status for them when compared to UV.
      b. This higher energy level allows them to penetrate deep into the tissue, causing ionization of the molecules they encounter.
      c. These are sources of **ionizing radiation**, and were predicted to be mutagenic by Herman Muller and Lewis Stadler in the 1920s.

2. As the X-rays penetrate the cells, electrons are ejected from the atoms of the molecules encountered by the radiation.
   a. Ions are left along the path of this high-energy ray, which can initiate a variety of chemical changes either directly or indirectly, to the DNA.
   b. This can cause alteration of purines and pyrimidines, resulting in a point mutation.
   c. They are also capable of breaking phosphodiester bonds, thus disrupting the physical integrity of the chromosomes.
3. The **target theory** was first proposed by J.A. Crowther and F. Dessauer in 1924.
   a. This theory suggests that there are one or more sites, or targets, within cells and that a single event of radiation will bring about a damaging effect.
   b. This theory suggests that X-rays interact directly with the DNA.
4. There are two other observations concerning irradiation effects as well.
   a. The first one states that results from varying organisms illustrate that the intensity of the dose does seem to make a difference in the mutagenic effect.
      - This is observed in *Drosophila*, but mammals such as mice and humans do not follow this suggestion.
      - Repair of the damage seems to occur in-between irradiation.
      - Therefore, several smaller doses are not as potent as one large dose.
   b. A second observation is that there are certain portions of the cell cycle that are more susceptible to irradiation effects than others.
      - Since X-rays can deform chromosomes, the damage will occur more readily when the chromosomes are greatly condensed in mitosis.
      - Radiation is used in treatments of malignancy because tumor cells undergo mitosis at a much higher rate than do normal cells.

XI. Knockout Genes and Transgenes

A. **Gene knockout** is the process in which a gene, which is a replacement of the comparable gene, is inserted into an organism.
   1. The genetically altered organism is called a **knockout organism**, such as a "knockout mouse."

2. Knockout mice are used as models for studying human genetic disorders such as cystic fibrosis and Duchenne muscular dystrophy.

B. A **transgene** is when a gene is inserted into an organism in addition to it normal copies.
   1. The organism is then called a **transgenic organism**.
   2. The gene may have undergone direct mutagenesis, or it may be a foreign gene isolated from another organism.
   3. This process is used more extensively than the knockout process because it is easier to perform.

XII. Transposable Genetic Elements

A. **Transposable genetic elements,** sometimes called **transposons**, are elements that move around the genome of an individual; thus they are mobile.

B. Barbara McCintock, who referred to them as "jumping genes," first discovered these over 50 years ago in maize, but nobody believed her.

C. The first observation at the molecular level came by **insertion sequences (ISs)** in *E. coli*, by Peter Starlinger and James Shapiro.
   1. They visualized a unique class of mutations affecting different genes in various bacterial strains.
   2. The phenotypic effect was heritable, but was determined to not be caused by a base-pair change characteristic of conventional gene mutations.
   3. These DNA segments were shown to be very short, not exceeding 2,000 base pairs.
   4. The analysis of the DNA sequences, revealed that the nucleotide sequence consisted of **inverted terminal repeats (ITRs)**.
      a. It was assumed that these had some important to the mobility of the DNA sequences.
      b. Others suggested that the IS termini are able to recognize the certain target sequences in DNA, during the process of insertion.
   5. Other investigations revealed that IS units are present in all wild-type *E. coli* chromosomes as well as in other autonomous segments of bacterial DNA called **plasmids**.

D. IS units also play an important role in the movement of **transposon (Tn) elements**, which are larger.
   1. The transposons in bacteria consist of IS units that contain genes whose functions are unrelated to the insertion process, within their internal DNA sequence.
      a. Tn elements are mobile in both bacterial and viral chromosomes, as well as in plasmids.
      b. The are able to exhibit movement from place to place within and between organisms.
      c. Susumu Mitsuhashi was the first to suggest that genes responsible for resistance to several antibiotics were mobile and could move between bacterial plasmids and chromosomes, in the mid-1960s.
   2. An example would be the **bacteriophage mu**, which can inserts its DNA consisting of over 35,000 nucleotides into various places along the *E. coli* chromosome.

XIII. Transposable Elements in Humans

A. The ***Alu* family** represents a class of mobile eukaryotic genetic units.
B. This family is found in mammals, including humans, but most interestingly is that they are found in all primates.
   1. These consist of a 200 – 300 base pair sequence, which is found interspersed throughout the genome.
   2. They constitute 3% of the entire genome.

Lecture Outline

Chapter Sixteen: Genetics of Bacteria and Bacteriophages

I. Bacterial Mutation and Growth

A. Environmental factors

1. Bacteriophage T1
2. *E. coli*
   a. Resistant
   b. **Adaptation hypothesis**
3. Salvador Luria and Max Delbruck
   a. **Spontaneous mutations**
   b. **Fluctuation test**

B. Primary source of genetic variation

1. Isolate mutant cells
2. **Minimal medium**
   a. **Prototroph**
   b. **Auxotroph**

C. Mutant growth

1. **Lag phase**
2. **Logarithmic (log) phase**
3. **Stationary phase**

D. Semisolid medium

1. **Serial dilution**
2. Number of bacteria

II. Genetic Recombination in Bacteria: Conjugation

A. Recombination in bacteria

1. **Conjugation**
2. *E. coli* K12
   a. Transparency 96, page 351, figure 16-3
   b. Strain A and strain B requirements
   c. Mixed
   d. Minimal medium

e. $1/10^{-}$ cells plated

B. Two different strains needed

1. F^{+} **cells**
2. F^{-} **cells**
3. Cell contact
   a. Bernard Davis
   b. U-tube
   c. F^{-} cells on one side
   d. Sample from each side
   e. Conclusion
   f. **F sex pilus**
4. **Fertility factor**
5. During conjugation
   a. Lederberg and Tatum

Strain A		Strain B
F^{+}	X	F^{-}
Donor		Recipient

   b. Isolation of F factor
   c. Contents of F factor
   d. 19 genes
6. **Plasmid**
7. Double-strand separates
   a. Transparency 97, page 353, figure 16-6
   b. Following exchange
   c. Both cells are F^{-}

C. Mapping of the *E. coli* chromosome

1. Cavalli-Sforza
   a. Rate of 10^{-1}
   b. Hayes
   c. These strains were soon designated as **Hfr**, **high-frequency recombination** strains.

2. If donor cell was an Hfr
3. Nature of recombination
   a. Nonrandom pattern of recombination
   b. Alteration of the F factor
4. Ellie Wollman and Francois Jacob
   a. Hfr and F^- strains together
   b. Blender
   c. **Interrupted mating technique**
      1. Look at figure 16-7 in the text
      2. First 8 minutes
      3. After 10 minutes
      4. By 15 minutes
      5. Within 20 minutes
   d. Order of transfer of genes
5. Linear transfer
   a. Look at figure 16-8 in the text
   b. Not long enough for transfer of entire chromosome
6. Not always transferred in same order
   a. Transparency 98, page 355, figure 16-9
   b. Point of origin (O)
7. Point of origin differed from strain to strain
   a. Position of the F factor
   b. Genes adjacent to *O*
   c. The F factor
   d. Recipient cells do not usually become F^+

D. F^- X F^- matings
   a. No genetic recombination
   b. Conversion of F^+ cells into Hfr cells

E. **F^+ state**
   1. Transparency 99, page 357, figure 16-11
      a. Bacterial genes

b. F'

2. Initiates conjugation with F^- cells

a. F factor is transferred

b. Genes being duplicated

c. **Merozygote**

III. The Rec Proteins and Bacterial Recombination

A. *Rec* genes

1. *RecA*

2. *RecB, recC, and recD*

B. *Rec* gene products

1. **RecA protein**

2. **RecBCD protein**

3. **RecA-mediated recombination**

4. Affinity for binding one of the two strands of DNA

a. DNA-protein complex

b. Migrates along chromosome

c. Invading DNA pairs with and displaces its counterpart

d. Genetic recombination.

e. RecBCD enzyme facilitates the "unwinding" and "cutting".

IV. Plasmids

A. **Plasmids**

B. Classification

1. The F factor

2. **R** and the **Col plasmids**

3. The R plasmid

a. Multiple drug resistance

b. 1950s in Japan

c. *Shigella*

d. Resistant cells

4. Recombinant DNA research

V. Bacterial Transformation

A. **Transformation**

1. Transparency 100, page 359, figure 16-14
2. Two main categories
   a. **Competence**
   b. Limited number of receptor sites
   c. 10,000 to 20,000 base pairs
   d. Active process
   e. Inhibit energy production
3. Nucleases
4. Transforming DNA
   a. Recombinant region
   b. **Heteroduplex** regions
   c. Following semiconservative replication
   d. Following cell division

B. Adjacent genes

1. **Cotransformation**
2. *Linked*
3. If two genes are not linked

VI. The Genetic Study of Bacteriophages

A. **Transduction**

1. Mediated by bacteriophages
2. Following infection

B. **T-even phage series**

1. Transparency 101, page 361, figure 16-15
   a. 150 average sized genes
   b. Icosahedral protein coat
   c. Tail
   d. Six fibers
2. First step in infection is binding of the tail fibers
   a. Central core penetrates cell wall

b. DNA extruded from head

c. All synthesis is inhibited

d. **Latent period**

3. Transcription of viral genes

a. Host cell's ribosomes

b. Numerous enzymes

c. **Lysozyme**

4. Pool of viral DNA molecules

5. Assembly of mature viruses

a. Three major pathways

b. The head

c. The tail

d. Infection occurs over and over

e. **Plaque**

C. **Plaque assay**

1. Bacteriophages used for investigation

2. Over 10^{10} viruses produced

3. Figure 16-16 in the text

a. Serial dilution

b. Melted agar

c. Solid agar

d. Viral plaques

D. Symbiotic relationship

1. **Lysogeny**

2. Bacterial chromosome replication

3. No new viruses produced

4. Under certain stimuli

5. Scientific terms

a. **Prophage**

b. **Temperate**

c. **Virulent**

d. **Lysogenic**

e. **Episome**

VII. Transduction: Virus-Mediated Bacterial DNA Transfer

A. *Salmonella typhimurium*

B. Auxotrophic strains LA-22 and LA-2

1. LA-22 and LA-2
2. Prototrophs (*phe*$^-$ *trp*$^+$ *met*$^+$ *his*$^+$) were recovered
3. Experiments utilizing the U-tube
4. Prototrophs were recovered
   a. Presence of LA-2 cells is essential
   b. **Filterable agents (FAs)**
5. Three main observations
6. Prophage (P22)

C. Normal lytic cycle

1. Following infection
2. During bacteriophage assembly
3. Sometimes, only bacterial DNA is packaged
   a. Ability for infection does not depend on capsid
   b. Bacterial DNA is injected into bacterium
      1. DNA can remain in the cytoplasm
      2. **Abortive transduction**
      3. Most common form
   c. **Complete transduction**
   d. **Generalized transduction**

VIII. Mutation and Recombination in Viruses

A. Mutations often affect morphology of plaques

1. T2 plaques on plates of *E. coli* strain B
   a. Larger with a sharp outer perimeter
   b. Isolated and allowed to reinfect
   c. Inherited trait
   d. *Rapid lysis (r)*

e. Wild-type phages

2. *Host range (h)*

a. Can extend the range of hosts

b. Wild-type T2 phages

c. Adsorption and infection of the B-2 *E. coli*

B. Recombination between bacteriophages

1. Two mutant strains of bacteriophages infecting same bacterial culture simultaneously

a. **Mixed infection experiments**

b. More viral particles than bacterial cells

2. T2/*E. coli* system

a. No recombination

b. Recombinant h^+r^+ and hr were detected

c. Percentage of recombinant plaques

C. **Negative interference**

1. The phage chromosomes involve a breakage and reunion

2. Facilitated by nucleases

3. Following infection

a. Pool of chromosomes

b. Double infection

c. Recombinant chromosomes

4. Replication and exchange events with each other

a. Packaged into the head randomly

b. Parental and recombinant genotypes are produced

5. **Intragenic recombination**

IX. Other Strategies for Viral Reproduction

A. **Bacteriophage ϕX**

1. Single circular strand of DNA

a. Serves as a template

b. **Replicated form (RF)**

c. 50 progeny

d. DNA strands are produced and packaged

2. DNA viruses versus RNA viruses

3. **RNA replicase**

B. Viral reproduction in animal cells

1. **Reverse transcriptase**

2. RNA serves as the genetic material

a. Genetic information flow could only occur from DNA to RNA

b. **Oncogenic**

3. Isolation of reverse transcriptase

a. RNA oncogenic viruses

b. **Retroviruses**

4. (+) RNA strand as a template

a. Synthesizes DNA

b. Synthesizes complementary strand

c. **Provirus**

d. DNA is transcribed into RNA

e. Mature viruses

f. **Transformed**

Lecture Content

Chapter Sixteen: Genetics of Bacteria and Bacteriophages

I. Bacterial Mutation and Growth

A. There are certain environmental factors that induce changes in bacteria, which lead to their adaptation to the new conditions.
   1. For example, *E. coli* are sensitive to infection by bacteriophage T1.
   2. If a plate of *E. coli* is sprayed with T1, many of the cells will be lysed for the production of new T1 progeny, but there will be a few left on the plate, which are resistant to the infection of T1.
      a. When these cells are isolated and established in pure culture, they are all resistant to T1 infection.
      b. The **adaptation hypothesis**, derived to explain this situation, requires the interaction of the phage and bacterium, which means that the phage "induces" the resistance to the bacteria.
   3. In 1943, Salvador Luria and Max Delbruck presented the first evidence that the bacteria are capable of spontaneous mutation.
      a. **Spontaneous mutations** provided an alternative model to the explanation for the origin of T1 resistant *E. coli*.
      b. The test that was used in this experiment was called the **fluctuation test**.

B. This test marked the initiation of modern bacterial genetic studies, and because of this, spontaneous mutation is now considered to be the primary source of genetic variation in bacteria.
   1. Selection techniques are utilized to isolate the mutant cells that spontaneously change from the parental strain.
      a. Almost any desired characteristic can be induced and isolated.
      b. All mutations are expressed in the descendents of the mutant cells.
   2. **Minimal medium** is medium in which there are simple components incorporated, including a carbon source and a variety of ions.
      a. For a bacterium to grow on this medium, it must be able to synthesize all essential organic compounds, and is known as a **prototroph**.

b. If a bacterium loses through mutation the ability to synthesize one or more of the organic components, it is known as an **auxotroph**.

C. An innoculum of bacteria are placed in a liquid culture medium and a growth pattern is graphed in order to study mutant growth quantitatively.
   1. The **lag phase** is exhibited first, and it is a period of initial slow growth.
   2. The **logarithmic (log) phase** is exhibited next, and is a period of rapid growth.
      a. During this growth period, the cells divide many times with a fixed time interval between cell divisions.
      b. The cell density can reach to approximately 10^9 cell/ml, before the nutrients and oxygen become limited.
   3. At this time, the cells enter the **stationary phase**, which is when they have achieved their maximum cell density and cannot replicate further.

D. Following the incubation of the cells in the liquid medium, they are placed on a semisolid medium in a Petri dish.
   1. If the number of colonies produced is too many to count, then they can be diluted by a technique called **serial dilution**.
   2. This can allow for the number of bacteria in the original sample to be determined.

II. Genetic Recombination in Bacteria: Conjugation

A. Joshua Lederberg and Edward Tatum initiated studies showing that recombination occurred in bacteria.
   1. This process is known as **conjugation**, which is when the genetic information from one bacterium is passed to another bacterium.
   2. The initial experiments included two multiple-auxotroph strains of *E. coli* K12.
      a. Transparency 96, page 351, figure 16-3.
      b. Strain A required methionine and biotin to be present in the medium for growth, while strain B required threonine, leucine, and thiamine for growth.
      c. The two strains were grown separately for several generations, and then placed together and mixed and allowed to grow for several generations.
      d. The cells were then plated on minimal medium and analyzed for growth.
         - Cells that grew on the minimal medium had to be prototrophs.

- There had to be some sort of genetic exchange and recombination between the two mutant strains in order to produce prototrophs.

e. The prototrophs in this experiment were recovered at a rate of $1/10^7$ cells plated.

B. It soon became evident that two different strains were needed for the unidirectional transfer of genetic material.

1. **F^+ cells** (F stands for "fertility") serve as donors in this exchange.
2. **F^- cells** serve as the recipients during this exchange.
3. Cell contact is essential for chromosome transfer to occur.
   a. Bernard Davis showed this in his experiment with the U-tube.
   b. He placed a sintered glass filter with a pore size small enough to allow the passage of water, but to small to allow the passage of bacteria, in the bottom of a U-tube.
   c. He then placed all the F^+ cells on one side, and the F^- cells on the other.
   d. Following many generations, a sample was extracted from each side of the U-tube was examined and plated on a minimal medium, resulting in zero prototrophs.
   e. This concluded that physical contact was necessary for conjugation.
   f. The physical interaction in the initial step of conjugation is established by a structure called the **F sex pilus**, which results between two individual bacteria.
4. Evidence was then provided that these F^- cells contained an independent **fertility factor**, which they pass through the pilus during conjugation.
   a. Studies by William Hayes, Lederberg, and Luca Cavalli-Sforza established that fertile cells could lose donor ability.
   b. However, donor ability can be reestablished when these cells are grown with fertile cells.
   c. This concluded that the **F factor** is responsible for donor ability, and that it can be lost and regained.
5. During conjugation, the F factor is passed to all recipient cells.
   a. Lederberg and Tatum's initial crosses were designated as:

Strain A		Strain B
F^-	X	F^+
Donor		Recipient

b. Following their experiments, the F factor was isolated.

c. The F factor consists of a circular double-stranded DNA molecule, containing about 100,000 nucleotide base pairs.

d. These code for 19 genes, including a gene for the formation of the pilus.

6. The F factor is actually a genetic unit referred to as a **plasmid**.

7. During conjugation, the double-stranded F factor separates and one strand moves through the pilus to the recipient cell.

a. Transparency 97, page 353, figure 16-6.

b. Following exchange, the two strands replicate forming a double-stranded plasmid in each of the cells.

c. The final result is that both cells are F^+.

C. Soon, discoveries were made that clarified how genetic recombination occurred, which aided in the mapping of the *E. coli* chromosome.

1. In 1950, Cavalli-Sforza treated an F^+ strain with nitrogen mustard, a chemical known to induce mutations.

a. The result was an altered strain that underwent recombination at a rate of 10^{-4}, 1000 times faster than in the original F^- strains.

b. In 1953, Hayes demonstrated a similar result with the elevated frequency of recombination.

c. These strains were soon designated as **Hfr, high-frequency recombination** strains.

2. It was also discovered that if the donor cell were an Hfr, the recipient cells would sometimes display recombination, but would never become Hfr, which meant that they would remain F^- cells.

3. The most significant characteristic of the Hfr strain is the nature of recombination.

a. There is a nonrandom pattern of recombination shown to vary from Hfr strain to Hfr strain.

b. Hayes hypothesized that some physiological alteration of the F factor had occurred, resulting in the production of Hfr strains in *E. coli*.

4. Ellie Wollman and Francois Jacob explained the difference between Hfr and F^- and showed that Hfr strains allowed for genetic mapping of the chromosome.

a. They incubated Hfr and F^- strains together, and removed samples at various time intervals.

b. They placed these samples in a blender, which separated conjugating bacteria, resulting in the termination of chromosome transfer.

c. This process is called **interrupted mating technique**, and demonstrates that specific genes of the Hfr strain were transferred and recombined sooner than others.

- Look at figure 16-7 in the text for a graph representing the time intervals and the genes transferred.
- During the first 8 minutes, no genetic recombination was observed.
- After 10 minutes however, recombination of the *azi* gene was detected.
- By 15 minutes of mixture, 70% of the recombinants were azi^+; 30% were ton^s; but none were lac^+ or gal^+.
- Within 20 minutes, the lac^+ was found, and within 25 minutes the gal^+ was transferred.

d. Wollman and Jacob correlated the length of time with the order of the transfer of genes.

5. They determined that the transfer of genes was in fact linear.

a. Look at figure 16-8 in the text.

b. Conjugation apparently does not last long enough for the transfer of the entire chromosome.

6. Wollman and Jacob soon discovered that although the genes are always transferred linearly, they are not always transferred in the same order.

a. Transparency 98, page 355, figure 16-9.

b. The major difference between each Hfr strain was the point of origin (O) and the direction in which the chromosome entered from that point during conjugation.

7. They postulated that since the chromosome was circular, containing no free ends, the point of origin differed from strain to strain.
   a. During conjugation between Hfr and F^- strains it is the position of the F factor that determines the initial point of transfer.
   b. The genes that are adjacent to *O*, are the genes that are transferred first.
   c. The F factor then becomes the last part that can be transferred.
   d. Since conjugation rarely lasts long enough for the entire chromosome to be transferred, the recipient cells do not usually become F^+.

D. Recombination occurs less frequently in the crosses between F^+ X F^- matings, and random gene transfer is involved.
   a. When F^+ and F^- cells are mixed, conjugation occurs readily, and the F^- cells receive the F factor, but without genetic recombination.
   b. However, at a low frequency in a population of F^+ cells, the F factor integrates spontaneously at a random point into the bacterial chromosome, resulting in the conversion of the F^- cells into Hfr cells.

E. Edward Adelberg discovered in 1959, that the F factor could lose its integrated status, causing reversion to the **F^- state**.
   1. Transparency 99, page 357, figure 16-11.
      a. The F factor frequently carries several adjacent bacterial genes along with it in this situation.
      b. Adelberg labeled this situation F'.
   2. An F' bacterium behaves like an F^+ cell, in that it initiates conjugation with F^- cells.
      a. When this occurs, the F factor is transferred, along with the chromosomal genes.
      b. This results in the donor cell genes being duplicated in the recipient cell since they are also present in the recipient cell's chromosome.
      c. This creates a partially diploid cell called a **merozygote**.

III. The Rec Proteins and Bacterial Recombination

A. Many insights have been gained by the discovery of mutations in a group of genes named *rec*.
   1. The first mutant gene, *recA*, was found to diminish genetic recombination in *E. coli* 100-fold.
   2. Other mutations, *recB, recC, and recD* reduce recombination by over 100 times.

B. The *rec* gene products have been isolated and determined to be involved in the process by which DNA of the donor and recipient is recombined.
   1. The first product is called the **RecA protein**.
   2. The second product is more complex and is known as the **RecBCD protein**.
   3. Figure 16-12 in the text illustrates the general scheme of the **RecA-mediated recombination** process.
   4. The RecA protein has been shown to have a high affinity for binding one of the two strands that make up the DNA double helix.
      a. This binding creates a DNA-protein complex that then invades and probes DNA of the recipient chromosome.
      b. The complex then migrates along the chromosome until the complementary region of the ssDNA is found.
      c. Once the complementary region is found, the invading DNA pairs with and displaces its counterpart in the chromosome.
      d. The RecA protein facilitates the pairing and displacement of the chromosome, resulting in genetic recombination.
      e. The RecBCD enzyme is also important in this process, it facilitates the "unwinding" and "cutting" of the DNA to aid in the displacement.

IV. Plasmids

A. **Plasmids** are genetic structures containing one or more genes.
   1. Replication of plasmids depends on the same enzymes that replicate the chromosome of the cell.
   2. The plasmids are distributed to daughter cells along with the host chromosome during cell division.

B. Plasmids are classified according to their genetic information.

1. The F factor confers fertility and also contains genes needed for the production of the sex pilus.
2. Other examples of plasmids include the **R** and the **Col plasmids**.
   a. These plasmids confer multiple resistance to antibiotics and the ability to release toxins known as colicins, respectively.
   b. Figure 16-3 in the text illustrates and R plasmid, which contains both r-determinants (for resistance) and RTFs, which facilitate plasmid transfer.
3. The R plasmid is often present in two to three copies per cell.
   a. The R plasmid provides means for rapid conferral of multiple drug resistance to cells, previously sensitive to antibiotics.
   b. The phenomenon became apparent in the late 1950s in Japan, when multiple resistant strains of the bacterium *Shigella* were found in the guts of humans.
   c. *Shigella* became resistant to many of the antibiotics following conjugation with *E. coli*, where the R factor originated.
   d. The resistant cells, following treatment with antibiotics, become the dominant flora of the gut in these humans.
4. Plasmids have become very important in recombinant DNA research.
   a. Specific genes can be inserted into a plasmid, which can then be inserted into a bacterial cell.
   b. As the cell replicates, the foreign gene is treated like one of the cell's own, and is replicated as well.

V. Bacterial Transformation

A. **Transformation** provides a mechanism for the recombination of genetic information in certain bacteria, and can be used to map bacterial genes.
   1. Transparency 100, page 359, figure 16-14.
   2. The steps for transformation can be divided into two main categories: (1) entry of DNA into a recipient cell, and (2) recombination of the donor DNA with its homologous region in the recipient chromosome.
      a. Only those cells in a particular physiological state, referred to as **competence**, can take up the donor DNA.

b. There are a limited number of receptor sites on the surface of the bacterial cell.

c. The most effective length of transforming DNA is about 10,000 to 20,000 base pairs.

d. The passage across the cell wall and membrane is an active process; therefore, it requires energy and specific transport molecules to proceed.

e. Substances that inhibit energy production will also inhibit the transformation process.

3. During entry, one of the two strands of the double helix is digested by nucleases.

a. The intact single strand of DNA then aligns with its complementary region of the bacterial chromosome.

b. The segment then replaces its counterpart in the chromosome with the aid of several enzymes.

4. The transforming DNA must be derived from different strains of bacteria bearing genetic variation, for recombination to be detected.

a. The recombinant region contains one host strand and one mutant strand, following integration into the chromosome.

b. Since the strands are from different sources, the helical regions that develop are referred to as **heteroduplex** regions.

c. Following one round of semiconservative replication, one chromosome is restored to its identical configuration, while the other one contains the mutant gene.

d. Then, following cell division, one mutant and one nonmutant cell are produced.

B. Genes that are adjacent, or very close to one another, on the bacterial chromosome may be carried on the DNA piece that is the effective size of 10,000 to 20,000 base pairs.

1. A single event may result in the **cotransformation** of several genes simultaneously.

2. When genes are close enough together to be cotransformed, they are said to be *linked*.

3. If two genes are not linked, simultaneous transformation can occur only as a result of two independent events involving two separate segments of DNA.
   a. The probability of two independent events occurring simultaneously is equal to the product of the two individual probabilities.
   b. Therefore, the frequency for two unlinked genes to be transformed simultaneously is much lower than if they are linked.

VI. The Genetic Study of Bacteriophages

A. There is yet another mode involving genetic transfer between bacteria, **transduction**.
   1. Transduction is mediated by bacteriophages.
   2. Following infection, bacterial viruses are capable of either lysing the bacterial host or inserting their DNA into the host chromosome, where it may remain genetically quiescent.

B. Phage T4, is a member of the **T-even phage series**.
   1. Transparency 101, page 361, figure 16-15.
      a. The phage contains double-stranded DNA in an amount sufficient to encode for more than 150 average sized genes.
      b. An icosahedral protein coat making up the head, or capsid, of the virus encloses the genetic material.
      c. The tail contains a collar and a contractile sheath that surrounds a central core, and is connected to the head of the virus.
      d. The tail consists of six fibers that protrude from it, and each contain binding sites that specifically recognize unique areas of the external surface of the *E. coli* cell wall, on their tips.
   2. The first step in infection is the binding of the tail fibers to the specific areas on the cell wall.
      a. The contraction of the tail sheath, which utilizes ATP, causes the central core to penetrate the cell wall.
      b. The DNA is then extruded from the head and moves across the cell wall and membrane into the bacterial cytoplasm.

c. All synthesis of DNA, RNA, and protein is inhibited, and the production of viral macromolecules begins.

d. The **latent period** is initiated, in which the production of viral components occurs prior to the assembly of virus particles.

3. Transcription of viral genes by the host cell's RNA polymerase initiates RNA synthesis.

a. Gene products are produced in a copious amount using the host cell's ribosomes.

b. Numerous enzymes participate in the assembly of mature bacteriophages, but are not structural components themselves.

c. **Lysozyme**, a late gene product, digests the bacterial cell wall, which leads to the release of the mature phages once they are assembled.

4. Semiconservative DNA replication begins prior to the synthesis of most viral gene products, leading to a pool of viral DNA molecules available to be packaged into the phage heads.

5. William Wood, Robert Edgar, and others have studied the assembly of mature viruses immensely.

a. There are three major pathways that occur: (1) DNA packaged into heads, (2) assembly of tails, and (3) synthesis and assembly of tail fibers.

b. The head is first assembled, and then DNA is packaged into it.

c. The complex then combines with the tail, and only then are the tail fibers added.

d. When these phages are transferred to a lawn of bacteria, infection occurs over and over again.

e. The multiple infection cycles lead to a clear area where many bacteria have been lysed, which is called a **plaque**.

C. A **plaque assay** is a technique routinely used to determine the number of bacteriophages in an original sample.

1. During infection of bacteria, enormous quantities of bacteriophages may be obtained for investigation.

2. There are often over 10^{10} viruses produced per milliliter of culture medium.

3. Figure 16-16 in the text illustrates the steps in a plaque assay.
   a. First, a serial dilution of the original virally infected bacterial culture is performed.
   b. Then, a 0.1-ml sample is added to a small volume of melted agar, to which a few drops of healthy bacterial culture have been added.
   c. This solution is then evenly distributed on a solid agar in a Petri dish and allowed to solidify.
   d. Viral plaques will occur at each place where a single virus infects one bacterium in the lawn.
      - If the dilution factor is too low, the plaques are plentiful and can take over the entire lawn.
      - If the dilution factor is increased, the plaques can be counted and the density of the viruses in the initial culture can be estimated.

(plaque number/ml) X (dilution factor)

D. Since the 1920s, it has been known that a virus can enter a bacterial cell and establish a symbiotic relationship with it.
   1. Following entry into the cell, the viral DNA is integrated into the bacterial chromosome, a step characterizing the developmental stage known as **lysogeny**.
   2. Then, with each bacterial chromosome replication, the viral DNA is also replicated and passed to the daughter cells following cell division.
   3. There are no new viruses produced, nor is there lysis of the bacterial cell.
   4. Under certain stimuli, such as chemical or ultraviolet light treatment, the viral DNA may lose its integrated status and initiate replication, phage reproduction, and lysis of the bacterial cell.
   5. There are several scientific terms used to describe this relationship between the phage and the bacteria.
      a. When the viral DNA is incorporated into the bacterial chromosome, it is called a **prophage**.
      b. Viruses that can either behave as a prophage or lyse the cell, are referred to as **temperate**.
      c. Viruses that can only lyse the cell are known as **virulent**.

d. A bacterium harboring a prophage is said to be **lysogenic**, capable of being lysed.

e. The viral DNA, which can either replicate in the bacterial cytoplasm or as part of the bacterial chromosome, is known as an **episome**.

VII. Transduction: Virus-Mediated Bacterial DNA Transfer

A. In 1952, Lederberg and Norton Zinder were investigating recombination factors in the bacterium *Salmonella typhimurium*.

1. Their investigations revealed a mechanism, different than the F factor, causing recombination.
2. They had discovered **transduction**.

B. Lederberg and Zinder mixed auxotrophic strains LA-22 and LA-2 of *Salmonella* together and recovered prototrophs.

1. LA-22 was unable to synthesize phenylalanine and tryptophan (*phe⁻ trp⁻*), while LA-2 could not synthesize methionine and histidine (*met⁺ his⁻*).
2. Prototrophs (*phe⁻ trp⁻ met⁺ his⁺*) were recovered at a rate of 1 in 100,000 cells.
3. Experiments utilizing the Davis U-tube soon showed that conjugation could not be the form of recombination occurring.
   a. The two auxotrophic strains were separated by the sintered glass filter, which prevented the cells to come in contact with each other.
   b. However, the different strains were allowed to grow in a common medium.
   c. When samples were removed and plated on minimal medium, prototrophs were observed, but only if the samples were taken from one side of the tube.
4. If the sample was taken from the side of the tube containing the LA-22, prototrophs were recovered.
   a. The presence of the LA-2 cells on the other side of the tube was essential for the recombination.
   b. Since it was not known how this recombination occurred, they called the genetic information responsible for the recombination, **filterable agents (FAs)**.
5. There were three main observations that made it clear that this form of recombination was different than any other form known of.

a. The FA would not pass across a filter with a pore diameter of less than 100nm.
b. If the FA were tested in the presence of DNase, it would not be destroyed; therefore FA was not naked DNA.
c. Finally, the LA-2 cells produced the FA only when they were grown in association with LA-22 cells.

6. The observations were explained by the existence of a prophage (P22), present in the LA-2 strain.
   a. Rarely, the P22 prophages enter the lytic phase, reproduced, and lysed some of the LA-22 cells.
   b. The phage is much smaller than the bacterium, and was able to cross the filter and lyse some of the LA-2 cells.
   c. During the lysis process, the P22 phages produced in LA-2 often acquired a region of the LA-2 chromosome, sometimes consisting of the *phe*$^+$ and the *trp*$^+$ genes, along with their own genetic material.
   d. Then, the phages passed back across the filter, reinfected the LA-22 strain, and produced prototrophs.

C. The initial discovery of transduction involved lysogenic bacteria, but the same process is known to occur during the normal lytic cycle as well.
   1. Following infection, the bacterial chromosome is degraded into small pieces.
   2. During the bacteriophage assembly, a small piece of bacterial DNA can become packaged along with the viral chromosome in the head, resulting in possible transduction.
   3. Sometimes, only bacterial DNA is packaged.
      a. This rarely occurs, but the ability for infection does not depend on the contents of the capsid; therefore, these phages can initiate infection on unlysed bacteria.
      b. When this occurs, bacterial DNA is injected into the bacterium, which can either remain in the cytoplasm, or become integrated into the bacterial chromosome.

- If the DNA remains in the cytoplasm, it does not replicate, but it may still remain in one of the daughter cells following cell division.
- When this occurs, the cell is partially diploid for the transduced genes, resulting in the phenomenon known as **abortive transduction**.
- This is the most common form of generalized transduction.

c. If the bacterial DNA recombines with the homologous region of the bacterial chromosome, the transduced genes are replicated as part of the chromosome and are transmitted to each daughter cell, in a process known as **complete transduction**.

d. Both abortive and complete transductions are subclasses of the **generalized transduction**.

VIII. Mutation and Recombination in Viruses

A. Phage mutations often affect the morphology of the plaques produced.

1. In 1946, Alfred Hershey observed unusual T2 plaques on plates of *E. coli* strain B.
   a. These plaques were larger and displayed a sharp outer perimeter.
   b. When the viruses were isolated and allowed to reinfect *E. coli* B cells; the plaque morphology was identical.
   c. The plaque phenotype was obviously an inherited trait.
   d. Hershey named this mutant *rapid lysis (r)* because the plaques were larger, apparently resulting from a more rapid life cycle of the phage.
   e. Wild-type phages undergo an inhibition of reproduction at a particular size of the plaque; however, the *r* mutant seems to overcome this inhibition.
2. Luria discovered another bacteriophage mutation, *host range (h)*.
   a. This mutation can extend the range of hosts the phage can infect.
   b. Wild-type T2 phages can infect *E. coli* B, but not B-2.
   c. This mutation allows for adsorption and infection of the B-2 *E. coli* cells.

B. Several research teams demonstrated that recombination could also occur between bacteriophages.

1. The experiments used to show this consisted of two mutant strains of bacteriophages allowed to infect the same bacterial culture simultaneously.

a. These experiments are called **mixed infection experiments**.
b. There were many more viral particles than bacterial cells, to ensure simultaneous infection of most cells by both viral strains.

2. For example, one study used the T2/*E. coli* system, where the viruses were either h^-r or hr^+ genotype.
   a. The two parental genotypes would be the only expected progeny, if no recombination occurred.
   b. However, the recombinant h^+r^- and *hr* were detected.
   c. The percentage of recombinant plaques divided by the total number of plaques reflects the relative distance between the genes.

C. A greater than expected frequency of double exchanges is observed in a three-point analysis, which is known as **negative interference**.
   1. There is evidence that supports the model in which recombination between phage chromosomes involves a breakage and reunion, similar to that of eukaryotic crossing over.
   2. This process is facilitated by nucleases, which nick and reseal DNA strands.
   3. Following infection, the chromosomes of each phage begin replication.
      a. A pool of chromosomes develops during this stage, which accumulates in the cytoplasm of the bacteria.
      b. When double infection occurs, the chromosomes of different genotypes are present in the pool.
      c. Genetic exchange between the two parental types will occur, resulting in recombinant chromosomes.
   4. Each of the recombinant chromosomes can undergo replication and exchange events with each other and the remaining parental types.
      a. The chromosomes are then removed randomly from the cytoplasm and packaged into the head of the phage.
      b. In this case, both parental and recombinant genotypes are produced.
   5. It is also possible to have **intragenic recombination**, in which exchange occurs, within the region of one single gene.

IX. Other Strategies for Viral Reproduction

A. There are other forms of reproduction utilized by phages, such as the **bacteriophage ϕX**.
   1. This bacteriophage injects only a single circular strand of DNA, called the plus (+) strand, into the host cell.
      a. This strand then serves as a template for the synthesis of the complementary negative (-) strand.
      b. A circular double-stranded DNA molecule called the **replicated form (RF)** is the result.
      c. This produces about 50 progeny, which serve as templates for the production of only (+) strands.
      d. Hundreds of these DNA strands are produced, and are packaged into protein capsids.
   2. DNA viruses can us the DNA polymerase of the host cell to replicate their nucleic acid, but there is no comparable enzyme for RNA-containing viruses to replicate.
   3. In this case, the RNA virus utilize an RNA-dependent RNA polymerase known as **RNA replicase** for replication.

B. Another type of viral reproduction occurs in animal cells rather than bacteria.
   1. Howard Temin and David Baltimore demonstrated an enzyme capable of synthesizing DNA on an RNA template called **reverse transcriptase**.
   2. RNA serves as the genetic material of certain animal tumor viruses, is initially transcribed into DNA, which then serves as a template for replication and transcription of RNA during infection, according to Temin's proposal.
      a. Many people did not believe this because it was previously thought that genetic information flow could only occur from DNA to RNA.
      b. The viruses that are included in this category are called **oncogenic** because they can induce malignant growth.
   3. Reverse transcriptase was isolated independently by Temin and Baltimore.
      a. It has been found in all RNA oncogenic viruses.
      b. These viruses are called **retroviruses** because the "reverse" the flow of genetic information.

4. The enzyme uses the infecting (+) RNA strand as a template.
   a. It synthesizes DNA in the 5'-3' direction.
   b. Another enzyme then uses this DNA strand as a template and synthesizes the complementary strand, which creates a double-stranded DNA molecule.
   c. This molecule is capable of integrating into the genome of the host cell, forming a **provirus**.
   d. If the proviral DNA is transcribed into RNA, (+) RNA strands are produced and may be translated into viral proteins.
   e. Packaging of other newly formed RNA molecules into the viral capsid occurs to form mature viruses.
   f. Such as host cells is said to be **transformed**.

Lecture Outline

Chapter Seventeen: DNA Biotechnology: Techniques and Analysis

I. Recombinant DNA Technology: An Overview

A. **Recombinant DNA**

B. Seven basic steps

1. DNA is purified
2. **Restriction endonucleases**
3. **Vectors**
4. **Clones**
5. Passed to all daughter cells
6. Recovered from host cell
7. Isolation of gene product

II. Constructing Recombinant DNA Molecules

A. Restriction endonucleases

1. Werner Arber, Hamilton Smith, and Daniel Nathans
2. Restrict or prevent viral infection
3. Cut both strands of DNA
4. ***Eco*RI**
   a. Figure 17-1 in the text
   b. Overhanging single-stranded tails
   c. Recombinant molecules
   d. **DNA ligase**
   e. Figure 17-3 in the text

B. Fragments of DNA joined to a vector

1. Vectors
2. Derived from plasmids and bacteriophages
3. Plasmids
   a. Single plasmid
   b. Copies of cloned DNA
   c. pUC18
      1. Large DNA inserts

2. 500 copies per cell
3. **Polylinker site**

4. Bacteriophages
   a. **Phage lambda**
   b. Dispensable genes
   c. Replaced with foreign DNA
   d. Reproduction
   e. Cloned DNA
5. Advantage with phage vectors

III. Cloning in E. coli Host Cells

A. Prokaryotic and eukaryotic cells
   1. *E. coli* K12
   2. Many vectors
   3. Steps
      a. Transparency 102, page 379, figure 17-9
      b. Isolate DNA and treat with restriction enzymes
      c. Ligation of fragments
      d. Heat-shocked
      e. Replication
      f. Colonies of genetically identical clones

B. Phage containing foreign DNA

IV. Cloning Eukaryotic Host Cells

A. *Saccharomyces cerevisiae*

B. **Yeast artificial chromosome (YAC)**

V. Constructing Libraries of Cloned DNA Sequences

A. Library of cloned DNA

B. **Genomic library**
   1. An entire genome or sequences on a single chromosome
   2. At least one copy of all sequences in the genome
   3. Construction of these libraries
   4. Required number of clones

a. Number of clones in a library

$$N = \frac{\ln(1 - P)}{\ln(1 - f)}$$

b. Human genome

c. Plasmid vector

d. Phage vector

C. **cDNA library**

1. Advantages

a. Contains genes that are expressed in a particular cell type or tissue

b. Do not contain introns

c. Amino acid sequence

2. Steps

a. Transparency 103, page 380, figure 17-11

b. Isolation of poly A-containing mRNA

c. **Complementary DNA (cDNA)** molecules

d. dT primer

e. **Reverse transcriptase**

f. The RNA strand is removed

g. DNA duplex

h. S1 nuclease

VI. Recovering Cloned Sequences from a Library

A. Genomic and cDNA libraries

B. **Probe**

C. Screening a plasmid library

1. Transparency 104, page 382, figure 17-12

a. Replica of the colonies

b. Lyse the bacteria

c. Nucleic acid probe

d. Added to a solution containing the filter

e. A DNA-DNA hybrid molecule

f. Washing

2. **Plaque hybridization**

a. Phage carrying DNA inserts
b. Infection of bacteria
c. Plaques
d. Addition of a probe

3. More efficient method

D. Another method

1. **Chromosome walking**
2. Transparency 105, page 383, figure 17-13
   a. DNA fragment is subcloned
   b. Determine degree of overlap
   c. Subfragment used as a probe
   d. **Open reading frame (ORF)**
   e. The ORF
   f. Cystic fibrosis and muscular dystrophy
3. Limitations

VII. PCR-Based DNA Cloning

A. **Polymerase chain reaction (PCR)**

1. Kary Mullins
2. PCR
   a. Information about nucleotide sequence
   b. **Oligonucleotides**
   c. Hybridization of primers
   d. DNA polymerase
3. Figure 17-14 in the text
   a. DNA is amplified and denatured
   b. Primers are annealed to single-stranded DNA
   c. DNA polymerase
4. **Cycle**
   a. 4-5 minutes
   b. Twenty-five cycles

B. Advantages of PCR

1. Faster
2. Computer software
3. Small DNA samples
4. DNA can be partly degraded

C. Limitations to PCR

1. Requirements for some information about nucleotide sequence
2. Serious difficulties with contamination

VIII. Analysis of Cloned Sequences

A. **Restriction map**

1. Length of a cloned insert and location of the restriction enzyme sites
2. Steps
   a. Transparency 106, page 386, figure 17-15
   b. DNA segment of 7.0 kb
   c. Restriction enzymes
   d. **Gel electrophoresis**
   e. Calculation of sizes of fragments
   f. Fragments analyzed
      1. *Hin*dIII used
      2. *Sal*I used
3. One cutting site for each restriction enzyme
   a. Two possible maps
   b. Results from two enzymes together
4. Refine genetic maps
   a. Accuracy of genetic maps
   b. Restriction enzyme cutting sites are inherited
5. **Restriction fragment length polymorphisms** or **RFLPs**
6. Hybridization experiments

B. **Southern blot**

1. Steps
   a. Transparency 107, page 388, figure 17-17
   b. Cloned DNA is cut into fragments

c. DNA is denatured

d. Buffer solution

e. DNA fragments become immobilized on membrane

2. Hybridization with probe

a. Complementary DNA fragments

b. Washed

3. Other purposes

4. Transcriptionally active

a. RNA is extracted

b. Pattern transferred to a sheet of membrane

c. Hybridization with a single-stranded DNA probe

d. **Northern blot**

e. **Western blot**

5. Northern blots

C. **DNA sequencing**

1. Four reactions

a. Contents of each reaction tube

b. Insertion of a dideoxynucleotide

c. Analog lacks a 3' hydroxyl group

d. Gel electrophoresis

e. Transparency 108, page 389, figure 17-19

f. Sequence

2. Automated DNA sequencers

a. Fluorescent dyes

b. Colored pattern of peaks

c. Look at figure 17-20 in the text

3. Organization of genes

a. Genes and proteins are colinear molecules

b. Regulatory regions that flank prokaryotic and eukaryotic genes

4. Computer analysis

Lecture Content

Chapter Seventeen: DNA Biotechnology: Techniques and Analysis

I. Recombinant DNA Technology: An Overview

A. **Recombinant DNA** is the creation of a new combination of DNA molecules that are not found together naturally, generally reserved for DNA molecules produced from different biological sources.

B. There are seven basic steps involved in this procedure.

1. First, the DNA is purified from the cells or tissues being used.
2. Then, DNA fragments are generated using enzymes called **restriction endonucleases**, which recognize and cut DNA molecules at specific nucleotide sequences.
3. These fragments are joined together to serve as **vectors**.
4. The recombinant DNA molecule, a vector with an inserted DNA fragment, is then transferred to the host cell, which replicates and produces dozens of identical copies, or **clones**.
5. The recombinant DNA molecule is passed on to all daughter cells formed from cell division.
6. The DNA, which has been cloned, can be recovered from the host cell, purified, and then analyzed.
7. Finally, the cloned DNA can be transcribed, its mRNA translated, and the gene product can be isolated and used for research or commercial sales.

II. Constructing Recombinant DNA Molecules

A. The most important part of DNA technology is the class of enzymes known as restriction endonucleases.

1. Werner Arber, Hamilton Smith, and Daniel Nathans won the 1978 Nobel Prize for their work with restriction enzymes.
2. These enzymes called restriction enzymes because they restrict or prevent viral infection by degrading the invading viral DNA.
3. These enzymes, which are isolated from bacteria, recognize a specific nucleotide sequence and cut both strands of DNA within that sequence.
4. One of the first restriction enzymes discovered was ***Eco*RI**, isolated from *E. coli*.

a. Figure 17-1 in the text shows this enzyme's recognition sites and points of cleavage.
b. The DNA fragments produced have overhanging single-stranded tails, which can reanneal with complementary single-stranded tails on other DNA fragments.
c. DNA fragments from two sources, if mixed under proper conditions, can form recombinant molecules by hydrogen bonding their sticky ends.
d. The enzyme T4 **DNA ligase** can be used to link the fragments covalently, resulting in the formation of recombinant DNA molecules.
e. Figure 17-3 shows common restriction enzymes and their recognition sequences.

B. Fragments of DNA can enter a host cell only when they are joined to a vector.
1. Vectors are essentially carrier DNA molecules.
2. A number of vectors are currently in use, including those derived from plasmids and bacteriophages.
3. Plasmids are naturally occurring, extrachromosomal, double-stranded DNA molecules, which replicate autonomously within bacterial cells.
a. Only a single plasmid can enter a host cell, but many plasmids can increase their number so that the host cell can contain up to 1000 copies.
b. Plasmids allow more copies of cloned DNA to be produced.
c. As a vector, a plasmid, such as pUC18, has several useful properties.
- Since the plasmid is small, it can carry large DNA inserts.
- pUC18 can replicate to form about 500 copies per cell, producing many clones of the inserted DNA fragments.
- A region called a **polylinker site** in the pUC18 plasmid contains a large number of unique restriction sites.

4. Bacteriophages are viruses that infect and kill bacterial cells, but can also be used as a vector.
a. One of the most widely used is the **phage lambda**.
b. The middle one-third of the lambda chromosome consists of a cluster of genes that are dispensable.

c. This region can be replaced with foreign DNA, without affecting the ability of the phage to infect cells and form plaques.
d. Lambda vectors containing inserted DNA can be introduced into bacterial host cells, where they reproduce and form many particles of infective phage, each which carries a DNA insert.
e. The cloned DNA can be recovered from the plaques that are formed by the phage during replication.

5. An advantage with phage vectors is that they are able to carry much longer fragments of DNA than plasmids.

III. Cloning in E. coli Host Cells

A. There are a variety of prokaryotic and eukaryotic cells that can be used as hosts for the replication of recombinant vectors.
   1. One of the most commonly used host strains is *E. coli* K12.
   2. Strains such as K12 are genetically well characterized, and they can serve as host cells for many vectors.
   3. There are several steps required to create recombinant DNA molecules in *E. coli* host cells.
      a. Transparency 102, page 379, figure 17-9.
      b. The first step is to isolate the DNA to be cloned, and treat it with a restriction enzyme to create fragments ending in a specific sequence.
      c. Then, the fragments are ligated to plasmid molecules that have been cut with the same restriction enzyme, resulting in a recombinant vector molecule.
      d. The bacterial cells are then heat-shocked to facilitate the uptake of the recombinant vector into the cell.
      e. The recombinant molecules are then allowed to replicate and form dozens of copies by culturing the bacterial cells.
      f. The bacterial cells are allowed to grow on a nutrient plate, where they can form colonies of genetically identical clones, which can then be screened to identify those that have taken up the recombinant plasmids.

B. Phage containing foreign DNA can also be grown in *E. coli*, resulting in plaques representing a cloned descendent of a single ancestral phage.

IV. Cloning Eukaryotic Host Cells

A. There are several cloning systems using eukaryotic vectors as host cells as well, including one based on the yeast, *Saccharomyces cerevisiae*.
   1. Although yeast is a eukaryotic organism, it can be grown and manipulated in much the same way as bacterial cells.
   2. Vectors for growth in yeast host cells have been produced by combining bacterial plasmid sequences with those of yeast plasmids.

B. Another type of yeast vector is the **yeast artificial chromosome (YAC)**.
   1. This chromosome is illustrated in figure 17-10 of the text.
   2. The YAC contains telomeres at each end, an origin of replication, and a yeast centromere.
   3. The YAC also contains two selectable markers and a cluster of restriction sites for DNA inserts.
   4. Segments of DNA more than 1 megabase long can be inserted into these YACs.
      a. This allows for the cloning of large pieces of DNA into these vectors.
      b. This has been very useful in the human genome project.

V. Constructing Libraries of Cloned DNA Sequences

A. A library of cloned DNA can represent either genomic DNA cut into fragments with restriction enzymes, or DNA copies of the mRNAs found in a particular cell or tissue type.

B. One type of library is called a **genomic library**.
   1. A genomic library can represent an entire genome, or the sequences contained on a single chromosome.
   2. These libraries contain at least one copy of all sequences in the genome.
   3. These libraries are constructed by extracting the DNA from cells or tissue, cutting the DNA with restriction enzymes, and then ligating the fragments into vectors.
   4. The number of clones required to carry all sequences in a genome is dependent on (1) the average size of the cloned inserts carried by the vector and (2) the size of the genome to be cloned.
      a. The number of clones in a library can be calculated as

$$N = \frac{\ln(1 - P)}{\ln(1 - f)}$$

b. N is the number of required clones, P is the probability of recovering a given sequence, and f represents the fraction of the genome in each clone.

c. The human genome consists of 3.0×10^6 kb of DNA, with an average size of 17 kb for the cloned inserts; therefore, the library would need about 8.1×10^5 phages.

d. If a plasmid vector, which can carry inserts of about 5 kb, were used, several million clones would be needed.

e. Therefore, for this example, a phage vector would be the best choice for the library.

C. A second type of library is a **cDNA library**, which represents the set of genes actively transcribed in a particular cell type.

1. cDNA libraries offer several advantages over the genomic libraries.

a. For example, the cDNA library will contain only those genes that are expressed in a particular cell type or tissue at a specific time.

b. Also, the clones in a cDNA library do not contain introns, instead they only represent the coding regions of the genes.

c. This makes it easier to derive the amino acid sequence of the gene and to deduce the function of the gene product.

2. There are several steps that must be followed to create a cDNA library.

a. Transparency 103, page 380, figure 17-11.

b. First, the poly A-containing mRNA molecules must be isolated from the cell type or tissue.

c. This mRNA is used to synthesize **complementary DNA (cDNA)** molecules, which are then cloned to create the cDNA library.

d. Next, a poly dT primer is used to pair with the poly A residues.

e. These serve as the starting point for the synthesis of a complementary DNA strand using the enzyme **reverse transcriptase**, resulting in an RNA-DNA double-stranded duplex molecule.

f. The RNA strand is then removed, and the single-stranded DNA is used as the template to make the DNA double-stranded molecule, by using the enzyme DNA polymerase I.

g. The 3'-end of the DNA single strand will often loop back on itself to create a primer for the synthesis of the second strand, which results in a DNA duplex with the strands joined together at one end.

h. This hairpin loop can then be opened using the enzyme S1 nuclease, which produces a double-stranded DNA molecule that can be cloned into a plasmid or phage vector.

VI. Recovering Cloned Sequences from a Library

A. A genomic or cDNA library can contain up several thousand or several hundred thousand clones, which must be identified and selected for.

B. Many procedures use a **probe**, which is any piece of DNA or RNA that has been labeled and is complementary to some part of a cloned sequence present in the library, to select clones from the library.

1. These are often used to identify complementary nucleic acid sequences present in one or more clones.
2. They can be single- or double-stranded molecules; however, they are also single-stranded when used in hybridization reactions.
3. The probes are often radioactive polynucleotides, but other methods can include chemical or color reactions to identify the location of a specific clone.
4. Probes can be derived from a variety of sources, including even related genes isolated from other species if the DNA sequence has been conserved.

C. In order to screen a plasmid library, the clones are grown on nutrient plates, forming hundreds or thousands of colonies.

1. Transparency 104, page 382, figure 17-12.
   a. A replica of the colonies on the plate is made by gently pressing a nylon or nitrocellulose filter onto the surface of the plate, resulting in the transfer of bacterial cells to the filter.

b. The filter is then transferred through solutions to lyse the bacteria, denature the double-stranded DNA, and convert it to single strands, and bind the strands to the filter.

c. The filter now has bound DNA from the colonies that are screened by incubation with a nucleic acid probe.

d. The probe, which had been denatured to form single strands, is added to a solution containing the filter.

e. A DNA-DNA hybrid molecule will form between the probe and the cloned DNA if the DNA sequence of any of the cloned DNA on the filter is complementary to the probe.

f. The unbound and excess probe is then washed away, and the filter is assayed to detect any hybridization.

- If a radioactive probe was used, decay in probe molecules bound to the DNA on the filter, will expose the film and produce dark spots representing colonies containing the cloned gene of interest.
- If a nonradioactive probe was used, there is a chemical reaction that emits photons of light to expose the photographic film and reveal the location of colonies carrying the gene of interest.

2. A slightly different method, called **plaque hybridization**, is used to screen a phage library.

a. A solution of phage carrying DNA inserts is spread over the lawn of bacteria growing on a plate.

b. The phages infect the bacteria on the plate, forming plaques as they replicate.

c. The plaques, clear spots on the plate, represent the progeny of a single phage, as well as its clone.

d. The plaques are then transferred to nylon or nitrocellulose membrane, the phage DNA is denatured, and then a probe is added.

3. Many more plaques can be screened on a single filter, making this a more efficient method for screening large genomic libraries.

D. Another method is used, when the approximate location of a gene is known, and it is possible to clone the nearby sequences.

1. This method is known as **chromosome walking**, which is the isolation of adjacent clones from a library.
2. Transparency 105, page 383, figure 17-13.
   a. First, the end piece of a cloned DNA fragment is subcloned, and used as a probe in order to recover overlapping clones from a library.
   b. The overlapping clones are then analyzed by restriction mapping to determine the degree of overlap.
   c. A subfragment from one end of the overlapping clone is used as a probe to recover another set of overlapping clones, and the analysis is then repeated.
   d. The gene in question is identified by nucleotide sequencing of the recovered clones, and by searching for a potential gene-coding sequence, or **open reading frame (ORF)**.
   e. The ORF is a stretch of nucleotides that begins with a start codon that is followed by amino acid-encoding codons, and ends with one or more stop codons.
   f. This method has been used to identify genes for cystic fibrosis and muscular dystrophy.
3. There are many limitations to chromosome walking in complex eukaryotic organisms.
   a. If a probe contains a repetitive sequence such as an *Alu* sequence, it can hybridize to other clones in the genomic library.
   b. Most of these clones will not be adjacent to the clone from which the probe was derived, resulting in the termination of the walk.
   c. A technique to overcome this is called **chromosome jump**, and is often used to skip over the region containing the repetitive sequence and continue the walk.

VII. PCR-Based DNA Cloning

A. The **polymerase chain reaction (PCR)**, which is a rapid, cell-free method of DNA cloning, was developed in 1986.
   1. Kary Mullis was awarded the Nobel Prize in 1993 for developing the PCR technique.

2. PCR allows the direct amplification of specific target DNA sequences within a population of DNA molecules, and can be used on fragments of DNA that are infinitesimally small in quantity.
   a. Some information about the nucleotide sequence of target DNA is required for PCR.
   b. This information is used to synthesize two primers 15-30 nucleotides in length, called **oligonucleotides**, that are then added to a denatured DNA sample.
   c. The primers hybridize to a complementary sequence that flanks the sequence to be amplified.
   d. A heat-stable from of DNA polymerase is then used to synthesize the complementary strands of the target DNA.
3. There are three basic steps in the PCR reaction, which are illustrated in figure 17-14 in the text.
   a. First, DNA is amplified and denatured; the DNA does not have to be purified, and it can come from any number of sources.
   b. Then, the primers are annealed to the single-stranded DNA.
      - The primers are synthetic oligonucleotides, which anneal to the sequences flanking the segment to be amplified.
      - Usually there are two different primers used, which each have a sequence that is complementary to one of the two strands of DNA being amplified.
      - The primers then align themselves with their 3' ends facing each other.
      - This process usually occurs at lower temperatures around 50 to 70°C.
   c. Finally, a heat-stable form of DNA polymerase is added to the reaction mixture and DNA synthesis is carried out at temperatures around 70-75°C, resulting in a double-stranded copy of the target DNA.
4. Each of the three steps, **denaturation** of the double-stranded product, **annealing** of the primers, and **extension** by polymerase, is referred to as a **cycle**.
   a. Each cycle takes approximately 4-5 minutes to complete.
   b. Twenty-five cycles result in more than a 1 million-fold increase in the amount of DNA.

B. There are several advantages of PCR over the host cell-based cloning system.
   1. The PCR reaction is fast and can be carried out within a few hours, as compared to the host cell-based cloning, which usually takes weeks to complete.
   2. The PCR primers can also by designed by computer software, and commercial synthesis of the oligonucleotides is also fast and economical.
   3. PCR is very sensitive, and can therefore be used to amplify target DNA from extremely small DNA samples, even from a DNA sample of a single cell.
   4. This reaction can also be used on samples of DNA that have partly degraded, contaminated with other materials, or even embedded in a medium that would make DNA extraction for conventional cloning difficult or even impossible.

C. There are also some limitations to PCR.
   1. First of all, there are requirements for some information about the nucleotide sequence of the target DNA.
   2. Also, minor contamination of the sample with DNA from other sources can cause serious difficulties.

VIII. Analysis of Cloned Sequences

A. One of the first steps in characterizing a DNA clone is the construction of a **restriction map**, which is a compilation of the number, order, and distance between restriction enzyme cutting sites along a cloned segment of DNA.
   1. The maps provide information about the length of a cloned insert, and the location of the restriction enzyme sites within the cloned DNA.
      a. This information can be used for subcloning fragments of a gene, or for comparing its internal organization with that of other cloned sequences.
      b. Restriction maps can also be used to compare a gene and its cDNA to identify exons and introns in the genomic copy of the gene.
      c. The map units are expressed in base pairs (bp).
   2. There are certain steps that must be followed in order to construct a restriction map.
      a. Transparency 106, page 386, figure 17-15.
      b. For this map, a cloned DNA segment of 7.0 kb is used.

c. Three samples of the cloned DNA are digested with restriction enzymes: one is digested with *Hin*dIII, one with *Sal*I, and one with both *Hin*dIII and *Sal*I.
d. The fragments, created by digestion with these enzymes, are separated by **gel electrophoresis**, and appear as bands when the DNA is stained with ethidium bromide and visualized under ultraviolet light.
e. The sizes of the fragments can be calculated by comparing them to a set of standards run in adjacent lanes.
f. These fragments are then analyzed.
    - When the *Hin*dIII is used, two fragments are produced, one of 0.8 kb and one of 6.2 kb; confirming that the cloned insert is 7.0 kb, and indicating that there is only one cutting site for this enzyme located at 0.8 kb from one end.
    - When the *Sal*I is used, two fragments are produced, one of 1.2 kb and one of 5.8 kb, indicating that there is one cutting site for this enzyme located 1.2 kb from the one end of the insert.

3. The results indicate that there is one cutting site for each restriction enzyme, but the relationship between the two sites is unknown.
    a. This results in two possible maps.
        - In one map, the *Hin*dIII site is located 0.8 kb from one end, and the *Sal*I is located 1.2 kb from the same end.
        - In the second map, the *Hin*dIII site is located 0.8 kb from one end, and the *Sal*I is located 1.2 kb from the other end.
    b. The next step is to consider the results from the two enzymes together.
        - The first map predicts that there will be three fragments generated, 0.4, 0.8, and 6.2 kb in length.
        - The second map predicts that the three fragments generated will be 0.8, 1.2, and 5.0 kb in length.
        - The fragment pattern observed in this example indicates that the first map model is correct.
4. Restriction maps can also be used to refine genetic maps.

a. The accuracy of genetic maps depends on the frequency of recombination between genetic markers, as well as on the number of markers used in constructing the map.

b. Restriction enzyme cutting sites are inherited, and can therefore be used as genetic markers, reducing the distance between markers, increasing their accuracy, and for providing reference points for the correlation of genetic and physical maps.

5. If a restriction site maps close to a mutant gene, it can be used as a marker in diagnostic tests, these sites are known as **restriction fragment length polymorphisms** or **RFLPs**.

6. The DNA inserts cloned into vectors can be used in hybridization experiments as well, to characterize the identity of specific genes, to locate coding regions or flanking regulatory regions within cloned sequences, and to study the molecular organization of genomic sequences.

B. A widely used method for detecting the formation of hybrids is known as the **Southern blot** method, which separates DNA fragments by gel electrophoresis, transfers them to filters, and then screens the fragments with probes.

1. There are certain steps that must be followed for the Southern blot method.

a. Transparency 107, page 388, figure 17-17.

b. First, cloned DNA is cut into fragments by one or more restriction enzymes, which are then separated by gel electrophoresis.

c. The DNA within the gel is denatured by adding an alkaline solution, and then transferred to a sheet of DNA-binding membrane.

d. The sheet is placed on top of the gel, while a buffer solution flows through the gel and membrane by capillary action.

e. The DNA fragments move out of the gel and become immobilized on the membrane.

2. The DNA fragments that are bound to the membrane are hybridized with a labeled single-stranded DNA probe.

a. Only the DNA fragments that are complementary to the nucleotide sequence of the probe will hybridize.

b. The unbound probe is then washed away and the hybridized fragments are then visualized on a piece of film.

3. Southern blots serve many other purposes as well, for instance, they can be used to map restriction sites within and near a gene, for the identification of DNA fragments carrying a single gene from a mixture of many fragments, and for the identification of related genes within different species.
4. Another use for Southern blot is to determine whether a cloned gene is transcriptionally active in a given cell or tissue type, by probing for the presence of complementary RNA.
   a. RNA is first extracted from several cell or tissue types and then fractioned by gel electrophoresis.
   b. The pattern of RNA bands is then transferred to a sheet of membrane as in the Southern blot.
   c. The membrane is then hybridized to a single-stranded DNA probe, which is derived from the cloned gene, resulting in complementary RNA exhibiting a band on the film.
   d. Since this is the inverse of the Southern blot procedure, it is appropriately called a **Northern blot**.
   e. A **Western blot** is another method, which is similar to these, except it involves proteins.
5. Northern blots provide information about the expression of specific genes, and are used to study the patterns of gene expression in both embryonic and adult tissues.
   a. They can also be used to detect alternatively spliced mRNAs and multiple types of transcripts derived from a single gene.
   b. They also provide information about transcribed mRNAs, where a relative measurement of transcriptional activity can be determined.
   c. In conclusion, northern blots can be used to characterize and quantify the transcriptional activity of a specific gene in different cells, tissues, and even different organisms.

C. The most widely used method of **DNA sequencing** is based on the elongation of single-stranded DNA templates by the enzyme DNA polymerase.
   1. This method requires four reactions to be performed.
      a. Each reaction tube contains a DNA template, a base-specific analog called a **dideoxynucleotide**, the normal deoxynucleotides, and DNA polymerase.
      b. While the DNA synthesis is occurring, the DNA polyermase occasionally inserts a dideoxynucleotide analog into a growing strand of DNA in place of a deoxynucleotide.
      c. The analog lacks a 3' hydroxyl group; therefore, it cannot participate in the formation of a 3' bond, this results in the termination of DNA synthesis, with fragments differing in their length at their 3' ends.
      d. The fragments from each reaction are then separated from each other via gel electrophoresis in four adjacent lanes, and the visualization is a series of bands forming a ladderlike pattern.
      e. Transparency 108, page 389, figure 17-19.
      f. The sequence can be read from bottom to top, corresponding to the 5' to 3' sequence of the DNA strand that is complementary to the template.
   2. Automated DNA sequencers are used for large-scale DNA sequences.
      a. These sequencers use fluorescent dyes, of four different colors.
      b. The result is a film with colored pattern of peaks that can be read to provide the sequence.
      c. Look at figure 17-20 in the text.
   3. DNA sequencing provides useful information about the organization of genes, as well as the nature and number of mutational events, which alter both genes and gene products.
      a. This information has confirmed the conclusion that genes and proteins are colinear molecules.
      b. It has also been used to study the organization of regulatory regions that flank prokaryotic and eukaryotic genes, as well as to infer the amino acid sequence of proteins.

4. Computer analysis can be used to determine whether a cloned segment of DNA contains all or just part of a gene.
   a. This is performed by searching for exon/intron junctions.
   b. It can also be done by comparing DNA sequences and their inferred amino acid sequence with information in various databases that contain sequences of previously identified genes and proteins.

Lecture Outline

Chapter Eighteen: DNA Biotechnology: Applications and Ethics

I. Mapping Human Genes

A. Variations

1. Frequency
2. Single nucleotide
3. Chromosomes can be distinguished
   a. Figure 18-1 in the text
   b. Chromosomes A and B
   c. Southern blot method
4. **Restriction length polymorphisms (RFLPs)**
   a. RFLPs
   b. Inherited
   c. Map specific regions

B. Chromosomal loci location

1. Linkage
2. Most cases
3. Probability analysis
   a. Occurring by chance
   b. **Logarithm of the odds** or **lod score**
4. **Centimorgan (cM)**
   a. Thomas H. Morgan
   b. Recombination frequency of 1%
   c. 1 to 3 million nucleotides

C. **Type I neurofibromatosis (NF1)**

1. Characteristics
   a. Autosomal dominant
   b. 1 in 3000
   c. Nervous system defects
2. Mapping the NF1 gene
   a. Multigenerational families studied

b. **Exclusion map**

c. Indication of non-linkage

d. Evidence for linkage

e. Centromere of chromosome 17

f. 17q11.2

g. Locus identified in 1990

3. Amino acid sequence

a. 2485 amino acids

b. Signal transduction

c. **Neurofibromin**

4. **Positional cloning**

II. Diagnosing and Screening for Genetic Disorders

A. Pre-natally

1. **Amniocentesis**

a. Amniotic fluid

b. Chromosomal or single-gene disorders

2. **Chorionic villus sampling (CVS)**

a. Fetal chorion

b. Cytogenetic, biochemical, and recombinant DNA-based testing

3. Prenatal detection of genetic disorders

4. Cloned DNA sequences

B. Sickle-cell anemia

1. Autosomal recessive

a. β-globin protein

b. Elimination of a restriction enzyme cutting site

c. RFLP patterns

2. *Mst*II

a. Transparency 109, page 402, figure 18-6

b. Normal β-globin gene present

c. Mutant allele present

d. Southern blot hybridization

3. Example
4. Restriction enzyme analysis

C. **Allele-specific oligonucleotides (ASO)**

1. Increased resolution and wider application
   a. Only with its complementary sequence
   b. ASOs in association with
   c. Transparency 110, page 403, figure 18-7
   d. White blood cells
   e. Amplification, spotting onto filters, hybridization with ASO
   f. Genotype
   g. Homozygous normal (AA), heterozygous (Aa), and the homozygous mutant (aa)
   h. Probe for mutant allele
2. Direct synthesis of ASOs
3. Cystic fibrosis
   a. **Cystic fibrosis transmembrane conductance regulator (CFTR)**
   b. Oligonucleotides
   c. Nylon filter
   d. Affected individuals, heterozygotes, and normal homozygous

D. **DNA chips**

1. Glass
2. Fields contain linker molecules
3. One nucleotide
4. Testing for nucleotide sequences
   a. DNA is extracted and cut
   b. Fluorescent dye, melted, and pumped into chip
   c. Binding
   d. Laser scanner
   e. Software program

III. Gene Therapy

A. **Gene therapy**

1. Transferring of a normal allele
   a. Vector or gene transfer system
   b. Several methods
2. Retroviral vector
   a. Cluster of genes removed and human clone is inserted
   b. Replication-deficient
   c. Integration into chromosome
3. **Severe combined immunodeficiency (SCID), familial hypercholesterolemia, and cystic fibrosis**

B. SCID
1. **Adenosine deaminase (ADA)**
2. Isolation of white blood cells
   a. Mixed with genetically modified retrovirus
   b. Virus infects the T cells
   c. Injected into the patient
3. 1990

C. First-generation vectors
1. Integration can only occur if replicating
2. Inactivation or mutation
3. Cannot carry very large sequences
4. Infectious virus

D. Ethical guidelines
1. Interests of the patient must be taken under consideration
2. **Germ-line therapy** and **enhancement therapy**

IV. DNA Fingerprints

A. **DNA fingerprinting**

B. **Minisatellites**
1. Clusters of nucleotides
   a. 14 and 100 nucleotides
   b. **Variable-number tandem repeats (VNTRs)**
2. Southern blotting results in specific band patterns

a. DNA fingerprint
b. Always the same
c. Unique pattern
d. Small and old samples of material

C. Criminal trials
1. Standard forensic tests
2. Interpretation

V. Genome Projects

A. Methods for generating and mapping mutants
1. Mutants must be isolated
2. Direct identification of genes
a. Genetic and physical maps
b. Genomic map

B. **Bottom-up method**
1. *E. coli* K12
2. Restriction mapping, computer analysis, and identification of overlapping clones
a. Transparency 111, page 409, figure 18-15
b. **Contig**
c. Physical map
3. Sequencing of the clones
a. 1997
b. 4,638,858 nucleotides and 4300 genes

C. **Top-down approach**
1. **Yeast artificial chromosome (YAC)** vectors
a. Six to eight chromosome bands
b. Contigs constructed
2. Studying individual genes or regions
a. Stretch of a unique sequence of DNA
b. **Sequence tagged site (STS)**
c. Determination of the location of gene

D. **Shotgun method**

1. *Haemophilus influenzae and Mycoplasma genitalium*
2. Genomic library
   a. Isolated and sequenced random fragments
   b. 8472 fragments
3. *H. influenzae*
4. Shortens the time required

E. **Human Genome Project**
1. Human Genome Organization (HUGO)
2. High-resolution genetic maps
   a. Look at figure 18-7 in the text
   b. Identified genes, RFLPs, STSs, and other markers
   c. 1995
   d. 2 million base pairs
3. Construction of physical map
   a. Top-down and bottom-up approaches
   b. Ultimate goal
4. STS-based physical map
5. Sequence the 3.2 billion nucleotides in the human genome

F. The Ethical, Legal, and Social Implications (ELSI) Program
1. How the information would be used
2. Impact of genetic information on individuals, the privacy and confidentiality of genetic information, the impact of genome information and technology on medical practice, and reproductive decision making
3. Four main areas of concern
   a. Privacy and fairness in the use and interpretation
   b. Transfer of genetic knowledge from the research laboratory to clinical practice.
   c. Informed consent for participants in genetic research
   d. Public and professional education
4. There are many other issues still

G. *Haemophilus influenzae* and *Mycoplasma genitalium*

1. Sequence of the yeast genome 1996, *E. coli* 1997
2. *M. genitalium*
   a. 482 genes
   b. 54% of the genome functions in three processes
3. Human disorders and human biology
   a. Previously unknown mechanisms of mutations
   b. Increases in gene dosage
   c. Different kinds of mutations in single gene
   d. Mutations in members of a gene family

VI. Biotechnology

A. Recombinant DNA technology companies

B. Human insulin
   1. Protein hormone that regulates sugar metabolism
      a. Diabetes.
      b. Frequency
   2. Transparency 112, page 413, figure 18-18
      a. Two polypeptide chains, A and B
      b. 21 and 30 amino acids.
      c. Synthetic genes for the A and B subunits
      d. **Fusion polypeptide**
      e. β-galactosidase and one insulin subunit
      f. Extracts then purified and treated with cyanogen bromide
      g. Intact active insulin molecule
      h. Packaged for use
   3. Table 18.3 in the text

C. First generation of recombinant proteins
   1. Disadvantages
   2. Second generation methods
   3. Deficiency in alpha-1-antitrypsin
      a. Production of alpha-1-antitrypsin
      b. Transgenic sheep developed

c. Production of milk with high concentrations of the alpha-1-antitrypsin

D. Plants

E. Vaccine

1. Stimulate immune system
2. Two main types
   a. **Inactivated vaccine**
   b. **Attenuated vaccines**
3. **Subunit vaccine**
   a. Acts as an antigen
   b. Hepatitis B
   c. Cloned into yeast-expression vectors
   d. Extracted and purified
4. Hepatitis B surface protein in plants
   a. Plant-produced vaccines
   b. Inexpensive, less purification, and no injections
5. Genetically engineered potatoes
   a. Small quantities of potatoes consumed
   b. Developed antibodies
6. Recombinant DNA technology

Lecture Content

Chapter Eighteen: DNA Biotechnology: Applications and Ethics

I. Mapping Human Genes

A. There are variations in nucleotide sequences that occur throughout the human genome.
   1. The frequency of these is approximately 1 in every 200 nucleotides.
   2. When a single nucleotide is altered, it can create or destroy restriction enzyme sites.
   3. When this occurs on one of two homologous chromosomes, the chromosomes can be distinguished by their pattern of restriction fragments on a DNA blot.
      a. Figure 18-1 in the text illustrates an example of this.
      b. Chromosomes A and B exhibit different fragments when they are both cut with *Bam*HI.
      c. A probe is then added and the Southern blot method is used to visualize the fragments of these chromosomes.
   4. The different DNA fragment lengths, created by the cutting of a restriction enzyme, are referred to as **restriction fragment length polymorphisms (RFLPs)**.
      a. RFLPs are common, and represent variations acquired by changes in a single nucleotide pair, or by deletions or insertions of one or more nucleotide pairs.
      b. These are then inherited as codominant alleles.
      c. They can be used to map specific regions on individual chromosomes, and as markers to follow the inheritance pattern of genetic disorders.

B. In order to use RFLPs to determine chromosomal loci for genetic disorders, a multigenerational family in which both a chromosomally assigned RFLP marker and the genetic disorder are cosegregating must be used.
   1. When the loci of the RFLP and the disorder are near each other on the same chromosome, they will exhibit linkage.
   2. In most cases, linkage is not apparent.
   3. Probability analysis is used to determine whether an RFLP marker and a genetic disorder are linked.

a. The probability that the observed pattern of inheritance could occur by chance alone, assuming that the marker and the gene are unlinked, is calculated first.
b. The probabilities are then expressed as the **logarithm of the odds** or **lod score**.
c. A lod score of 3 indicates that the odds are 1000:1 in favor of linkage, while a lod score of 4 means that the odds are 10,000:1 in favor of linkage.

4. The unit of linkage used in mapping human chromosomes is known as the **centimorgan (cM)**.
   a. This unit of measure is named after a geneticist named Thomas H. Morgan.
   b. One centimorgan is equal to a recombination frequency of 1% between two loci.
   c. In humans, a centimorgan is equivalent to approximately 1 to 3 million nucleotides of DNA.

C. The search for the chromosomal locus of the gene for **type I neurofibromatosis (NF1)**, provides an example for the use of RFLP analysis in gene mapping.
   1. There are many characteristics for this disorder.
      a. It is inherited as an autosomal dominant condition.
      b. Its incidence is approximately 1 in 3000.
      c. It is associated with a range of nervous system defects.
   2. There were a series of steps involved in the mapping of the NF1 gene.
      a. First, multigenerational families were used to compare the inheritance of NF1 with dozens of RFLP markers, where each RFLP marker represented a specific human chromosome or chromosome region.
      b. This step resulted in the formation of an **exclusion map**.
      c. This indicated which RFLPs were not linked to the disease, and therefore, which chromosomes and chromosome regions did not carry the NF1 locus.
      d. Evidence for linkage was also produced from this work, it indicated that chromosomes 5, 10, and 17 were candidates for carrying the NF1 gene.
      e. The next stage of experiments focused on these chromosomes, and produced evidence suggesting that the disorder was closely linked to an RFLP known to reside near the centromere of chromosome 17.

f. More than 30 RFLP markers from this region where used to analyze 13,000 individuals from NF1 families, and the gene was mapped to region 17q11.2.

g. A collection of genomic clones was then used and the locus was identified in 1990 by chromosome walking and DNA sequencing.

3. Following the identification of the gene, the amino acid sequence was determined.

a. It was found to include 2485 amino acids.

b. The NF1 gene product was found to be similar to proteins that play a role in signal transduction.

c. It was soon determined that the gene product, **neurofibromin**, was involved in the transduction of intracellular signals and the regulation of a gene that controls cell division.

4. The mapping and cloning of the gene for NF1 described, is an example of **positional cloning**.

a. This method is a recombinant DNA-based method, and is different than the methods used to map from an identified gene-product and gene locus.

b. In positional cloning, the gene can be mapped, isolated, and cloned with no prior knowledge of the gene product.

II. Diagnosing and Screening for Genetic Disorders

A. Much of the diagnosis for genetic diseases is performed pre-natally.

1. There are two main methods used for pre-natal diagnosis, one of them is **amniocentesis**.

a. Amniocentesis requires a needle to withdraw amniotic fluid.

b. The fluid retrieved, can be analyzed for chromosomal or single-gene disorders.

2. The second common method used for pre-natal diagnosis is **chorionic villus sampling (CVS)**.

a. A catheter is inserted into the uterus, and is used to retrieve a small tissue sample of the fetal chorion.

b. The tissue is then used for cytogenetic, biochemical, and recombinant DNA-based testing.

3. The use of these methods in combination with recombinant DNA technology has proven to be a highly sensitive and accurate tool for prenatal detection of genetic disorders.
4. The use of cloned DNA sequences allow for the direct examination of the genotype, which expands the range of prenatal testing.

B. Sickle-cell anemia is a disorder in which prenatal testing is often utilized.
   1. Sickle-cell anemia is an autosomal recessive disorder, common in people with family origins in areas of West Africa, the Mediterranean basin, and parts of the Middle East and India.
      a. It is caused by a single nucleotide change involving the β-globin protein.
      b. The mutation eliminates a restriction enzyme cutting site for the restriction enzymes *Mst*II and *Cvn*I.
      c. The characteristic RFLP patterns can be used to diagnose sickle-cell anemia, prenatally, as well as for the identification of the genotypes of parents and other family members.
   2. The fetal cells, obtained from either amniocentesis or CVS, have the DNA extracted from them and digested with a restriction enzyme such as *Mst*II.
      a. Transparency 109, page 402, figure 18-6.
      b. When the normal β-globin gene is present, the enzyme cuts twice producing two small DNA fragments.
      c. When the mutant allele is present, the second *Mst*II site is destroyed, producing one large restriction fragment.
      d. The fragments are then separated by gel electrophoresis and visualized by Southern blot hybridization.
   3. In this example, both parents are heterozygous for the mutation.
      a. The first child is homozygous normal, since there are only two small bands.
      b. The second child, who produces one large band, is homozygous for the mutant allele.
      c. Amniocentesis shows that the fetus has one large band and two small bands, indicating that he/she is heterozygous for the mutant allele, which means that he/she will be unaffected, but will be a carrier for the allele.

4. Restriction enzyme analysis can detect only about 5 to 10% of all nucleotide substitutions.

C. **Allele-specific oligonucleotides (ASO)** use synthetic probes to distinguish between alleles that differ by as little as a single nucleotide.
   1. The use of ASOs offer increased resolution and a wider application, when compared to restriction enzyme analysis.
      a. An ASO will hybridize only with its complementary sequence, and not with other sequences that might vary by as little as a single nucleotide.
      b. Using ASOs in association with PCR allows for a different method of screening for sickle-cell anemia.
      c. Transparency 110, page 403, figure 18-7.
      d. The DNA from white blood cells is extracted and denatured.
      e. A region of the β-globin gene is then amplified by PCR, and spotted onto filters, with each filter being hybridized to an ASO.
      f. The genotype can then be read directly from the filters.
      g. The homozygous normal (AA) produces a dark spot, the heterozygous (Aa) produces a light spot and a dark spot, while the homozygous mutant (aa) produces no spot, since it will not bind to the probe.
      h. When a probe is used for the mutant allele, the pattern is reversed.
   2. ASOs can be synthesized directly from the wild type and mutant copies of the gene when the nucleotide sequence of the normal gene is known, and where the molecular nature of the mutant gene is known.
      a. This allows for the screening of heterozygous carriers of the genetic disorder.
      b. Cystic fibrosis is often screened using this method.
   3. In cystic fibrosis, 70% of all mutant alleles are represented by a deletion called delta 508.
      a. Cystic fibrosis is an autosomal recessive disorder, associated with a defect in a protein called the **cystic fibrosis transmembrane conductance regulator (CFTR)**, and affects approximately 1 in 2000 individuals of northern European descent.

b. Allele specific oligonucleotides are made by PRC of cloned samples of the normal allele and the mutant allele, in order to detect heterozygous carriers.
c. The DNA prepared from the while blood cells of an individual to be tested is applied to a nylon filter and hybridized to each of the ASOs.
d. In affected individuals, only the ASO made from the mutant allele will hybridize; in heterozygotes, both ASOs will hybridize; while in the normal homozygous, only the ASO from the normal allele will hybridize.

D. DNA probes similar to the allele-specific nucleotides are being coupled with the technology of the semiconductor industry to produce **DNA chips**.
1. The chips are made of glass, and are about one-half inch square, divided into fields.
2. Each of the fields contain linker molecules to which DNA probes, about 20 nucleotides in length, are attached.
3. Along a single row of fields, the sequence of the probe differs by one nucleotide.
4. Testing for nucleotide sequences is fairly easy using these DNA chips.
a. First, the DNA is extracted from cells and cut with one or more restriction enzymes.
b. The fragments, which result from the cutting, are then tagged with fluorescent dye, melted into single strands, and pumped into the chip.
c. The fragments with nucleotide sequences that exactly match the probe sequence will bind, while the other sequences that do not match are washed off of the chip.
d. The chip is then exposed to a laser scanner, which reads the chip and detects the patter of fluorescence.
e. A linked software program then analyzes the patter of fluorescence, and the data are presented in the form of a nucleotide sequence.

III. Gene Therapy

A. **Gene therapy** is a process that is now being used to treat genetic disorders.
1. Gene therapy transfers a normal allele into a somatic cell that carries one or more mutant alleles.

a. The delivery of the structural genes and their regulatory sequences is accomplished using a vector or gene transfer system.
b. There are several methods for the transferring genes, including, the use of viruses as vectors, the chemically assisted transfer of genes across cell membranes, and the fusion of cells with artificial vesicles containing cloned DNA sequences.

2. The most common method of gene transfer is the use of a retroviral vector such as Maloney murine leukemia virus.
   a. A cluster of genes is removed from the virus, and the cloned human gene is inserted.
   b. Following packaging into a viral protein coat, the recombinant vector can infect the cells, but is replication-deficient because of the missing viral genes.
   c. The viral genome carrying the human gene moves into the nucleus, integrating into a chromosome, once it is in the cell.
3. There are several heritable disorders that are currently being treated with gene therapy, such as **severe combined immunodeficiency (SCID), familial hypercholesterolemia,** and **cystic fibrosis**.

B. An individual affected with SCID, has no functional immune system and usually dies from otherwise minor infections.
   1. An autosomal form of SCID is caused by a mutation in the gene that codes for the enzyme **adenosine deaminase (ADA)**.
   2. The treatment begins with the isolation of a subpopulation of white blood cells (T cells) from the patient.
      a. The cells are mixed with a genetically modified retrovirus carrying a normal copy of the human ADA gene.
      b. The virus infects the T cells, inserting a functional copy of the ADA gene into the cell's genome.
      c. These genetically modified T cells are then grown in the laboratory and a billion or so of them are injected into the patient.
   3. Gene therapy began in 1990, with a girl suffering from SCID.

a. Three years after her treatment began, she exhibited the normal ADA gene in more than 50% of her T cells; she is now living a normal life.
b. However, this is not always the case, many of the gene therapy trials fail, due to inefficient vectors.

C. The first-generation vectors have several drawbacks, which limit their widespread use.
1. First, integration of the retroviral genome into the host cell genome can only occur if the host cells are replicating.
2. Secondly, insertion of the viral genomes into the host chromosome can sometimes inactivate or mutate an indispensable host gene.
3. Thirdly, retroviruses have a low capacity, and cannot carry very large inserted sequences.
4. Finally, there is always the possibility of producing an infectious virus if a recombination event occur between the vector and retroviral genomes already present in the host cell.
5. It is because of these reasons, that other viral vectors and strategies are being developed.

D. Presently, there are well-established ethical guidelines for gene therapy.
1. This experimental form of gene therapy is undertaken only after extensive reviews have been made, and the interests of the patient are taken under consideration.
2. There are two types of gene therapy that are currently not under use because of the ethical issues involved, **germ-line therapy** and **enhancement therapy**.
a. Germ-line therapy is not under use because unlike somatic gene therapy, it affects individuals of future generations, without their consent.
b. Enhancement therapy is used to enhance human potential, rather than to treat a disease, which causes ethical issues to arise, and the thought of a "superhuman."

IV. DNA Fingerprints

A. **DNA fingerprinting** is a method used to identify individuals and establish degrees of genetic relatedness between individuals.

B. One of the most useful forms of restriction fragment length polymorphisms is the use of **minisatellites**, which are variations in the number of tandemly repeated DNA sequences between two restriction enzyme sites.
   1. These sequences are actually clusters of nucleotides from 2 to 100 nucleotides in length, and they are widely dispersed in the human genome.
      a. Each repeat typically contains between 14 and 100 nucleotides, and the number of repeats at each locus ranges from 2 to more than 100.
      b. These loci are known as **variable-number tandem repeats (VNTRs)**.
   2. A pattern of bands is produced with the VNTR sequences are cut with restriction enzymes and visualized by Southern blotting.
      a. This pattern is an example of a DNA fingerprint.
      b. They are equivalent to fingerprints because the pattern of bands is always the same for a certain individual, no matter where the DNA is extracted.
      c. Each person's pattern is unique; therefore, this type of analysis is very useful in identification.
      d. The analysis can also be performed on very small samples of material, as well as very old samples of material.

C. DNA fingerprinting has been used in criminal trials since 1988.
   1. Standard forensic tests employing four to six different probes are used in developing a detailed DNA fingerprint profile.
   2. The results of the DNA fingerprint are then interpreted using statistics, probabilities, and population genetics.
      a. The frequency of each VNTR allele in the population is calculated, then multiplied together to give a combined frequency, which results in a calculation of how often the observed DNA fingerprint might occur in the general population.
      b. For example, if the frequency of locus 1 is determined to be 1 in 333, and the frequency of locus 2 is 1 in 83, then the combined frequency, equal to the product of their individual frequencies, would be 1 in 28,000.

c. This might not hold up in court, but if a third and fourth locus were added, then the frequency gets less and less, pointing out the certain individual as being the one in question.

V. Genome Projects

A. Over the past 90 years, geneticists have developed methods for generating and mapping mutants.
   1. One drawback is that the genes can only be characterized when mutants are isolated, and at least one mutation must be present for each gene.
   2. Recombinant DNA techniques are now being used to identify all the genes directly, in an organism's genome.
      a. A genomic library is established, and overlapping clones are assembled to create genetic and physical maps to encompass the entire genome.
      b. The final step involves the sequencing of the entire genome, resulting in a genomic map, with all the genes identified by their location and their nucleotide sequence.

B. The *E. coli* genome was sequence with the use of the **bottom-up method**.
   1. Yuji Kohara and his colleagues cloned a genomic library of the *E. coli* K12 strain using lambda vectors.
   2. Restriction mapping, computer analysis, and identification of overlapping clones by the presence of restriction sites characterized the clones from this library.
      a. Transparency 111, page 409, figure 18-15.
      b. The contiguous segment that resulted is referred to as a **contig**, when covered by the overlapping clones.
      c. The contigs were then arranged in a physical map that covered the entire 4700 kb of DNA in the *E. coli* genome.
   3. The second stage involved the sequencing of the clones in the contig library.
      a. The nucleotide sequencing of two different strains of *E. coli* was completed early in 1997.
      b. The genome was discovered to consist of 4,638,858 nucleotides, and approximately 4300 genes.

C. The *Drosophila* genome project used a method called the **top-down approach**.

1. A cloned genomic library was constructed using **yeast artificial chromosome (YAC)** vectors.
   a. The average YAC covers about six to eight chromosome bands.
   b. The contigs were constructed by identifying the YACs that had two or more chromosome bands in common.
2. YACs are broken down by restriction digestion and subcloned into other vectors in order to study individual genes or regions.
   a. Each *Drosophila* gene was defined as a stretch of a unique sequence of DNA, which should occur only once in the haploid genome.
   b. Any short DNA segment within a gene can be used as a marker, and is known as a **sequence tagged site (STS)**.
   c. It is therefore not necessary to know the nature of the gene, the gene product, or its phenotype, in order to determine the location of the gene.

D. A third method, which is used on prokaryotes, is the **shotgun method**.
   1. The shotgun method was used on both *Haemophilus influenzae and Mycoplasma genitalium*.
   2. To sequence the *M. genitalium* genome, a genomic library was created from restriction digests of DNA.
      a. Then, randomly selected fragments were isolated and sequenced.
      b. The nucleotide sequence of 8472 fragments was then analyzed by a software program, and organized into a complete genome sequence.
   3. This method was also utilized to sequence the *H. influenzae* genome.
   4. The shotgun method shortens the time required for sequencing a genome, and bypasses the need of a prepared physical map.
   5. Work is now being done to see if this method can apply to other genomes as well.

E. The **Human Genome Project** is an international effort to determine the entire sequence of the human haploid genome.
   1. The project began in the United States in 1990, but many other countries have since joined in, being coordinated by an international organization, the Human Genome Organization (HUGO).

2. The first stage of the project involved the construction of high-resolution genetic maps for each chromosome.
   a. Look at figure 18-7 in the text.
   b. The maps were constructed by using identified genes, RFLPs, STSs, and other markers.
   c. By 1995, genetic maps for each chromosome, incorporating about 15,000 markers, were complete.
   d. There is an average distance of 2 million base pairs of DNA between markers, which aids in the sorting out and organization of the contigs generated by the physical mapping.
3. The second stage of the project involves the actual construction of the physical map.
   a. Both the top-down and the bottom-up approaches are being used.
   b. The ultimate goal is to create a physical map of the entire genome, consisting of 30,000 STSs spaced at intervals of about 100kb.
4. An STS-based physical map covering approximately 95% of the genome has recently been created.
   a. The STSs in the map are each composed of 200 to 500 nucleotides, and are all stored in databases as a nucleotide sequence.
   b. These sequences can then be downloaded and used as probes.
   c. Any of the sequences can be generated by PCR.
5. The ultimate goal of the entire project is to sequence the 3.2 billion nucleotides in the human genome by the year 2005.
   a. The last stage of the project, involving the establishment of six centers in the United States to improve technology related to sequencing, has already began.
   b. It is expected that by the year 1999, regions on the eight chromosomes will be completely sequenced.
   c. Europe is currently involved in a similar project, but with other regions of the genome.

F. The Ethical, Legal, and Social Implications (ELSI) Program was established to address certain concerns about the Human Genome Project.

1. Following the initiation of the project, many scientists raised questions about what the information would be used for, as well as how the society and individuals could be protected.
2. The ELSI program began by considering a number of issues, such as the impact of genetic information on individuals, the privacy and confidentiality of genetic information, the impact of genome information and technology on medical practice, and reproductive decision making.
3. The ELSI program is currently focusing on four main areas of concern.
   a. The first is privacy and fairness in the use and interpretation of genetic information.
   b. The second is the transfer of genetic knowledge from the research laboratory to clinical practice.
   c. The third is the issues of informed consent for participants in genetic research.
   d. And the final concern is public and professional education.
4. There are still many issues that must be addressed by ELSI, concerning the results of the Human Genome Project.

G. The two genomes of two bacteria, *Haemophilus influenzae* and *Mycoplasma genitalium*, have already been completely sequenced.
   1. The sequence of the yeast genome was reported in 1996, while *E. coli* was reported in 1997.
   2. The sequence of the *M. genitalium* genome is of great interest, since it is the smallest genome of any living organism.
      a. This genome contains only 482 genes.
      b. Altogether, 260 of the 482 genes, or 54% of the genome, function in three processes, translation, DNA replication, and signal transduction, leaving less than half the genome for all other functions.
   3. Several insights into human disorders and human biology have already been gained, even in the early stages of the Human Genome Project.
      a. For example, previously unknown mechanisms of mutations, like the expansion of trinucleotide repeats, have been useful information about the

nature of several neurodegenerative diseases, including Huntington disease, myotonic dystrophy, and spinal/bulbar muscular atrophy.

b. Increases in gene dosage due to the duplication of small regions of chromosomes have also been identified, and they have been associated with certain disorders such as Charcot-Marie-Tooth syndrome.

c. It has also been discovered that different kinds of mutations in a single gene can give rise to different disorders.

d. Finally, it has also been reported that mutations in members of a gene family can give rise to diseases with related phenotypes.

VI. Biotechnology

A. Recombinant DNA technology companies produce products such as hormones, clotting factors, herbicide-resistant plants, enzymes for food production, and vaccines.

B. In 1982, human insulin was the first human gene product manufactured using recombinant DNA and licensed for therapeutic use.

1. Insulin is a protein hormone that regulates sugar metabolism.

a. An inability to produce insulin results in diabetes.

b. Diabetes, in its severe form, affects more than 2 million individuals in the United States.

2. Transparency 112, page 413, figure 18-18.

a. The functional protein consists of two polypeptide chains, A and B.

b. The A polypeptide chain has 21 amino acids, whereas the B subunit has 30 amino acids.

c. Synthetic genes for the A and B subunits were created by oligonucleotide synthesis, and each oligonucleotide was then inserted into a vector at a position adjacent to the gene encoding the bacterial form of the enzyme, β-galactosidase.

d. Following insertion, the vector was transferred into a bacterial host, where the β-galactosidase gene and the synthetic oligonucleotide were transcribed and translated as a unit, forming a **fusion polypeptide**.

e. The fusion polypeptide consisted of the amino acid sequence for β-galactosidase fused to the amino acid sequence for one of the insulin subunits.
f. The bacterial extracts were then purified and treated with cyanogen bromide, to cleave the fusion protein from the β-galactosidase.
g. Each subunit was produced separately, then they were mixed together, causing the two subunits to fuse, forming an intact active insulin molecule.
h. This purified insulin is then packaged for use by those with diabetes, which take insulin injections one or more times a day.

3. Table 18.3 in the text lists examples of other engineered proteins, which are used for therapeutic reasons.

C. The first generation of recombinant proteins were produced by bacterial hosts.
1. There were many disadvantages to having bacterial hosts produce eukaryotic proteins.
a. For example, bacterial cells are sometimes unable to process and modify eukaryotic proteins, and they cannot add sugars and phosphate groups that are often needed for full biological function.
b. Eukaryotic proteins produced in bacterial hosts often cannot form the proper three-dimensional structure, resulting in the product being inactive.
2. To overcome these disadvantages, second generation methods were produced, that used eukaryotic hosts instead of bacterial hosts.
3. A deficiency in the enzyme alpha-1-antitrypsin is associated with a heritable form of emphysema.
a. In order to produce the alpha-1-antitrypsin, the human gene was cloned into a vector at a site adjacent to a promoter sequence from sheep that regulates the expression of milk-associated proteins; the gene adjacent to this promoter is expressed only in mammary tissues.
b. The fusion gene was then microinjected into sheep zygotes, which were then implanted into foster mothers, resulting in a transgenic sheep that developed normally.

c. After the transgenic sheep developed, and mated, they produced milk that contained high concentrations of the alpha-1-antitrypsin, which could then be isolated and utilized by humans.

D. In plants, vectors have been used to transfer traits for herbicidal resistance to crop plants.

E. Vaccine production is one of the most beneficial applications of recombinant DNA technology.

1. Vaccines stimulate the immune system to produce antibodies against a disease-causing organism and thereby confer immunity against the disease.
2. There are two main types of vaccines in use.
   a. One is the **inactivated vaccine**, which is prepared from killed samples of the infectious virus or bacteria.
   b. The second is the **attenuated vaccines**, which are the live viruses or bacteria that are no longer able to reproduce and cause disease when present in the body.
3. A new type of vaccine, called a **subunit vaccine**, consists of one or more surface proteins of the virus or bacterium.
   a. The protein acts as an antigen to stimulate the immune system to make antibodies against the virus or bacterium.
   b. One of the first licensed subunit vaccines is for a surface protein of hepatitis B, a virus that causes liver damage and cancer.
   c. The gene for hepatitis B protein was cloned into a yeast-expression vector, which was produced within a yeast host cell.
   d. The protein is then extracted and purified and then able to be used by humans.
4. Currently, scientists are trying to produce the hepatitis B surface protein in plants.
   a. Plant-produced vaccines offer several advantages over bacterial-produced vaccines.
   b. They would be inexpensive, less purification would be necessary, and injections would not be required.

5. There have been recent clinical trials that have used genetically engineered potatoes that carry recombinant bacterial antigens.
   a. Human volunteers ate small quantities of these potatoes.
   b. These volunteers did develop antibodies to the antigens, which establishes the validity to this method.
6. Recombinant DNA technology has changed the rate at which new plants and animals are being developed; the transfer of genes between species has also altered the types of changes that can be made.

Lecture Outline

Chapter Nineteen: Genetics of Cancer and Immunology

I. Genes and Cancer

A. Development of cancer

1. Two main properties
   a. Large-scale mutational events
   b. Small-scale mutational events
2. Research

B. Single gene mutations

1. Families with high frequencies of cancer
   a. **Retinoblastoma (RB)**
   b. 1 to 20,000 individuals
   c. Two forms
2. Predisposition to retinoblastoma
   a. Autosomal dominant
   b. Both eyes
   c. Other types of cancer
3. Spontaneous
   a. Not a familial form
   b. One eye
4. **Wilms tumor (WT)** and **Li-Fraumeni syndrome**

C. Alfred Knudson

1. Familial predisposition
   a. If normal allele were to mutate
   b. Only one mutational event is required
   c. Does not always happen
   d. Recessive trait.
2. Sporadic retinoblastoma
   a. Lower frequency
   b. Later age

II. Tumor Suppressor Genes

A. Two ways to regulate mitosis
   1. **Tumor suppressor genes**
   2. **Proto-oncogenes**

B. RB gene
   1. pRb gene product
      a. Regulation of this product
      b. Adding or removing phosphate groups
      c. Phosphorylation
   2. Part of the cell cycle control
      a. G0 or G1
      b. Initiation of the S phase
      c. Mitosis
      d. Experiments

C. Breast cancer
   1. *BRCA1* gene
      a. Autosomal dominant
      b. Increased risk for ovarian cancer
   2. *BRCA2* gene
   3. Inherited cases of breast cancer
   4. G1/S boundary
      a. Same pathway
      b. RAD51
   5. Role in DNA repair
      a. Sporadic cases of breast cancer
      b. Three mutations

D. *p53* gene
   1. Normal control
   2. Normal cells
      a. UV radiation
      b. Cells that have no functional p53 protein

c. Guardian of the genome

III. Oncogenes

A. **Oncogenes**

1. Peyton Rous
   a. **Sarcoma** from chickens
   b. Tumors resulted
   c. "Filterable agent"
2. **Rous sarcoma virus (RSV)**
   a. **Reverse transcriptase**
   b. **Provirus**
   c. New virus particles
   d. **Retroviruses**
3. *src* gene
   a. Induction of sarcoma formation
   b. **Acute transforming viruses**
   c. **Nonacute** or **nondefective** viruses

B. Acute transforming viruses

1. Transparency 113, page 427, figure 19-2
2. *v-onc*, *c-onc*, or proto-oncogene
   a. *v-onc*
   b. *v-src*
   c. *c-src*
3. More than 20 oncogenes identified

C. Three mechanisms

1. Single nucleotide change
   a. Transparency 114, page 429, figure 19-4
   b. Normal *ras* protein
   c. Oncogenic mutations of the *ras*
   d. Somatic mutation
   e. Single amino acid substitution
2. Overexpression.

a. New promoter

b. New upstream regulatory sequences

c. Amplification of proto-oncogene

3. Protein products of proto-oncogenes

IV. A Genetic Model for Colon Cancer

A. Cancer is a multistep process

1. Colon cancer

a. Malignant tumors

b. **Familial adenomatous polyposis (FAP)** and **hereditary nonpolyposis colorectal cancer (HNPCC)**

c. Spontaneous forms

2. Interaction of genetic and environmental factors

B. Number and nature of genetic steps

1. Transparency 115, page 430, figure 19-5

a. Five to seven genes

b. Benign growth

c. Specific sequence

2. *APC* gene

a. Individual with FAP

b. Spontaneous cases

c. Accumulation of other mutations

d. K-*ras* oncogene

e. Other genes in figure 19-5

f. *DCC*, *DPC4*, and *JV18-1*

3. Loss or inactivation of the *p53*gene

C. Metastasis

1. One or two mutations

2. Familial predisposition cases

D. Another inherited form of colon cancer

1. Hereditary nonpolyposis colorectal cancer (HNPCC)

2. *hMSH2*

3. *hMLH1*
4. Two other DNA repair genes
5. Genomic-wide genetic instability
6. Screening of individuals

V. Gatekeeper Genes and Caretaker Genes

A. Two pathways of colon cancer in the FAP and HNPCC
   1. FAP
      a. Accumulation of mutations
      b. Malignant tumor
   2. HNPCC
   3. Mutations in two different gene types
   4. **Gatekeeper genes**
      a. *FAP* gene
      b. Inhibit cell growth
      c. A few genes serve as gatekeeper genes
   5. **Caretaker gene**
      a. Maintain integrity of genome
      b. Genetic instability

VI. Genomic Changes and Cancer

A. Relationship between alterations and development of cancer
B. Selected cases
   1. Leukemias
      a. **Chronic myelogenous leukemia (CML)**
      b. **Philadelphia chromosome**
      c. Janet Rowley
   2. White blood cells
C. Exact location of breakpoints
   1. Look at figures 19-6 and 19-7 in textbook
      a. Proto-oncogene *c-abl* and the gene *bcr*
      b. *c-abl* gene is moved
      c. Hybrid *bcr/c-abl* gene

2. Malignant transformation

VII. The Immune System

A. Genomic alterations

1. Association with the immune system
2. Several billion combinations of DNA sequences

B. Effective barrier

1. **Antibodies**
2. **Antigens**
3. **Epitope**

VIII. Genetic Diversity in the Immune System

A. Production of antibodies in humans

1. Five classes of **immunoglobulins (Ig)**
2. **IgG**
3. **IgA**
4. **IgM**
5. **IgD**
6. **IgE**

B. A typical IgG molecule

1. Transparency 116, page 433, figure 19-8
2. **Heavy chain (H)**
   a. **Variable region (V_H)**
   b. **Constant region (C_H)**
   c. Chromosome 4
3. **Light chain (L)**
   a. Variable region
   b. Constant region
   c. Two different types
      1. **Kappa chain**
      2. **Lambda chain**
4. Disulfide bonds
5. **Antibody combining site**

C. Billions of combinations of antibodies
   1. Genetic recombination
   2. Susumu Tonegawa
      a. L-chains in embryonic cells and antibody-producing cells
      b. Isolated and characterized cloned DNA fragment
      c. L-chain in embryo DNA and in the antibody-producing DNA

D. Coding sequences for kappa and lambda
   1. **L-V (leader-variable)** regions, **J (joining)** regions, and a **C (constant)** region
   2. Each light chain
   3. One of the L-V regions is randomly joined to one of the J genes
      a. Transparency 117, page 435, figure 19-10
      b. Excised and destroyed
      c. L-V-J region becomes an exon
      d. Stable and passed on to all progeny
   4. Diversity in the kappa L-chain
   5. V (variable), D (diversity), J (joining), and C (constant)
      a. 300 different V genes, 10 to 50 different D genes, four different J genes, and five different C genes
      b. V region combines with a D sequence and a J sequence
   6. V-D-J composite
      a. Can be joined by any one of the C segments
      b. Two main ways for joining
         1. Transcription and splicing
         2. Second round of recombination
   7. H-chain proteins

E. Organ transplants between two unrelated individuals
   1. Interaction between cell-surface antigens of donor and immune system of recipient
   2. **Histocompatability antigens**
   3. **HLA (human leukocyte antigen) complex**

F. Four closely linked genes

1. Most highly polymorphic gene systems
2. Recombination events are rare
   a. **Haplotype**
   b. Two HLA haplotypes
   c. Homozygosity is rare

G. Matching of HLA haplotypes
1. Identical twins
2. Survival of transplanted organs
3. Drugs used to improve survival
   a. **Cyclosporine**
   b. Inactivates a T-cell subpopulation

IX. Disorders of the Immune System

A. Genetically determined immunodeficiency disorders

B. **X-linked agammaglobulinemia**
1. Male with B cells and plasma cells absent
2. Lack of circulating antibodies
3. Normal T cell level
4. Bone marrow transplants

C. **T-cell immunodeficiency**
1. B cells are not affected, T cell levels decrease
2. Increase in viral infections
3. Buildup of purines
4. Autosomal dominant trait

D. **Severe combined immunodeficiency syndrome (SCID)**
1. Do not have antibody-mediated or cell-mediated immunity
2. X-linked and autosomal forms.
3. X-linked forms
   a. No antibody production
   b. David (the boy in the bubble)
4. Autosomally inherited SCID form
   a. Neither antibody-mediated nor cell-mediated immunity

b. Recurring infections and gene therapy

E. **Human immunodeficiency virus (HIV)**

1. RNA molecule serves as genetic material
   a. T4 cells
   b. RNA is transcribed in a DNA molecule
2. New viral particles
   a. New round of T-cell infection
   b. Increased susceptibility to infection as well as some forms of cancer
   c. Premature death
3. HIV transmission

Lecture Content

Chapter Nineteen: Genetics of Cancer and Immunology

I. Genes and Cancer

A. A small number of genes must be mutated in order to bring about the development of cancer and maintain the growth of malignant cells.

1. Cancer has two main properties, uncontrolled cell division and the ability to spread or metastasize, which are the results of genetic alterations.

a. The alterations can be large-scale mutational events such as chromosome loss, rearrangement, or the insertion of foreign DNA sequences in human chromosomes.

b. Small-scale alterations may also be involved, including changes in the nucleotide sequence, or more subtle modifications that alter only the amount of a gene product that is present or the time over which the gene product is active.

2. Research has been focused on two main questions, whether or not there are mutant alleles that predispose an organism or specific cell types to cancer, and how many mutational events are necessary to cause cancer.

B. Single gene mutations indeed predispose cells to becoming malignant.

1. Families with high frequencies of certain types of cancer have been studied extensively.

a. One example of such a predisposition is the inheritance of **retinoblastoma (RB)**, which is a cancer of the retinal cells of the eye.

b. This form of cancer occurs in 1 to 20,000 individuals, and usually is present in the ages between 1 and 3 years.

c. There are two forms of retinoblastoma known.

2. One form of retinoblastoma is a predisposition to retinoblastoma, which is the cause for approximately 40% of all cases.

a. This form is inherited as an autosomal dominant trait, although the disorder itself is recessive.

b. Individuals who inherit the RB allele are predisposed to develop eye tumors, which usually occur in both eyes.

c. Family members who inherit the mutant allele are predisposed to develop other types of cancer as well, including osteosarcoma, even if they do not develop retinoblastoma.

3. The second form of retinoblastoma accounts for approximately 60% of all cases.
   a. This is not a familial form, and tumors develop spontaneously.
   b. This form is characterized by the formation of tumors in only one eye, and the onset generally occurs at a much later age than the familial form.
4. Table 19.1 in the text lists other types of dominant predispositions to cancer, including **Wilms tumor (WT)**, a cancer of the kidney, and **Li-Fraumeni syndrome**, a rare condition that predisposes to a number of different cancers.

C. Alfred Knudson and his colleagues have developed a model that requires the presence of two mutant copies of the RB gene in the same retinal cell for tumor formation to occur, by studying the two different types of retinoblastoma.
1. Within the familial predisposition, one mutant RB allele is inherited and carried by all the cells of the body.
   a. If the normal allele were to mutate in a retinal cell, formation of retinal tumors will result.
   b. Therefore, individuals carrying the inherited single mutant of the RB gene are predisposed to develop RB because only one mutational event is required to cause tumor formation.
   c. This does not always happen; in fact 10% of those who inherit the mutant allele do not develop RB.
   d. This model explains how retinoblastoma can be inherited as a recessive trait, but act as a dominant trait, in that it predisposes individuals to cancer.
2. In the case of sporadic retinoblastoma, both copies of the RB gene must undergo mutation in the same retinal cell in order to produce a tumor.
   a. Two mutational events in the same cell occur at a much lower frequency than just one mutation.
   b. Therefore, the sporadic cases develop at a later age and usually in only one eye.

II. Tumor Suppressor Genes

A. There are two ways that mitosis can be regulated, by genes that normally function to suppress cell division, and by genes that normally function to promote cell division.
   1. The first group is called **tumor suppressor genes**, which inactivate or repress passage through the cell cycle, resulting in cell division.
      a. These genes and their products must be absent or inactive for cell division to result.
      b. On the other hand, if they become permanently inactivated or deleted by mutation, control over cell division is lost, and the mutant cell begins to proliferate uncontrollably.
   2. The second class of genes are known as **proto-oncogenes**, which function to promote normal cell division.
      a. These genes can be turned "on" or "off," when they are "on" they promote cell division, and they must be inactivated to halt cell division.
      b. If they become permanently switched on, then uncontrolled cell division occurs, leading to tumor formation.
      c. **Oncogenes** are the mutant forms of proto-oncogenes.

B. The RB gene is located on chromosome 13 and encodes a protein of 928 amino acids.
   1. The pRb gene product is found in all cell and tissue types, and is found in both resting and proliferating cells.
      a. The regulation of this product occurs by reversible chemical modifications to the RB gene product, and not by transcriptional control.
      b. Adding or removing phosphate groups regulates the activity of the pRb protein.
      c. The pRb is phosphorylated in the S phase and the G2/M junction of the cell cycle, but is not phosphorylated in the G0 and G1 stages of the cell cycle.
   2. The observation that this phosphorylation occurs in synchrony with the cells cycle, suggests the pRb might be part of the cell cycle control at G1.
      a. In cells that are at G0 or G1, pRb is not phosphorylated, and the protein is active and stops passage through the cell cycle.

b. Following the initiation of the S phase, pRb experiences the addition of a phosphate group and becomes inactive.
c. Cells pass through the S phase and the subsequent events leading to mitosis, when the pRb activity is suppressed.
d. There have been experiments that have confirmed this hypothesis, in which normal copies of the RB gene have been introduced into cultured tumor cells, resulting in the production of pRb and the termination of cell division.

C. There is a small portion of breast cancer cases, especially those in young women, which are related to the inheritance of dominant genes that confer a predisposition to the disease.
1. The *BRCA1* gene, which is located on the long arm of chromosome 17, is associated with a predisposition to breast cancer when mutated.
a. This is inherited as an autosomal dominant trait.
b. Approximately 90% of women with a mutant *BRCA1* gene will develop breast cancer; they also have an increased risk for ovarian cancer.
2. The *BRCA2* gene, located on the long arm of chromosome 13, is also inherited as an autosomal dominant predisposition to breast cancer, but there is not an increased risk for ovarian cancer.
3. Together, these genes contribute to a large majority of inherited cases of breast cancer, but have little or nothing to do with sporadic cases of breast and ovarian cancer.
4. Both the *BRCA1* and *BRCA2* genes are expressed in rapidly dividing cells, especially at the G1/S boundary in the cell cycle.
a. The common pattern of expression, the similar phenotype of the mutant alleles, and the experimental evidence from knockout mice, has indicated that both genes may work in the same pathway.
b. The *BRCA1* and *BRCA2* proteins both bind to another protein, RAD51, which is involved in the repair of breaks in the double-stranded DNA, which may explain their normal function as tumor suppressor genes.
5. The role of both the *BRCA1* and *BRCA2* gene products in DNA repair may also explain why they are not associated with sporadic cases of breast cancer.

a. At least three mutations must occur in a breast cell for it to become cancerous in the sporadic cases.
b. For this to occur, both copies of *BRCA1* and *BRCA2* would need to be mutant, and at least one other mutation a the cell cycle regulatory gene would have to occur.

D. The *p53* gene is a type of tumor suppressor gene, which encodes a nuclear protein that acts as a transcription factor.
1. The *p53* gene normally controls the passage of the cell from G1 into the S phase of the cell cycle.
a. Mutations in this gene can result in a wide range of cancers, including breast, lung, bladder, and colon cancers.
b. It is estimated that over half of the cancers are associated with mutations in the *p53* gene.
2. Normal cells contain low concentrations of p53 protein, but the concentration rises dramatically after irradiation of cells by ultraviolet radiation.
a. Following irradiation with UV radiation, cells normally arrest in the G1 phase to allow for repair of damaged DNA caused by the UV radiation.
b. The cells that have no functional p53 protein move directly from G1 into the S phase without repairing their DNA damage, resulting in a high frequency of mutation.
c. Studies of this gene have led to the conclusion that *p53* controls the passage through the cell cycle to ensure that DNA damage is repaired before the cell enters the S phase; therefore, *p53* is often referred to as a guardian of the genome.

III. Oncogenes

A. **Oncogenes**, the mutant form of proto-oncogenes, induce or maintain uncontrolled cellular proliferation associated with cancer.
1. Peyton Rous postulated the existence of specific genes associated with the transformation of normal cells into cancerous cells in 1910.
a. He used cells from a tumor of chickens known as **sarcoma**, and injected the cell-free extracts to into normal healthy chickens.

b. The result of this experiment was that the healthy chickens formed tumors.
c. Rous postulated the existence of a "filterable agent" that was responsible for transmitting the disease.

2. Investigators later showed that this filterable agent is a virus, known as the **Rous sarcoma virus (RSV)**.
   a. The RNA genome of the viruses is first transcribed into a single-stranded DNA molecule by the enzyme **reverse transcriptase**.
   b. The DNA is then converted into a double-stranded DNA molecule that integrates into the genome of the infected cell forming a **provirus**.
   c. The viral DNA can be transcribed to produce RNA at a later time, and is packaged into viral proteins to form new virus particles.
   d. RSV and related viruses are known as **retroviruses** because they "reverse" the normal flow of genetic information.
3. The tumor-forming ability in the RSV results from a single gene, the *src* gene, acquired from the host genome.
   a. This gene is responsible for the induction of sarcoma formation in the chickens, and is designated as an oncogene.
   b. Retroviruses that carry oncogenes are known as **acute transforming viruses**.
   c. Other retroviruses, which do not carry oncogenes, but are able to induce the activity of cellular genes that brings about tumor formation, are known as **nonacute** or **nondefective** viruses.

B. Acute transforming viruses acquire oncogenes from the host's genome during the process of infection, when a portion of the viral genome and host genome are exchanged.
   1. Transparency 113, page 427, figure 19-2.
   2. The oncogenes carried by retroviruses are called *v-onc*, whereas the normal cellular version of the gene is called a *c-onc*, or proto-oncogene.
      a. Retroviruses that carry the *v-onc* are able to infect and transform a specific type of host cell.

b. For example, the oncogene in RSV captured from the chicken genome is called *v-src*, and confers the ability to transform chicken cells following injection.

c. The normal version of the same gene is called *c-src*.

3. There have been more than 20 oncogenes identified in retroviral genomes, and over 50 oncogenes identified overall.

C. There are at least three mechanisms that explain the conversion of proto-oncogenes into oncogenes, including point mutations, translocations, and overexpression.

1. The *ras* gene family encodes a protein of 189 amino acids that is involved in the transduction of signals across the plasma membrane, illustrates an example of how a single nucleotide change can convert a *c-onc* sequence into the oncogenic version.

a. Transparency 114, page 429, figure 19-4.

b. The normal *ras* protein functions as a molecular switch, that alternates between the "on" and "off" positions.

c. When oncogenic mutations of the *ras* force the gene to become stuck in the "on" position, the cell is stimulated for growth.

d. In some tumors, this is a result of a somatic mutation, and does not involve a retrovirus.

e. All mutant *ras* gene products have a single amino acid substitution at position 12 or 61, that is caused by a single nucleotide change in the *ras* gene.

2. There are at least three separate mechanisms of proto-oncogene activation associated with overexpression.

a. First, the *c-onc* may acquire a new promoter, which causes an increase in the level of transcript production or activating a silent locus.

- This is the case in avian leukosis.
- Strong viral promoters become integrated upstream from a proto-oncogene, causing an increase in mRNA production and in the amount of the gene product.

b. The second mechanism of overexpression involves the acquisition of new upstream regulatory sequences, including enhancers.

c. The third mechanism involves the amplification of the proto-oncogene.

3. The protein products of proto-oncogenes are found in, and are associated with, the plasma membrane, cytoplasm, and nucleus.

IV. A Genetic Model for Colon Cancer

A. It is clear that cancer is a multistep process, resulting from a number of specific genetic alterations.

1. The study of colon cancer offers several advantages over studies of tumors such as Wilms tumor and retinoblastoma.

a. First, malignant tumors of the colon and rectum develop from preexisting benign tumors, and tumors of all stages of development are available for study.

b. Secondly, there are two forms of genetic predisposition to colon cancer known: an autosomal dominant trait known as **familial adenomatous polyposis (FAP)**, and a genetically complex trait known as **hereditary nonpolyposis colorectal cancer (HNPCC)**.

c. There are also several spontaneous forms of colon cancers.

2. This array of causes allows for the study of the interaction of genetic and environmental factors in the genesis of tumors.

B. The number and nature of the genetic steps involved in changing normal intestinal epithelial cells into tumor cells has been defined through an analysis of mutations in tumors.

1. Transparency 115, page 430, figure 19-5.

a. Mutations in five to seven genes are required to induce malignant growth.

b. When fewer changes are present, benign growth or intermediate stages of tumor formation results.

c. The order of mutations usually follows a specific sequence, which suggests that both the accumulation of mutations and the order in which they occur are important in the development of colon cancer.

2. A mutation in the *APC* gene, a tumor suppressor gene located on the long arm of chromosome 5, converts normal epithelium into benign tumors, called adenomas.

a. An individual with FAP, inherits a single *APC* mutation, and develops hundreds or even thousands of benign adenomas in the colon and rectum.
b. Evidence suggests that spontaneous cases are the result of clones of cells with a single cell mutational event.
c. Accumulation of other mutations can also result in cancerous tumors.
d. A mutation in one copy of the K-*ras* oncogene is enough to cause progression from an early adenoma to an intermediate adenoma, which is a benign tumor with many fingerlike villous outgrowths.
e. Other genes in figure 19-5, are all tumor suppressor genes, but both alleles of these genes must be mutated for tumor progression.
f. The 18q21 region contains a number of tumor suppressor genes involved in colon cancer, including *DCC, DPC4,* and *JV18-1*; and mutations in at least one of these genes results in the formation of late adenomas.

3. Finally, a mutation involving the loss or inactivation of the *p53*gene causes the transition to a cancerous cell.
   a. Mutations in the *p53* gene are pivotal to the development of a number of cancers.
   b. The *p53* gene product has DNA-binding properties, and mutations in this gene may alter DNA binding to confer a new function of the gene product.

C. The process of metastasis occurs after the formation of cancerous cells and involves an unknown number of mutational steps.
   1. Current studies suggest that one or two mutations may be sufficient to allow tumor cells to detach and spread to secondary sites.
   2. In cases where there is a familial predisposition to colon cancer, the first mutation is inherited, and the rest occur as a result of the action of environmental factors.

D. Recent work on another inherited form of colon cancer has suggested that mutations in DNA repair genes may be important in destabilizing the genome, and allowing mutations to accumulate.
   1. Hereditary nonpolyposis colorectal cancer (HNPCC) has been mapped by linkage analysis to loci at 2p16 and 3p21.

a. This is one of the most common forms of human hereditary disorders, affecting approximately 1 in 200 individuals.
b. A cascade of mutations in short, tandemly repeated microsatellite sequences located throughout the genome are a result of mutations at these two loci.

2. A DNA repair gene called *hMSH2* is associated with HNPCC.
   a. This gene is located on chromosome 2.
   b. The inactivation of this gene causes a rapid accumulation of mutations, resulting in the development of colorectal cancer.
3. Another DNA repair gene associated with HNPCC is called *hMLH1* and is located on chromosome 3.
4. There are at least two other DNA repair genes related to *hMLH1 and hMLH2*, but they have not yet been mapped.
5. Genomic-wide genetic instability is promoted by mutations in any of these repair genes, accelerating the rate at which mutations accumulate, with colon cancer as one of the outcomes.
6. The discovery of the DNA repair genes and their mutations will make it possible to screen individuals with a family history of colon cancer to identify those who have inherited a mutant allele, and are thus at a high risk for colon cancer.

V. Gatekeeper Genes and Caretaker Genes

A. Insights to the nature of genes that control cancer predisposition are offered by the two pathways to colon cancer in the FAP and HNPCC.
   1. In FAP, an inherited or acquired mutation in the *APC* gene causes the formation of thousands of adenomas.
      a. The adenomas then progress slowly into a malignant condition by the accumulation of mutations in other genes.
      b. This results in a high risk that at least one will progress to form a malignant tumor.
   2. In HNPCC, adenomas form at slow rate during the aging process, however mutations do accumulate at a magnitude of two to three times faster than in normal cells.

3. It was these differences that led to the idea that mutations in two different gene types cause predisposition to cancer.
4. One of these types of genes is known as **gatekeeper genes**.
   a. The *FAP* gene is an example of a gatekeeper gene; in fact, most tumor suppressor genes are gatekeeper genes.
   b. They inhibit cell growth or promote cell death.
   c. In different types of cells, only a few genes serve as gatekeeper genes, and if both copies are mutated, a specific cancer develops.
5. The other type of gene that causes predisposition to cancer is known as a **caretaker gene**.
   a. These genes maintain the integrity of the genome, such as DNA repair genes.
   b. Mutation of the caretaker genes does not promote tumor formation directly, but leads to genetic instability, which increases the rate of mutation for all genes.

VI. Genomic Changes and Cancer

A. Even though alterations in chromosome number and/or structure are associated with many forms of cancer, the relationship between these alterations and the development of cancer is not clear.
B. In selected cases, the relationship between the chromosome aberration and the development and/or maintenance of the cancerous state is known.
   1. Leukemias exhibit the clearest connection between chromosome aberrations and cancer.
      a. **Chronic myelogenous leukemia (CML)** is one of the best studied examples of the association between chromosome rearrangement and cancer development.
      b. The translocation involved in this type of leukemia was originally described as an abnormal chromosome 21, and was called the **Philadelphia chromosome**.
      c. Janet Rowley demonstrated that the Philadelphia chromosome results from the exchange of genetic material between chromosomes 9 and 22.

2. This translocation is only seen in white blood cells, and the observations indicate that the translocation is a primary and causal even in the generation of CML, which may occur from a single cell bearing this translocated chromosome.

C. The exact location of the breakpoints on chromosomes 9 and 22, were established by examination of a large number of cases involving the Philadelphia chromosome.

1. Look at figures 19-6 and 19-7 in the textbook.
   a. By using recombinant DNA techniques in association with genetic mapping studies, the chromosomal maps were created, revealing that the proto-oncogene *c-abl* maps to the breakpoint region on chromosome 9, and the gene *bcr* maps near the breakpoint on chromosome 22.
   b. Most, if not all, of the *c-abl* gene is moved to a region within the *bcr* gene during the translocation event.
   c. This results in the formation of a hybrid *bcr/c-abl* gene that is transcriptionally active, producing a hybrid 200-kDa product, which has been implicated in the generation of CML.
2. In most cases, transcription results in the formation of a hybrid gene that is transcribed to form a gene product, which causes the cell to undergo a malignant transformation even though a second normal copy of the gene is present and active.

VII. The Immune System

A. Genomic alterations are also important events in the maturation and function of the immune system.

1. Alterations such as rounds of recombination associated deletion of DNA sequences in somatic cells, and a lack of precision in cutting and joining segments during excision are examples of alterations that occur in the immune system.
2. Genomic alterations associated with the immune system allow the body to produce several billion combinations of DNA sequences that can encode for antibodies and cell surface receptors.

B. The immune system is an effective barrier against the invasion of potentially harmful foreign substances such as viruses, bacteria, fungi, and parasites, which are eventually destroyed by the immune system.
   1. The response of the immune system usually involves **antibodies**, which are proteins produced and secreted by specific cells of the immune system.
   2. **Antigens** are agents that elicit antibody production.
   3. An **epitope**, is a distinctive structural feature of an antigen that stimulates antibody production.

VIII. Genetic Diversity in the Immune System

A. Antibodies in humans are produced by plasma cells, a type of lymphocyte, or white blood cell.
   1. There are five classes of antibodies, or **immunoglobulins (Ig)** that are known.
   2. The first class is **IgG**, which represents approximately 80% of all antibodies found in the blood.
      a. IgG is also the best-characterized antibody at this time.
      b. IgG is associated with immunological memory.
   3. The **IgA** class is found in breast milk, can cross plasma membranes, and is associated with immunological resistance to infections of the respiratory and digestive tracts.
   4. The **IgM** antibodies are usually the first to be secreted in response to an antigen, and are associated with the early stages of the immune response.
   5. There is little known about the **IgD** antibodies, except that they are associated with the surface of B cells and may regulate their action.
   6. The last class, **IgE**, is involved in fighting parasitic infections, as well as allergic responses.

B. A typical IgG molecule consists of two polypeptide chains, with each present in two copies.
   1. Transparency 116, page 433, figure 19-8.
   2. The larger or **heavy chain (H)** contains approximately 440 amino acids.
      a. The sequence of the first 110 amino acids at the N-terminus differs among heavy chains, and is known as the **variable region (V_H)**.

b. The remaining C-terminal amino acids are the same length in all H chains, and make up the **constant region (C_H)**.

c. The H chains are encoded by genes on the long arm of chromosome 4 in humans.

3. The **light chain (L)** is composed of 220 amino acids.

a. The first 110 amino acids make up the variable region.

b. The remaining amino acids at the C-terminus make up the constant region.

c. There are two different types of L chains.

- One type of chain is the **kappa chain**, which is encoded by genes on the human chromosome 2.
- The other type of chain is the **lambda chain**, which is encoded by the genes present on the human chromosome 22.

4. The functional IgG molecule is made up of two light and two heavy chains, which are held together by disulfide bonds.

5. The variable regions of both the heavy and the light chains, form the **antibody combining site**, which has a unique formation for a specific antigen.

C. There are billions of combinations of antibodies, and it is impossible for each combination to be coded directly within the genome.

1. The diversity of antibodies results from the genetic recombination between three clusters of antibody genes, the H-chain genes on chromosome 14, the kappa L genes on chromosome 2, and the lambda L genes on chromosome 22.

a. As antibody-forming B cells mature, DNA recombination rearranges these genes so that each mature B lymphocyte comes to encode, synthesize, and secrete only one specific type of antibody.

b. When a B cell is stimulated, by the presence of an antigen, it encodes an antibody against the antigen to divide and differentiate, resulting in populations of differentiated plasma cells, all of which synthesize and one type of antibody to interact with the antigen.

2. Susumu Tonegawa and his colleagues provided evidence for genetic recombination in antibody genes in the mid-1970s.

a. They compared the size of restriction fragments, and demonstrated that DNA segments coding for parts of the L chain gene are far apart in embryonic cells, but are adjacent in antibody-producing cells.
b. They isolated and characterized a cloned DNA fragment from an embryonic cell, and compared its organization with the equivalent region isolated from an antibody-producing cell.
c. The variable region of the L-chain in embryo DNA was separated by 4.5 kb of DNA from another part of the L chain, whereas in the antibody-producing DNA, the regions were joined forming a single transcription unit encoding a specific L chain.

D. The coding sequences for kappa and lambda have been isolated from the germ line and B cell DNA in a variety of higher organisms.
1. The basic organization is similar in most mammals, and consists of several elements, **L-V (leader-variable)** regions, **J (joining)** regions, and a **C (constant)** region.
2. Each light chain consists of 70 to 300 V-L segments, each with a different nucleotide sequence; the J region contains six different segments, while the C region only has one.
3. One of the 300 L-V regions is randomly joined by a recombination event to one of the six J genes to form a functional light chain gene, during the maturation of a B cell.
a. Transparency 117, page 435, figure 19-10.
b. The remaining gene segments are then excised and destroyed.
c. The newly created L-V-J region becomes an exon in a gene that includes the C region; then, this light-chain gene is transcribed and translated to form kappa L chains, which will become part of an antibody molecule.
d. The rearranged gene is stable and passed on to all progeny of the B cell.
4. Diversity in the kappa L-chain production is a result of combining any of the 300 V genes with any of the six J segments, which generates about 1800 different kappa genes.

5. The heavy-chain gene in humans extends over a large region of DNA and include for types of segments, V (variable), D (diversity), J (joining), and C (constant).
   a. There are 300 different V genes, 10 to 50 different D genes, four different J genes, and five different C genes, one for each class of immunoglobulins.
   b. Recombination randomly joins a V region with one of the D sequences and one of the J sequences, during B cell maturation.
6. This V-D-J composite lies adjacent to the C regions of the five classes of heavy chains.
   a. A V-D-J segment can then be joined by any one of the five C segments.
   b. There are two main ways in which this joining can occur.
      - One way is by transcription of a long mRNA molecule that begins at the 5'-end of the V region and terminates at the 3'-end of the C segments; splicing this pre-mRNA yields functional mRNA molecules.
      - The alternative method involves a second round of recombination, as well as excision of the H gene that places one of the C segments adjacent to the joined V-D-J segment, eliminating the other C segments.
7. A large number of H-chain proteins are generated due to the random recombination events that occur.
   a. The potential for overall antibody diversity is calculated by the combination of all heavy-chain genes (30,000) and all light-chain genes (3600).
   b. This results in 108 million possible antibody genes.

E. Organ transplants between two unrelated individuals are usually rejected within weeks, and are rejected even faster on the second try, indicating the role of the immune system with grafts.
   1. The interaction between the cell-surface antigens of the donor and the immune system of the recipient determines whether the graft will be accepted or rejected.
   2. These antigens are known as **histocompatability antigens**, which are encoded by 20 to 40 genes in laboratory mice.

3. There is a group of closely linked genes on chromosome 6 in humans, known as the **HLA (human leukocyte antigen) complex**, which plays a critical role in histocompatability transplants.

F. The four closely linked genes in the HLA complex are known as HLA-A, HLA-B, HLA-C, and HLA-D, which is subdivided into the HLA-DR, HLA-DQ, and HLA-DP genes.
   1. There are at least 23 alleles in the HLA-A, 47 in HLA-B, 8 in HLA-C, 14 in HLA-DR, 3 in HLA-DQ, and 6 in HLA-DP, making this one of the most highly polymorphic gene systems in the human genome.
      a. Each allele encodes a specific antigen identified by a letter and a number.
      b. For example, A6 is allele number 6 at the HLA-A locus.
   2. Because these genes are so closely linked, recombination events are extremely rare, and the allelic combination on a single chromosome tends to inherited as one codominant unit.
      a. A **haplotype** is the array of HLA alleles on a given copy of chromosome 6.
      b. Humans have two copies of every chromosome; therefore, they have two HLA haplotypes, which are fully expressed, since they are inherited in a codominant fashion.
      c. Since there is such a large number of alleles possible, it is rare that any one would be homozygous at any of the loci.

G. When a transplant is performed, the doctors try to match the HLA haplotypes of the donor and recipient.
   1. Identical twins will always have a perfect match, parents on the other hand, only have one haplotype, and siblings have a one-in-four chance of a perfect match.
      a. The order of preference for organ and tissue donors among relatives is an identical twin, a sibling, a parent, and then an unrelated donor.
      b. Since HLA allele frequency differs widely between racial groups, matches between groups is often difficult.
   2. The survival of transplanted organs is improved dramatically when HLA types are matched.

3. Currently, there are drugs used to improve the survival of transplants even when HLA matching is not perfect.
   a. The most widely used drug is **cyclosporine**, which was first isolated from a soil fungus.
   b. This drug selectively inactivates the T-cell subpopulation that is most active in tissue rejection, without damaging other cells.

IX. Disorders of the Immune System

A. Mutations that inactivate or destroy some component of the immune system cause genetically determined immunodeficiency disorders.

B. **X-linked agammaglobulinemia** is a genetic disorder in which B cells are missing.
   1. Affected individuals are almost always male, the age of onset is usually around 6 to 12 moths, both B cells and plasma cells are absent, or immature B cells are present.
   2. There is a complete lack of circulating antibodies, as well as a lack to produce antibodies, resulting in a high susceptibility rate for infections from microorganisms and bacteria.
   3. These individuals do have a normal T cell level, which can confer immunity to most viral infections.
   4. Bone marrow transplants are currently the best form of treatment for this disorder.

C. There is a genetically controlled form of **T-cell immunodeficiency**, where affected individuals have a deficiency of the enzyme nucleoside phosphorylase.
   1. The number of B and T cells is normal at birth, but the T cells gradually declines from there, whereas the B cells are not affected.
   2. An increase in viral infections is present with the absence of T-cell immunity, as well as a high risk for some forms of cancer.
   3. It has been suggested that the decline in T cells is caused by the buildup of purines as a result of the enzyme deficiency and that the resulting toxicity is what kills the T cells.
   4. This is inherited as an autosomal dominant trait, with the gene located on the long arm of human chromosome 14.

D. **Severe combined immunodeficiency syndrome (SCID)** is when T- and B-cell populations are absent or nonfunctional.
   1. Affected individuals do not have either antibody-mediated or cell-mediated immunity.
   2. There are X-linked forms as well as autosomal forms of SCID.
   3. In the X-linked forms, persistent infections begin about 6 moths after birth, with no T-cells present.
      a. There are normal or even elevated levels of B cells, but there is no antibody production in them.
      b. The longest surviving individual with this form of SCID was a boy named David, (the boy in the bubble).
   4. In the autosomally inherited SCID form, there is a deficiency in the enzyme adenosine deaminase (ADA), there are no T cells present, and the B cells are not functional.
      a. The result is again an individual with neither antibody-mediated nor cell-mediated immunity.
      b. Recurring infections lead to death by the age of 7 months, but many affected children are now receiving gene therapy to provide them with a normal copy of the gene.

E. AIDS is a collection of disorders that develop as a result of infection with the retrovirus known as the **human immunodeficiency virus (HIV)**.
   1. The virus consists of a protein coat, which encloses an RNA molecule that serves as the genetic material, and an enzyme reverse transcriptase.
      a. The virus selectively infects a subset of T lymphocytes known as T4 cells.
      b. Once the virus is inside the cells, the RNA is transcribed into a DNA molecule by reverse transcriptase, and the viral DNA is inserted into a human chromosome, where it may remain for months or years.
   2. Later, when the infected T cell participates in an immune response, the cellular DNAs are transcribed, and the viral RNA transcript is translated into viral proteins, forming new viral particles.

a. These viral particles bud off the surface of the T cell, rupturing and killing the cell, while setting off a new round of T-cell infection.
b. Over time, there is a decrease in the number of helper T cells, causing a decrease in the ability to mount an immune response, resulting in increased susceptibility to infection and increased risk of certain forms of cancer.
c. The outcome is premature death by any of a number of diseases that overwhelm the body and its compromised immune system.

3. HIV is transmitted through body fluids such as blood, semen, vaginal secretions, and breast milk.
   a. The virus is not viable for more than 1 to 2 hours outside the body, and cannot be transmitted through food, water, or casual contact.
   b. Treatment options are currently limited, and include drugs that are somewhat effective in slowing or stopping reproduction of HIV for an uncertain amount of time.

Lecture Outline

Chapter Twenty-one: Population Genetics

I. Foundation for the Modern Interpretation of Evolution

A. The modern interpretation of evolution

1. Population numbers remain relatively constant
   a. Competitive struggle for survival
   b. Theory of natural selection
2. **Population genetics**
3. Variation at the protein and DNA levels

II. Populations and Gene Pools

A. **Population**

1. **Gene pool**
2. Alleles of the gene

B. Measurement of allele and genotype frequencies

1. Gametes
2. Succeeding generation

C. Populations are dynamic

III. Calculating Allele Frequencies

A. Measurement for the frequency of an allele

1. Codominant fashion
2. **MN blood groups**

B. L^M and L^N

1. Production of a distinct antigen
2. M ($L^M L^M$), N ($L^N L^N$), or MN ($L^M L^N$)

C. Frequency of the M and N alleles

1. 100 individuals with 36 type M, 48 type MN, and 16 type N
2. 72 *M* alleles and 48 *M* alleles
3. *M* allele is (120/200) = 0.6 = 60%
4. *N* allele is (80/200) = 0.4 = 40%

D. Transparency 118, page 475, table 21.2

1. Two methods for computing M and N

2. Table 21.3 in the textbook

IV. The Hardy-Weinberg Law

A. One recessive allele

B. Mathematical model to calculate allele frequencies

1. **Hardy-Weinberg Law (HWL)**
2. Three important properties

C. Several assumptions

1. Large population
2. Random mating
3. No selection
4. No mutation, migration, or genetic drift

D. Ideal population

1. *A* allele is represented by p and the *a* allele is represented by q
   a. Transparency 119, page 475, figure 21-1
   b. $p + q = 1$
2. $p \times p = p^2$
   a. $(p \times q) + (p \times q) = 2pq$
   b. $q \times q = q^2$
3. The p^2 value is the probability that both gametes carry the *A* allele
   a. $2pq$ is the frequency of Aa heterozygotes and q^2 is the frequency of the homozygous recessive (*aa*)
   b. The distribution of homozygous and heterozygous genotypes is

$$p^2 + 2pq + q^2 = 1$$

4. A new population with 70% *A* and 30% *a*
   a. $p = 0.7$ and $q = 0.3$, and $p(0.7) + q(0.3) = 1$
   b. Transparency 120, page 476, figure 21-2
   c. New generation
   d. Frequency of *A* allele

$$p^2 + \tfrac{1}{2}(2pq)$$
$$0.49 + \tfrac{1}{2}(0.42)$$
$$0.49 + 0.21 = 0.70$$

   e. Frequency of *a* allele

$q^2 + \frac{1}{2}(2pq)$
$0.09 + \frac{1}{2}(0.42)$
$0.09 + 0.21 = 0.30$
or
$q = 1 - p$
$= 1 - 0.70 = 0.30$

5. Hardy-Weinberg conditions are assumed
6. **Genetic equilibrium**
7. Not all alleles are in equilibrium
   a. Dominant traits
   b. Genetic equilibrium and genetic variability
   c. Allelic frequencies remain unchanged

E. Equilibrium of genotypes
1. Heterozygotes must be identified phenotypically
2. MN blood group in the Australian aborigines
   a. Table 21.3 in the text
   b. Frequencies must be calculated

Expected frequency of type M: $= p^2$
$= (0.178)^2 = 0.032$
$= 3.2\%$

Expected frequency of type MN: $= 2pq$
$= 2\ (0.178)(0.822) = 0.292$
$= 29.2\%$

Expected frequency of type N: $= q^2$
$= (0.822)^2 = 0.676$
$= 67.6\%$

   c. **Expected frequencies** and **observed frequencies**
3. If the population is not in equilibrium
   a. 500 Australian aborigines and 500 Native Americans
   b. Frequency of the *M (p)* would be

$0.315 + \frac{1}{2}(0.324) = 0.477$

   c. Frequency for the *N (q)* would be

$0.361 + \frac{1}{2}(0.324)\ 0.523$

d. Expected frequencies MM (p^2) 22.8%, MN ($2pq$) 49.8%, and NN (q^2) 27.4%

e. Lack of random mating

4. How long for populations to reach equilibrium

a. One generation

b. Transparency 121, page 477, figure 21-3

c. Expected frequencies after one generation

V. Extensions of the Hardy-Weinberg Law

A. X-linked genes

1. Some species have two X chromosomes

2. Distributed unequally

3. Females carry two-thirds of all X-linked genes, while males carry one-third

B. Males only have one copy

1. X-linked color blindness has a frequency of 8% in males

2. Females have two doses

a. Frequency is 0.08 in males at equilibrium and females have q^2, or 0.0064

b. 800 out of 10,000 males are color-blind

3. Not in equilibrium

a. Not ascertained in only one generation

b. Frequency in females determines the frequency in males

c. Daughters

d. Overall frequency

e. Look at figure 12-4 in the textbook

C. Several alleles of a single locus

1. ABO blood group

a. Three alleles (I^A, I^B, and I^O)

b. *A* and *B* alleles are codominant and dominant over the *O* allele

c. Homozygous *AA* and heterozygous *AO* individuals are phenotypically identical

2. Another variable added to the H-W equation

a. Frequency of three alleles

$$p\,(A) + q\,(B) + r\,(O) = 1$$

distribution of the genotypes

$$(p + q + r)^2$$

b. Genotypes *AA*, *AB*, *AO*, *BB*, *BO*, and *OO*

$$p^2(AA) + 2pq(AB) + 2pr\ (AO) + q^2(BB) + 2qr(BO) + r^2(OO) = 1$$

3. Frequencies for the three alleles of the ABO system

a. A = 0.53, B = 0.13, and O = 0.26

b. Frequency of type O blood, r^2

$$r^2 = 0.26$$

$$r = (0.26)^{1/2}$$
$$= 0.51$$

c. Frequencies for the *A (p)* and *B (q)* alleles

d. Frequency of the *AA* genotype is p^2, and *AO* genotype is $2pr$

The frequency of $A + O = p^2 + 2pr + r^2$

This can be arranged to give

$$p = (\text{Frequency of } A + O)^{1/2} - r$$
$$= (0.53 + 0.26)^{1/2} - 0.51$$
$$= 0.89 - 0.51 = 0.38$$

e. Frequency for the *B* allele

$$p + q + r = 1$$

$$q = 1 - (p + r)$$

$$= 1 - (0.38 + 0.51)$$

$$= 1 - 0.89 = 0.11$$

VI. Using the Hardy-Weinberg Law: Calculating Heterozygote Frequency

A. Recessive phenotype

B. Albinism

1. Lack of pigment in their skin, hair, and irises

2. Homozygous for the recessive allele

$$(q^2)^{1/2} = (0.0001)^{1/2}$$

$$q = 0.01 \text{ or } 1/100$$

3. Frequency for p

$$p = 1 - q$$
$$= 1 - 0.01$$
$$= 0.99 \text{ or } 99/100$$

4. Frequency for heterozygotes

$$2pq = 2\,[(0.99)(0.01)]$$
$$= 0.02 \text{ or } 2\% \text{ or } 1/50$$

5. Heterozygotes for albinism are common

C. Frequencies of all three genotypes

1. Transparency 122, page 480, figure 21-5
2. Heterozygotes increase dramatically in a population
3. Heterozygotes constitute the major class of the population

VII. Factors That Alter Allele Frequencies in Populations

A. Reshuffling of the gene pool

1. Only a fraction of all possible genotypes are represented
2. **Mutation**
3. Randomly occur
4. The rate of mutations must be measured
   a. Most are recessive
   b. Dominant mutations
   c. Several conditions
5. Number of new mutant alleles per given number of gametes
   a. 2 out of 100,000 births exhibit a mutant phenotype
   b. 200,000 copies of the gene
   c. The mutation rate is 2/200,000, or 1/100,000
6. **Achondroplasia**
   a. Characteristics
   b. The mutation rate (μ) was calculated as: $1.4 \times 10^{-5} \pm 0.5 \times 10^{-5}$
   c. Change in frequency for each generation
   d. If normal d (q_0) exists with a frequency of 1.0, the frequency for the D (dwarf) (p_0) allele is 0

e. Rate of mutation from *d* to *D* is μ so in the next generation,

$$\text{Frequency of D} = p_1 = q_0\mu$$

f. Rate of mutation is constant

7. Mutation rate of 1.0×10^{-5}

a. Changes in allele frequency are very small

b. Figure 21-6 in the text

c. Mutation is a major force in creating genetic variability

B. Subpopulations

1. Different allele frequencies within subpopulations

a. **Migration**

b. Change in frequency of *A*

$$\Delta p = m(p_m - p)$$

2. Example: $p = 0.4$ and $p_m = 0.6$, and $m = 0.1$

a. Change in frequency of *A* in one generation

$$\begin{aligned}\Delta p &= m(p_m - p)\\ &= 0.1(0.6 - 0.4)\\ &= 0.1(0.2)\\ &= 0.02\end{aligned}$$

b. Frequency of *A* (p_1) in next generation

$$\begin{aligned}p_1 &= p + \Delta p\\ &= 0.40 + 0.02\\ &= 0.42\end{aligned}$$

3. Large change in the frequency of *A*

a. Equilibrium will be obtained when $p = p_m$

b. Change in allele frequency attributable to migration

c. Effect of migration

4. Flow of genes between two populations

a. American blacks have an almost 100% frequency for the Duffy blood group

b. U.S. whites have a frequency that is nearly 0%

c. The amount of gene flow into the black population can be estimated

d. The migration of the Fy^a allele is approximately 5% per generation

C. **Natural Selection**

1. There is differential survival and reproduction of some genotypes over others
   a. Consequence of the differential reproduction of genotypes
   b. Departure from one of the Hardy-Weinberg assumptions
2. Polygenic traits
   a. Body height and weight
   b. Three ways to classify selection
3. **Directional selection**
   a. Transparency 123, page 484, figure 21-12
   b. Extreme phenotypes selected for
   c. Example
   d. Upward selection
   e. Downward selection
   f. No more genetic variation
   g. In nature
4. **Stabilizing selection**
   a. Birth weight of humans
   b. Optimal birth weight is 7.5 pounds
   c. Maintenance of a population well adapted to its environment
   d. Individuals closer to average for a given trait will have a higher fitness
5. **Disruptive selection**
   a. *Drosophila* with high and low numbers of bristles
   b. Females from one strain and males from the other
   c. Two lines diverge rapidly
   d. In nature

D. Selection occurs when an advantage is present
   1. Relative strength of the selection
      a. Measure of fitness
      b. Comparing a particular genotype/phenotype combination
      c. Fitness is a relative concept
   2. **Selection coefficient (*s*)**
      a. $s = 0.01$

b. $s = 1.0$

c. Effect of selection on successive generations

$$q_n = \frac{q_0}{1 + n q_0}$$

n is the number of generations elapsing since p_0 and q_0

3. Example: $A\ (p_0) = a(q_0) = 0.5$

a. Transparency 124, page 485, figure 21-13

b. High percentage of *aa* genotypes

c. Halved in first two generations

d. Heterozygotes are not selected against

e. Difficulty in removing a recessive allele

4. s is less than 1.0

$$q_1 = \frac{q_0 - sq_0^2}{1 - sq_0^2}$$

E. Peppered moth *Biston betularia*

1. Light-colored moths

a. Industrialization

b. Dark-colored moth gained a selective advantage

c. A rapid shift in frequency of this phenotype occurred

2. The frequency of the light-colored forms increased again

3. Genotype frequencies can respond and quickly adjust to shifts in environment

F. Large sample size

1. 1000 heterozygotes (*Aa*) mating randomly results in approximately 25% *AA*, 50% *Aa*, and 25% *aa* genotypes in the next generation

a. Minor deviations from predicted ratio

b. Frequencies of *A* and *a* will remain equal

2. Population from only one set of heterozygous parents

3. One set of heterozygous parents producing two offspring

a. Large interbreeding populations are essential

b. Small populations

c. Degree of fluctuation increases as the population decreases

d. **Genetic drift**

4. Warwick Kerr and Sewall
   a. 100 lines with four males and four females
   b. Frequency of sex-linked bristle mutant *forked* (*f*) and wild-type (f^+) was 0.5
   c. Four females and four males were chosen to be the parents for succeeding generation
   d. After 16 generations, 70 lines experienced gene fixation
   e. Random fixation
   f. Elimination of alleles by chance alone
5. Three main ways for small populations to be created
   a. Splitting of a large population
   b. Epidemic occurring
   c. Emigrating of a small group into a new environment
6. Role of drift as evolutionary force
   a. Pingelap atoll in the western Pacific Ocean
   b. **Achromatopsia** affects 4 to 10% of population
   c. One of the 60 original survivors was heterozygous for this condition
   d. Initial gene frequency would have been 1/60
   e. Today, 7% of the current population are affected by this disorder
7. Another example involves the Dunkers
   a. Population has grown only from marriages within the group
   b. Frequencies of the ABO blood and MN blood group alleles are different from the rest of the U.S. population

G. Inbreeding or nonrandomly mating populations
   1. Small populations
   2. Large populations
   3. Restriction of mobility

H. Nonrandom mating
   1. **Assortative mating**
   2. **Inbreeding**
      a. Increases the chance for homozygosity of a recessive deleterious allele
      b. **Self-fertilization**

3. Figure 21-16 in the text
4. **Consanguineous marriages**
   a. **Coefficient of inbreeding**
   b. *F*
   c. Brother-sister couple has an *F* value of ¼
      1. When *F* = 1
      2. When *F* = 0
   d. Look at figure 21-17 in the text

I. Inbreeding results in homozygosity of certain recessive alleles
   1. Lowered fitness.
      a. **Inbreeding depression**
      b. Inbreeding in a large population
      c. Zoos are now employing DNA fingerprinting techniques
   2. Effects of inbreeding in humans
   3. Inbreeding is not always bad
   4. **Hybrid vigor**
      a. Corn
      b. Many hybrids are sterile
   5. Two ways to explain the hybrid vigor phenomenon
      a. **Dominance hypothesis**
         1. Reversal of inbreeding depression
         2. Deleterious recessive alleles are masked by the more favorable dominant alleles
      b. **Overdominance**
         1. The heterozygote is superior to either homozygote
         2. Biochemical diversity
         3. Cumulative effect of heterozygosity at many loci
      c. Most likely a combination of both the hypotheses

Lecture Content

Chapter Twenty-one: Population Genetics

I. Foundation for the Modern Interpretation of Evolution

A. Charles Darwin and Alfred Russel Wallace established the foundation for the modern interpretation of evolution.

1. Darwin and Wallace noticed that population numbers remain relatively constant in nature.

a. They also deduced that some form of competitive struggle for survival must have been occurring.

b. The theory of natural selection states, "...that any being, if it vary however slightly in any manner profitable to itself...will have a better chance of surviving."

2. As others began to study the process of evolution, it became apparent that the population rather than the individual had to be the unit studied, thus arose the discipline of **population genetics**.

3. Biochemical and molecular techniques have been utilized to measure variation at the protein and DNA levels directly, in order to test the theories and models of population genetics and natural selection.

II. Populations and Gene Pools

A. A **population** is a local group of a single species, within which mating is actually or potentially occurring.

1. The set of genetic information carried by all interbreeding members of the population is called the **gene pool**.

2. The gene pool consists of all the alleles of that gene that are present in the population.

B. Population genetics focuses on the measurement of allele and genotype frequencies in succeeding generations, rather than the distribution of genotypes from just one single mating.

1. Gametes produced from one generation form the zygotes of the next generation.

2. The next generation therefore, has a reconstituted gene pool that may differ from that of the preceding generation.

C. Populations are dynamic; they can grow and expand or diminish and contract through changes in birth or death rates, by migration, or by merging with other populations.

III. Calculating Allele Frequencies

A. When the mode of inheritance and the number of different alleles of a gene present in a population is known, the frequency of the allele can be measured.
   1. Some alleles are expressed in a codominant fashion, that is there is a direct relationship between the phenotypes and genotypes, with each phenotype having a unique genotype.
   2. An example of a codominantly inherited trait is the autosomally inherited **MN blood groups** in humans.

B. In this case, the gene *L* on chromosome 2 has two alleles, L^M and L^N, often referred to as *M* and *N*.
   1. Each of these alleles controls the production of a distinct antigen on the surface of red blood cells.
   2. The genotype of any individual may be type M ($L^M L^M$), N ($L^N L^N$), or MN ($L^M L^N$).

C. The frequency of the M and N alleles in a population can be determined simply by counting the number of individuals with each phenotype.
   1. For example, consider a population of 100 individuals of which 36 are type M, 48 are type MN, and 16 are type N.
   2. The 36 type M represent 72 *M* alleles, and the 48 MN heterozygotes represent and additional 48 *M* alleles, resulting in a total of 72 + 48 = 120 *M* alleles in a population of 200 alleles.
   3. The frequency of the *M* allele is then calculated as (120/200) = 0.6 = 60%.
   4. The frequency of the *N* allele is calculated as (80/200) = 0.4 = 40%.

D. Transparency 118, page 475, table 21.2.
   1. This table illustrates two methods for computing the M and N alleles in a hypothetical population of 100 individuals.
   2. Table 21.3 in the text lists the frequencies of M and N alleles measured in several human populations.

IV. The Hardy-Weinberg Law

A. When one allele is recessive, the heterozygotes would be phenotypically identical to the homozygous dominant individuals; when this is the case, the alleles can not be determined directly.

B. Godfrey H. Hardy and Wilhelm Weinberg developed a mathematical model that can be used to calculate the allele frequencies in a case such as this.

   1. This model is known as the **Hardy-Weinberg law (HWL)**, which under a set of assumptions predicts, that genotype and allele frequencies will remain constant from generation to generation.
   2. The HWL has three important properties to it.
      a. a. Allele frequencies predict genotype frequencies.
      b. At equilibrium, allele and genotype frequencies do not change from generation to generation.
      c. Equilibrium is reached in one generation of random mating.

C. There are several assumptions that must be made in order to utilize the Hardy-Weinberg law.

   1. The population is infinitely large, which in practical terms means that the population is large enough that sampling errors and random effects are negligible.
   2. Mating within the population occurs in a random fashion.
   3. There is no selective advantage for any genotype; that is, all genotypes produced by random mating are equally viable and fertile.
   4. There is an absence of other factors, including no mutation, no migration, and no genetic drift.

D. In an ideal population, suppose that a locus has two alleles, A and a.

   1. The frequency of the A allele in both sperm and eggs is represented as p, while the frequency of the a allele is represented as q.
      a. Transparency 119, page 475, figure 21-1.
      b. Since the sum of p and q equals 100% percent of the alleles for that gene in the population, $p + q = 1$.

2. Since random combination of gametes occurs within the population, the probability that the sperm and egg both contain the *A* allele is $p \times p = p^2$.
   a. The chance that the gametes will carry unlike alleles is $(p \times q) + (p \times q) = 2pq$.
   b. The chance that the individual will be homozygous recessive is simply $q \times q = q^2$.
3. These terms describe a characteristic of the H-W law, that the allele frequencies determine the genotype frequencies; therefore, the p^2 value is the probability that both gametes will carry the *A* allele, as well as the frequency of the *AA* homozygous genotype in the following generation.
   a. The $2pq$ describes the frequency of the Aa heterozygotes, while q^2 measures the frequency of the homozygous recessive (*aa*) zygotes.
   b. Thus, the distribution of homozygous and heterozygous genotypes in the next generation can be expressed as

$$p^2 + 2pq + q^2 = 1$$

4. Now consider a population in which 70% of the alleles for a given gene are *A*, and 30% are *a*.
   a. In this population, $p = 0.7$ and $q = 0.3$, and $p(0.7) + q(0.3) = 1$.
   b. Transparency 120, page 476, figure 21-2.
   c. In the new generation, 49% (p^2) of the individuals will be homozygous dominant, 42% ($2pq$) will be heterozygous, and 9% (q^2) will be homozygous recessive.
   d. The frequency of the *A* allele for the new generation can be calculated as

$$p^2 + \tfrac{1}{2}(2pq)$$
$$0.49 + \tfrac{1}{2}(0.42)$$
$$0.49 + 0.21 = 0.70$$

   e. The frequency of the *a* allele can be calculated as

$$q^2 + \tfrac{1}{2}(2pq)$$
$$0.09 + \tfrac{1}{2}(0.42)$$
$$0.09 + 0.21 = 0.30$$

or

$$q = 1 - p$$
$$= 1 - 0.70 = 0.30$$

5. If the Hardy-Weinberg conditions are assumed, then the frequencies of other genotypes can be calculated from knowing the frequency of only one genotype.
6. A population in the state of **genetic equilibrium** has the frequency of a given allele remaining constant from generation to generation.
7. Even though the hypothetical populations given as examples were at equilibrium, not all alleles in a population are.
   a. These examples demonstrate why dominant traits do not tend to increase in frequency as new generations are produced.
   b. The examples also demonstrate that genetic equilibrium and genetic variability can be maintained within a population.
   c. Once allelic frequencies are established in a population, they remain unchanged during equilibrium.

E. Another application of the Hardy-Weinberg law is to demonstrate whether genotypes in a given population are in equilibrium.
   1. In a natural population, any of the assumptions of the H-W law may not be met; therefore, the heterozygotes must be identified phenotypically.
      a. If a population does not fit the $p^2 + 2pq + q^2 = 1$ equation, then there must be some factor causing allelic shifts within each generation.
      b. These factors can include natural selection, migration, or even mutation.
   2. The MN blood group distribution in the Australian aborigines is a good example of this application.
      a. The expected frequencies of blood types M, MN, and N can be calculated from the values of allelic frequencies listed in table 21.3 in the text.
      b. In order to determine if the population is in equilibrium, the frequencies must be calculated.

Expected frequency of type M	$= p^2$ $= (0.178)^2 = 0.032$ $= 3.2\%$
Expected frequency of type MN	$= 2pq$ $= 2\ (0.178)(0.822) = 0.292$ $= 29.2\%$

Expected frequency of type N
$= q^2$
$= (0.822)^2 = 0.676$
$= 67.6\%$

c. By looking at the frequencies in Table 21.3 in the text, you can see that the **expected frequencies** are nearly identical to the **observed frequencies**, confirming that this population is in equilibrium.

3. If the H-W test demonstrates that the population is not in equilibrium, then one or more of the necessary conditions are not being met.
   a. Consider a hypothetical situation of a mixed population consisting of 500 Australian aborigines and 500 Native Americans, the frequencies for the *M* and *N* alleles are shown in Table 21.4 in the textbook.
   b. In this population, the frequency of the *M (p)* would be
   $$0.315 + \frac{1}{2}(0.324) = 0.477.$$
   c. The frequency for the *N (q)* allele would be
   $$0.361 + \frac{1}{2}(0.324)\ 0.523.$$
   d. If these frequencies were driven by random mating, the expected frequencies would be MM (p^2) 22.8%, MN ($2pq$) 49.8%, and NN (q^2) 27.4%.
   e. It is easy to see that the expected frequencies do not fit the observed, concluding that this population is in a state of nonequilibrium, brought about by a lack of random mating.
4. A common question asked about these populations is how long would it take for the populations to reach equilibrium.
   a. If the H-W equation were applied, it would illustrate that after one generation, the observed and expected genotype frequencies would converge.
   b. Transparency 121, page 477, figure 21-3.
   c. The transparency shows the expected frequencies after one generation.
   d. The observed genotype frequencies would be 22.8% for MM; 49.8% for MN, and 27.4% for NN, confirming that the allele frequencies for $M = 0.477$ and $N = 0.523$ equal those expected for a population in equilibrium.

V. Extensions of the Hardy-Weinberg Law

A. In considering genotype and allelic frequencies for autosomal loci using the Hardy-Weinberg equation, X-linked genes are not considered.

1. In species that have two X chromosomes, such as *Drosophila* and humans, the females carry two copies of all genes on the X chromosome, whereas males only carry one copy of all X-linked genes.
2. The genes on the X chromosome are therefore distributed unequally in the population.
3. In populations where there are equal numbers of males and females, the females carry two-thirds of all the X-linked genes, while males only carry one-third.

B. Because males only have one copy of all genes from the X chromosome, the phenotype reveals both dominant and recessive alleles, making the determination of the frequencies of the X-linked genes easy.
   1. For example, in Western Europe a form of X-linked color blindness occurs with a frequency of 8% in males.
   2. Since females have two doses of all genes on the X chromosome, the genotypic and allele frequencies can be calculated using the standard Hardy-Weinberg equation.
      a. The frequency for color-blindness is 0.08 in males at equilibrium, making the expected frequency for color-blindness in females equal to q^2, or 0.0064.
      b. This means that 800 out of 10,000 males are expected to be color-blind, while only 64 out of 10,000 females are expected to exhibit the trait.
   3. The population is not in equilibrium when the frequency of an X-linked allele differs between males and females.
      a. Equilibrium in each group will not be ascertained in only one generation as in the autosomal loci, but will approach equilibrium following a series of succeeding generations.
      b. The allelic frequency in females determines the frequency in males, since the single X chromosome is inherited maternally.
      c. Daughters on the other hand, inherit one X chromosome maternally and the other paternally, making the frequency an average of that found in both parents.
      d. The overall frequency in the population may remain constant, but the allelic frequencies will oscillate in the two sexes in each generation, with the

differences being halved in each succeeding generation until equilibrium is met.

e. Look at figure 12-4 in the textbook for a graphical representation of this situation.

C. It is common to find several alleles of a single locus present in a population.

1. The ABO blood group is an example of such a situation.

a. The locus *I* (isoagglutinin) has three alleles (I^A, I^B, and I^O), resulting in six possible genotypic combinations $I^A I^A$, $I^B I^B$, $I^O I^O$, $I^A I^B$, $I^A I^O$, and $I^B I^O$.

b. The *A* and *B* alleles are codominant and dominant over the *O* allele.

c. This results in the homozygous *AA* and heterozygous *AO* individuals being phenotypically identical, as well as the *BB* and *BO* individuals.

2. When another variable is added to the H-W equation, the genotype and allele frequencies for the situation involving three alleles can be calculated.

a. When a population is in equilibrium, the frequency of the three alleles can be described as

$$p\,(A) + q\,(B) + r\,(O) = 1$$

and the distribution of the genotypes will be given as

$$(p + q + r)^2$$

b. In this hypothetical population, the genotypes of *AA*, *AB*, *AO*, *BB*, *BO*, and *OO* will be found in the ratio

$$p^2(AA) + 2pq(AB) + 2pr\,(AO) + q^2(BB) + 2qr(BO) + r^2(OO) = 1$$

3. The frequencies for the three alleles of the ABO system can be estimated if the frequencies for the blood types of the population are known.

a. Consider the following blood types, A = 0.53, B = 0.13, and O = 0.26.

b. Since the *O* allele is recessive, the frequency of type O blood in the population is equal to the frequency of the recessive genotype, r^2, therefore,

$$r^2 = 0.26$$
$$r = (0.26)^{1/2}$$
$$= 0.51$$

c. The allele frequencies for the *A (p)* and *B (q)* alleles can be estimated using the estimated value for *r*.

d. The *A* allele is present in two genotypes, *AA and AO*; therefore, the frequency of the *AA* genotype is represented by p^2, and the *AO* genotype by $2pr$.

The frequency of $A - O = p^2 + 2pr + r^2$

This can be arranged to give

$$p = (\text{Frequency of } A + O)^{1/2} - r$$
$$= (0.53 + 0.26)^{1/2} - 0.51$$
$$= 0.89 - 0.51 = 0.38$$

e. Since the frequencies for *A (p)* and *O (r)* have been estimated, the frequency for the *B* allele can be estimated.

$$p + q + r = 1$$
$$q = 1 - (p + r)$$
$$= 1 - (0.38 + 0.51)$$
$$= 1 - 0.89 = 0.11$$

VI. Using the Hardy-Weinberg Law: Calculating Heterozygote Frequency

A. For a recessive phenotype, the frequency can be calculated by counting such individuals in a sample of the population.

B. Albinism is an example of an autosomal recessive trait, with an incidence of about 1/10,000, or 0.0001, in some populations.

1. Affected individuals are easily distinguished because they lack pigment in their skin, hair, and irises.
2. Since this is a recessive trait, affected individuals must be homozygous for the recessive allele, and their frequency in a population is represented as q^2, or

$$(q^2)^{1/2} = (0.0001)^{1/2}$$
$$q = 0.01 \text{ or } 1/100$$

3. Since $p + q = 1$, the frequency for p would be

$$p = 1 - q$$
$$= 1 - 0.01$$
$$= 0.99 \text{ or } 99/100$$

4. The frequency for the heterozygotes would be

$$2pq = 2\,[(0.99)(0.01)]$$
$$= 0.02 \text{ or } 2\% \text{ or } 1/50$$

5. These calculations show that the heterozygote for albinism is rather common in the population, even though the incidence for homozygous recessives is only 1/10,000.

C. The frequencies of all three genotypes can be estimated once the frequency of either allele is known.
   1. Transparency 122, page 480, figure 21-5.
   2. Note in the graph how fast the heterozygotes increase in a population as the values of p and q move away from zero.
   3. This confirms the conclusion that for a recessive trait like albinism, which is rare, the majority of those carrying the allele are heterozygous; and in cases where the frequencies of p and q are between 0.33 and 0.67, the heterozygotes actually constitute the major class of the population.

VII. Factors That Alter Allele Frequencies in Populations

A. The gene pool within a population is reshuffled each generation, producing new combinations in the genotypes of the offspring.
   1. Since the number of possible combinations is so large, it is correct to assume that the individuals alive at any given time represent only a fraction of all possible genotypes.
   2. **Mutation** creates new alleles, but in the absence of other forces mutation has a negligible effect on allele frequencies.
   3. Mutations, for our purposes occur at random, that is without regard for any possible benefit or disadvantage to the organism.
   4. The rate at which mutations are produced must be measured in order to determine if the mutation is a significant force in changing the allele frequencies.
      a. Most mutations are recessive, making it difficult to observe the rate of mutation directly in diploid organisms; therefore, indirect methods such as probability and statistics or large-scale screening programs must be employed.
      b. Dominant mutations on the other hand, can be measured by direct methods.
      c. There are several conditions that must be met to ensure accuracy when measuring the rate of mutation.

- The trait must produce a distinctive phenotype that can be distinguished from similar traits produced by recessive alleles.
- The trait must also be fully expressed or completely penetrant, so that the mutant individuals can be identified.
- Nongenetic agents such as drugs or chemicals must never produce an identical phenotype.

5. The mutation rates can be expressed as the number of new mutant alleles per given number of gametes.
   a. For instance, consider a given gene that undergoes mutation to a dominant allele, and 2 out of 100,000 births exhibit a mutant phenotype.
   b. The zygotes produced from the normal parents each carry two copies of the gene; therefore, we have actually surveyed 200,000 copies of the gene.
   c. If it is assumed that each of the affected births is heterozygous, then the mutation rate is 2/200,000, or 1/100,000.
6. An example of this sort of mutation in humans would be the dominant form of dwarfism known as **achondroplasia**.
   a. Affected individuals have an enlarged skull, short arms and legs, and can be diagnosed at birth.
   b. In a survey of approximately 250,000 births, the mutation rate (μ) was calculated as: $1.4 \times 10^{-5} \pm 0.5 \times 10^{-5}$.
   c. By knowing the rate of mutation, the change in frequency for each generation can be estimated.
   d. If only the normal *d* (q_0) exists, with a frequency of 1.0, then the frequency for the *D* (dwarf) (p_0) allele is 0.
   e. If the rate of mutation from *d* to *D* is μ, then in the next generation,

      $$\text{Frequency of D} = p_1 = q_0 \mu$$

   f. Even though the rate of mutation from generation to generation is constant, the rate of change in the mutant allele is initially high, but declines to zero at mutational equilibrium.
7. Consider a mutation rate of 1.0×10^{-5} for genes in the human genome.

a. Changes in the allele frequency brought about by mutation alone are very small, when the mutation rate is this low.

b. Figure 21-6 in the text, illustrates that if the population begins with only on allele (*A*) at a locus (p=1), and a mutation rate of 1.0 X 10^{-5} for *A* to *a* exists, it would take approximately 70,000 generations to reduce the frequency of *A* to 0.5.

c. This proves the thought that mutation is a major force in creating genetic variability, but when it comes to changing the allele frequency, by itself mutation does play a very critical role.

B. There are times when species become divided into subpopulations that to some extent are separated geographically.

1. Different mutation rates and selective pressures can establish different allele frequencies within these subpopulations.

a. **Migration** occurs when individuals move between these populations and breed.

b. For example, consider a pair of alleles, *A* (p) and *a* (q); the change in the frequency of *A* can be expressed as

$$\Delta p = m(p_m - p)$$

where p= the frequency of *A* in the existing population, p_m= the frequency of *A* immigrants, Δp= the change in one generation, and m= the coefficient of migration.

2. Consider an example where $p = 0.4$ and $p_m = 0.6$, and that 10% of the parents giving rise to the new generation are immigrants ($m = 0.1$).

a. The change in the frequency of *A* in one generation is

$$\begin{aligned}\Delta p &= m(p_m - p)\\ &= 0.1(0.6 - 0.4)\\ &= 0.1(0.2)\\ &= 0.02\end{aligned}$$

b. The frequency of *A* (p_1) in the next generation would be

$$\begin{aligned}p_1 &= p + \Delta p\\ &= 0.40 + 0.02\\ &= 0.42\end{aligned}$$

3. A rather large change in the frequency of A will occur in a single generation when either the m is large, or p is very different from p_m.
   a. With all other factors being equal, an equilibrium will be obtained when $p = p_m$.
   b. The change in allele frequency attributable to migration is proportional to the differences in the allele frequencies of the donor and recipient populations and to the actual rate of migration.
   c. The effect of migration can in fact substantially alter allele frequencies in populations.
4. Migration can also be thought of as the flow of genes between two populations that were once, but no longer geographically isolated.
   a. For instance, most American blacks are descendents from West Africa, where the frequency for the Duffy blood group allele Fy^a is almost 100%.
   b. In Europe, the source of most U.S. whites, the frequency is nearly 0%.
   c. By measuring the frequency of Fy^a or Fy^b among U.S. blacks, the amount of gene flow into the black population can be estimated.
   d. Figure 21-8 in the text illustrates the frequency of this allele in some regions of both Africa and the United States; it was estimated that the migration of the Fy^a allele is approximately 5% per generation.

C. **Natural Selection** is the principal force that shifts the allelic frequencies within large populations.
   1. At any given time, in any population, there are individuals with different genotypes, some of which are better adapted to the environment than others, leading to the differential survival and reproduction of some genotypes over others.
      a. Natural selection is therefore the consequence of the differential reproduction of genotypes, with the allelic frequencies changing over time as a result.
      b. Natural selection represents a departure from the Hardy-Weinberg assumption that all genotypes have equal viability and fertility.
   2. Traits that are polygenic, that is they are controlled by a number of genes, also respond to natural selection.

a. These types of traits include body height and weight, and often demonstrate a continuously varying distribution, which resembles a bell-shaped curve.
b. There are three ways in which selection for these traits can be classified, directional, stabilizing, and disruptive selection.

3. **Directional selection** often represents phenotypic extremes being selected for, and is important to plant and animal breeders.
   a. Transparency 123, page 484, figure 21-12.
   b. When the trait is polygenic, the most extreme phenotypes that the genotype can express will appear in the population only after a prolonged period of selection occurs.
   c. An example of directional selection is the long-running experiment at the State Agriculture Laboratory in Illinois to select for high and low oil content in corn kernels.
      - A population of 163 ears of corn was surveyed, and the 24 ears that were highest in oil content were used as the parents for the next generation.
      - The resulting generation with highest oil content were used for breeding, which is an example of upward selection, resulting in the oil content being raised about 4% after only 50 generations.
      - On the other hand, 12 ears with the lowest oil content were selected, and downward selection for this group lowered the oil content from 4% to less than 1% over the period of 50 generations.
   d. In upward selection, alleles for high oil content increase in frequency, replacing the alleles for low oil content, resulting in all individuals having the genotype for the highest oil content and expressing the most extreme phenotype for high oil content.
   e. In downward selection, the opposite occurs and the result is a population with a genotype and phenotype for the lowest oil content.
   f. There is no more genetic variation for oil content, when the lines fail to respond further to selection.
   g. In nature, directional selection occurs when the phenotypic extremes become selected for or against, which is usually a result of the environment.

4. **Stabilizing selection** favors intermediate types, with both extreme phenotypes being selected against.
   a. Mary Karn and Sheldon Penrose demonstrated stabilizing selection in association with birth weight of humans.
   b. They demonstrated that the optimal birth weight for humans is 7.5 pounds, and that infant mortality increases dramatically on either side of this weight.
   c. Stabilizing selection acts to maintain a population well adapted to its environment.
   d. Therefore, in this form of selection, individuals who are closer to the average for a given trait will have a higher fitness.
5. **Disruptive selection** can be viewed as the opposite of stabilizing selection, in that it selects against intermediates and for the both phenotypic extremes.
   a. John Thoday demonstrated disruptive selection in an experiment of *Drosophila* with high and low numbers of bristles.
   b. Mating was carried out between females from one strain and males from the other.
   c. The progeny were then selected for high and low bristle numbers, and mated for a number of generations, resulting in the two lines diverging rapidly.
   d. In nature, this is a situation that might exist for a population in a heterogeneous environment.

D. Selection occurs when a particular phenotype/genotype combination offers an advantage to organisms in competition with others.
   1. The relative strength of the selection varies with the amount of advantage provided.
      a. The probability that a particular individual will survive and leave offspring is a measure of its fitness, which refers to the total reproductive potential or efficiency.
      b. This concept is usually expressed by comparing a particular genotype/phenotype combination with one regarded as optimal.
      c. The environmental conditions change making the advantage conferred from a particular genotype change, resulting in fitness being a relative concept.

2. Fitness, mathematically, is the difference between the fitness of a given genotype and another, with the reference genotype being the **selection coefficient (*s*)**.
   a. The selection coefficient, *s*, equals 0.01 for a phenotype conferred by the genotype *aa*, when 99 of every 100 organisms reproduce successfully.
   b. If the genotype *aa* is a homozygous lethal and *AA* and *Aa* have equal fitness, $s = 1.0$.
   c. The effect of selection on successive generations, starting with any original frequencies of p_0 and q_0 in a population with $s = 1.0$, can be calculated as

$$q_n = \frac{q_0}{1 + n q_0}$$

   where n is the number of generations elapsing since p_0 and q_0.
3. Consider an example when $A\ (p_0) = a(q_0) = 0.5$.
   a. Transparency 124, page 485, figure 21-13.
   b. The frequency of the *a* allele is reduced rapidly at first because of the high percentage of *aa* genotypes.
   c. The frequency is halved in the first two generations, and is halved again by the sixth generation.
   d. The majority of the *a* alleles are by this time carried by heterozygotes, and since selection operates on phenotype and not on components of genotypes, the heterozygotes are not selected against.
   e. Successive generations result in slow subsequent reductions, which actually depend on the rate at which heterozygotes are removed from the population, resulting in a difficulty in removing a recessive allele from a population.
4. When s is less than 1.0, it is possible to calculate the effects of selection on each successive generation with any s, p_0 and q_0 values using the following formula

$$q_1 = \frac{q_0 - sq_0^2}{1 - sq_0^2}$$

E. A classic example of selection within a natural environment involves the peppered moth *Biston betularia*, in England.
   1. Before the year 1850, 99% of the moth population was light-colored, allowing for the nocturnal moths to rest undetected in the day on lichen-covered trees.

a. As industrialization occurred, toxic gases that killed the lichens growing on the trees and buildings were produced, resulting in the light-colored moths becoming easy prey for their predators.
b. The rare dark-colored moth soon gained a selective advantage because of their natural camouflage, which is created by single dominant allele, *C*.
c. A rapid shift in frequency of this phenotype occurred, probably in less than 50 generations.

2. The frequency of the non-melanic forms of the moth began to increase following the enactment of laws in the mid-1960s to restrict environmental pollution, resulting in the frequency of the dark-colored moth to decrease from over 90% in 1959, to just over 50% in 1985.
3. This illustrates that genotype frequencies can respond and quickly adjust to a shift in the environment.

F. A large sample size is important to the study of population genetics for the examination and predication of allele and genotype frequencies.

1. If a population consists of 1000 heterozygotes (*Aa*) mating randomly, the next generation will consist of approximately 25% *AA*, 50% *Aa*, and 25% *aa* genotypes.
   a. If the initial populations are large, then there will only be minor deviations from this predicted ratio.
   b. The frequencies of *A* and *a* in a cross such as this will remain about equal.
2. On the other hand, if the population is formed from only one set of heterozygous parents and they produce only two offspring, the allele frequency can change drastically.
   a. Look at table 21.7 in the textbook for the frequencies of the offspring in such a cross.
   b. In 10 of 16 times, the new allele frequencies will be altered.
   c. In 2 of 16 times, either the *A* or *a* allele will be eliminated in a single generation.
3. When only one set of heterozygous parents that produce two offspring, an extreme result is produced.

a. This type of cross illustrates the point that large interbreeding populations are essential to the Hardy-Weinberg equilibrium.
b. Small populations often experience random fluctuations in allelic frequencies that are possible by chance alone.
c. The degree of fluctuation will increase as the population size decreases.
d. These changes illustrate the concept known as **genetic drift**, which in the extreme case may lead to the chance fixation of one allele to the exclusion of another allele.

4. Warwick Kerr and Sewall set up an experiment illustrating genetic drift.
   a. They used 100 lines, each with four males and four females as the parents for the line.
   b. Within each of these lines, the frequency of the sex-linked bristle mutant *forked* (*f*) and its wild-type (f^+) was 0.5.
   c. In each generation, four females and four males were chosen to be the parents for the succeeding generation.
   d. After only 16 generations, 70 lines had experienced gene fixation, 29 in which only the *forked* allele was present and 41 in which only the wild-type allele was present.
   e. If there had been random fixation, then an equal number of lines should have become fixed for each allele.
   f. This experiment illustrates that alleles can spread through the population and eliminate other alleles by chance alone.
5. There are three main ways that small populations are created in nature.
   a. One way is by a large population being split by some natural event, resulting in at least two small isolated subpopulations.
   b. A second way is by an epidemic occurring, leaving a small number of survivors to constitute the breeding population.
   c. A third way is by a small group emigrating from a large group into a new environment.
6. The role of drift as an evolutionary force is best supported by allelic frequencies in some human isolates.

a. For example, the Pingelap atoll in the western Pacific Ocean has in the past been devastated by typhoons and famine, resulting in only a few survivors.
b. Today, 4 to 10% of the 2000 inhabitants are blind from infancy, caused by a rare autosomal recessive disorder known as **achromatopsia**.
c. It was found that one of the original survivors of a major typhoon was a chief who was heterozygous for the condition.
d. If he was the only carrier for this disorder, the initial gene frequency would have been 1/60, since there were only 60suriviors.
e. The average today is about 7% of the current population being affected by this disorder, and the frequency of the allele is the 0.26.

7. Another example of genetic drift in human populations involves the Dunkers, a small isolated religious group that emigrated from the German Rhineland to Pennsylvania.
   a. The religious beliefs of this group prohibit outside marriages, meaning that the population has grown only from marriages within the group.
   b. It is because of this, that the frequencies of the ABO blood and MN blood group alleles are so different between the Dunkers and the rest of the U.S. population.
      - The frequency of blood group A in the Dunkers is about 60%, whereas in the U.S. and German populations it is 45%.
      - The I^B allele is practically absent in the Dunker population.
      - Type M blood is found in about 45% of the Dunkers, compared with about 30% in the U.S. and German populations.
      - Since there is no evidence of these alleles having an advantage over any others, it is assumed that the observed frequencies are the result of chance events in a relatively small, isolated population.

G. The same effect as seen in genetic drift can occur in inbreeding or nonrandomly mating populations.
   1. In a small population, mates are more likely to be related to each other than in a large population.

2. In large populations, on the other hand, individuals tend to mate with those nearby, rather than those living at a great distance.
3. When the mobility of individuals is restricted, a pattern of nonrandom mating can cause genetic drift, and subdivide the population into smaller interbreeding subpopulations, differing from each other in the frequency of some alleles, leading to the chance elimination of alleles.

H. Nonrandom mating is very common, even between humans.
1. One form of nonrandom mating is termed **assortative mating**.
   a. This is when bonds are established by religious practices, physical characteristics, professional interests, etc.
   b. In nature, phenotypic similarity plays the same role.
2. Another form of nonrandom mating is termed **inbreeding**, where mating occurs between relatives.
   a. The sequences of inbreeding increase the chance that an individual will be homozygous for a recessive deleterious allele.
   b. The most extreme form of inbreeding is **self-fertilization**.
3. Figure 21-16 in the text illustrates the results of four generations of self-fertilization, with the original parent being heterozygous for one pair of alleles.
   a. By the fourth generation, 6% of the individuals are still heterozygous, and 94% are homozygous.
   b. However, the frequencies of *A* and *a* remain at 50%.
4. Inbreeding in humans is called **consanguineous marriages**.
   a. The probability that two alleles at the same locus in an individual are derived from a common ancestor can be determined using the calculation for the **coefficient of inbreeding**, devised by Sewall Wright.
   b. This is expressed as *F*, and can be defined as the probability that two alleles on a given gene in an individual are derived from a common allele in an ancestor.
   c. An F2 generation produced by a brother-sister couple, results in an *F* value of ¼.

- If $F = 1$, all genotypes are homozygous and both alleles are from the same ancestor.
- If $F = 0$, no alleles are derived from a common ancestor.

d. Look at figure 21-17 in the text for a pedigree of first- and second-cousin marriages.

I. Inbreeding results in the production of individuals who are homozygous for certain recessive alleles, which were previously masked in heterozygotes.

1. Many recessive alleles are deleterious, resulting in the inbreed population having a lowered fitness.
   a. **Inbreeding depression** is a measure of the loss of fitness caused by inbreeding.
   b. Inbreeding in a large population can result in high levels of inbreeding depression, which can be seen by examining the mortality rates in offspring of inbred animals in zoo populations.
   c. Many zoos are now employing DNA fingerprinting techniques to assure the two parents of a "captured" mate are not related.
2. The effects of inbreeding in humans are an increase in the risks for spontaneous abortions, neonatal deaths, and congenital deformities, as well as recessive genetic disorders.
   a. There are still some regions of the world that encourage men to marry their first-cousins.
   b. Matings such as these often result in the death of the offspring, but many of the parents just have more children to compensate for the ones lost to such disorders or abortions.
   c. On average, 2/3 of the resulting offspring are heterozygotes carrying a copy of the deleterious allele.
3. Inbreeding is not always bad, in fact inbreeding programs have been used for a number of domesticated plants and animals.
4. When members of two favorable lines are mated, they produce hybrid offspring that are more vigorous and more desirable than either of the parents; this phenomenon is known as **hybrid vigor**.

a. This has been used in many varieties of corn.

b. Many hybrids are sterile, and those that are fertile show subsequent declines in yield.

5. There are two ways in which hybrid vigor has been explained.

a. The first is known as the **dominance hypothesis**.

- This incorporates the obvious reversal of inbreeding depression, which inevitably must occur in outcrossing.
- Deleterious recessive alleles present in homozygous offspring are masked by the more favorable dominant alleles in the hybrids, which is thought to cause hybrid vigor.

b. The second way to explain hybrid vigor is known as **overdominance**.

- This holds that in many cases the heterozygote is superior to either homozygote.
- This may be related to the fact that the heterozygote has two forms of the gene product present, providing a form of biochemical diversity.
- Thus, the cumulative effect of heterozygosity at many loci accounts for the hybrid vigor.

c. Neither of these hypotheses has been proved, and it is most likely a combination of both the hypotheses that results in hybrid vigor.

Table of Contents

Introduction: Genetics Laboratory

The laboratory that is outlined here utilizes the lab book Laboratory Manual of Genetics, by A.M. Winchester and Peter J. Wejksnora. The genetics laboratory course will meet once a week throughout the semester. There will be thirteen labs, a midterm, a final exam, and a presentation day. The lab will be taught as a separate class from the lecture. The students will learn how to manipulate different strains of *Drosophila*. The objective of this laboratory course is to integrate the information that the students learn in lecture with actual lab experience.

The majority of the semester will be spent performing crosses on different *Drosophila* strains, but there will be other laboratories included as well. The students will be required to keep a stock of wild type female *Drosophila*. These flies will need to be cared for by each student separately. It will be required for the student to mate male and female wild type *Drosophila* and collect virgin females. The wild type virgin female stock vial will be the student's only source for this strain of *Drosophila*; therefore they must maintain it all through the semester.

The student will receive worksheets every lab period that must be returned the following lab meeting. Some of the labs will extend into the succeeding lab, and in such a case, the worksheets will be due the following lab from when the experiment is complete. The worksheets will constitute ten-percent of the laboratory grade. Students will be able to perform many types of tests in the lab. For example, they will be able to perform crosses between *Sordaria*, the Ames test, gel electrophoresis, DNA isolation, and many others.

The students will be required to keep a *Drosophila* journal throughout the semester. The journal should be kept in a two-inch binder and everything in it must be legible. The journal entries will be used to record every cross made with the *Drosophila*. The information that must be present in every journal is: the cross, the date, the number of parental flies, when the parental generation was removed, the number of male and female offspring, the phenotypes of every offspring, the suggested or actual genotype of the offspring, and whatever else the student feels is important to record. The journal will

be turned in once during the semester and again at the end of the semester. This journal will be worth fifteen-percent of the student's final grade.

There will be two examinations within this laboratory, a midterm and a final. The midterm will consist of questions from the first six laboratory exercises. The final will be a comprehensive final that will cover the entire semester. Each exam will consist of questions from the lab procedures performed by the student. The students will need to know the steps for every procedure executed in class, as well as the theories that are associated with them. The first half of both the midterm and the final, consist of discussion questions. The second half will consist of questions that refer to different laboratory stations. Due to the fact that this is a lab final and preparation time is needed to set it up, there will be no make-up exams. If a student misses an exam, he/she may hand in a ten to fifteen page research paper on a genetic disorder of his/her choice, to take the place of the exam.

Attendance will be required in the lab. Although there is no actual penalty for missing a laboratory session, attendance will be taken into consideration when the final grades are distributed. If a student must be absent, he/she may attend lab on another day. Changing labs will only be allowed once per student, and any more will create specific consequences. If a student is absent, and they have already used the one time to switch labs, he/she cannot turn in the worksheet for that lab. The only exception to this would be if the student informed me that he/she was going into the lab on another day and performing the experiment by his/herself. Some of the experiments call for dangerous chemicals to be used, if one of these labs is missed, the student must accept a zero for the worksheet that day.

The students will also be required to design a personal *Drosophila* cross for their *Drosophila* project. For this project, the student must chose a mutation in the *Drosophila* to work with. The mutation must be included in the *Drosophila* that are present in the lab. Students may not select mutations that have already been chosen. The students must get their projects approved before they begin. Two crosses must be made between the mutant *Drosophila* and wild type *Drosophila*; one with the male being the mutant and the other with the female being the mutant. An inter se cross and a backcross must also be created for the project. The crosses must be continued up to at least the third generation.

Each generation of each cross must consist of at least seventy-five progeny. This will require the student to either return to the lab every day to check the flies, or the students may take the flies home with them. Students will be given time to work on their project in the lab in the last three meetings, but the rest of the project must be completed outside of lab.

Every detail of every cross must be recorded in order to receive full credit on the project. The mutation that the student chooses must be researched. The report will need to contain the location of the gene causing the mutation, how prevalent the gene is in the *Drosophila* community, whether or not it is linked to any other gene(s), and how many alleles are present for that specific gene. The final report for the *Drosophila* project must be at least five pages long, double-spaced, and include charts of the data. Each student will make a presentation on the last day of lab concerning his or her individual project. This presentation will be considered when the grades for the project are assigned.

The final grades for the laboratory course will include the worksheets, the exams, the journal, and the final project. This course will aid the student in their understanding of genetics. It offers hands-on experience for many of the experiments that are discussed in the lecture. The journal and *Drosophila* project allow the student to understand exactly what is involved in scientific experiments.

Genetics Laboratory Syllabus

Lab	Topic	Chapter in Lab Book
1.	*Drosophila* Culture	1
	Mitosis: Onion Tip	2
2.	Meiosis: *Ascaris*	-Not in text-
3.	Probability	3
4.	Monohybrid Cross	4
5.	Dihybrid Cross	5
6.	Inheritance Related to Sex	6
7.	Midterm Exam	
8.	Linked Genes and Location of Genes on a Chromosome	8
9.	Restriction Digestion and Electrophoretic Separation	13
10.	Mapping Restriction Sites	13
11.	The Ames Test, Detecting Mutagens	17
12.	Phage Recombination	16
13.	Human Genetics	11
14.	Isolation of Eukaryotic DNA	18
15.	-----Presentations------	
16.	Final Exam	

Grading for Laboratory Course:

Worksheets	10%
Journal	15%
Project	20%
Midterm	25%
Final Exam	30%

Text: Laboratory Manual of Genetics Fourth Edition. A.M. Winchester and Peter J. Wejksnora. Wm. C. Brown Publishers. © 1996.

Genetics Laboratory Midterm

Name:

Lab Section:

Date:

I. Discussion Questions

1. What are some phenotypic differences between male and female fruit flies? List at least four.

2. What is the scientific name for the fruit fly we have been working with?
_____

3. Draw a diagram of the life cycle for these fruit flies.

4. Why is it important that the female flies used in a cross are virgins?
_____
_____
_____

5. What is a karyotype? _____

6. What did the drawing beans lab represent? _____
_____
_____
_____

7. What is a monohybrid cross; what is the expected ratio of phenotypes for the progeny of this kind of cross?_____

_____

8. What is a dihybrid cross; what is the expected ratio for the progeny of this kind of cross? _____

_____

9. How did we perform a monohybrid cross in lab? _____

_____

10. How did we perform a dihybrid cross in lab? _____

_____

11. What is an inter se cross? A backcross? _____

_____

_____

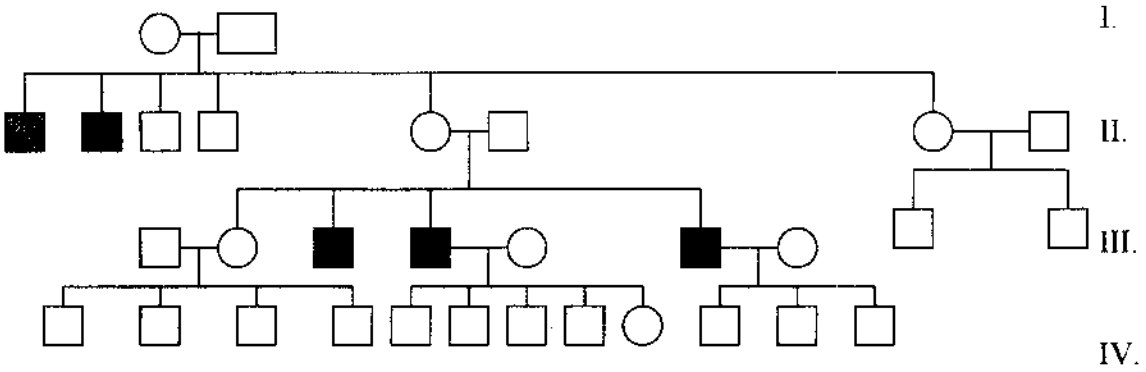
_____

12. Consider an experiment with the fruit flies. Suppose that wild type males and females were mated, and the resulting progeny consisted of 59 wild type males and 41 wild type females; do the data deviate significantly from the expected ratio? [Hint: Perform a chi-square].

11. What is an example of a human X-linked characteristic, besides hemophilia?

A human pedigree is illustrated below, showing the inheritance of hemophilia.

Answer questions 12 – by referring to this pedigree.



12. What is the probability that the only female in generation IV is a carrier for hemophilia? _____

13. What is the probability that she will have sons who are hemophiliacs?

_____

14. What is the probability that she will have daughters who are hemophiliacs?

_____

15. What is the probability that she will have daughters heterozygous for the gene for hemophilia?

_____

II. Stations

[Stations 1-3: Slide of Mitosis in Onion Cell]

1. What is the object in this slide? _____
2. What stage of the cell cycle does this cell represent, refer to the cell that is pointed too. _____
3. What stage of the cell cycle does this cell represent, refer to the cell that is pointed too. _____

[Stations 4 - 7: Slide of Cheek Cells Representing Barr bodies]

4. What is represented in this slide? _____
5. What is the dark spot in the cell? _____

6. Where is this dark spot found, and what is its function?_____

7. What is the stain used for this slide? _____

[Stations 8 – 10: *Drosophila* Examples]

8. Describe this fly; be sure to include eye color, sex, and wing type.

_____

_____

9. Describe this fly in the same manner._____

10. Show how the label would look for a cross between these two flies (You do not have to distinguish sex for the cross label). _____

Genetics Laboratory Final

Name:

Lab Section:

Date:

I. Discussion Questions:

1. What is meant by the term *linked genes*? _____
2. What genes did we use in the linked gene cross with *Drosophila*? _____
3. What is a three-point test, and what genes of what organism did we use to perform this test? _____
4. What are restriction enzymes? _____
5. Why did we use the procedure Gel Electrophoresis? _____
6. From the following data, construct a restriction site map.

*Eco*R1	5,	10		
Hin dIII	2,	6,	7	
*Sal*I	3,	4,	8	
*Eco*R1 + *Hin dIII*	2,	3,	3,	7
*Eco*R1 + *Sal*I	1,	3,	4,	7

7. What is phage recombination? _____
8. What phage did we use to examine recombination? _____
9. What were the two test performed in the Ames Test lab, and what was different about each of them? _____

   _____

   _____
10. Which compound inhibited growth the most from the Ames test? _____

11. What did the DNA look like when you isolated it? _____

_____

12. Name at least seven different human monogenetic characteristics that we studied in lab.

a. _____ b. _____ c. _____

d. _____ e. _____ f. _____

g. _____

13. Tell your phenotype for each characteristic listed in question 12.

a. _____ b. _____ c. _____

d. _____ e. _____ f. _____

g. _____

14. Why is it important to limit the amount of time the *Drosophila* are exposed to the Fly Nap ®? _____

15. Describe the anesthetizing procedure. _____

_____

_____

_____

16. Illustrate a crossover event between the following two chromosomes.

$$\frac{A \quad b}{A \quad B} \qquad \frac{A \quad B}{a \quad B}$$

17. Illustrate a double crossover event in the following chromosomes:

A	b	C
a	b	c

a	B	C
A	b	C

18. Consider a test cross of an organism with the genotype *AaBbCc* with the following results:

Genotype	Number of Progeny
AaBbCc	90
AaBbcc	10
aabbcc	90
aabbCc	10

a. What are the expected frequencies for each of the genotypes? _____

b. Do the observed frequencies show significant deviation from the expected? (Use chi-square analysis).

19. Describe the process used in the restriction digestion, electrophoretic separation, and mapping experiment. _____

_____

_____

_____

20. Briefly describe the steps used in the phage recombination lab.

_____

_____

_____

_____

_____

II. Stations

[Stations 1 - 8: *Sordaria* Plates]

1. What is the name of the organism on these plates? _____
2. What experiment did we use it for? _____
3. What is the object that holds the ascospores called? _____
4. Was there crossing-over within this example; what is the symbol for its genotype?

_____

5. Was there crossing-over within this example; what is the symbol for its genotype?

_____

6. What color of ascospore does the mutant form of this organism produce? _____
7. Approximately how many asci are found in each cluster? _____
8. Why is it important to heat and sterilize the inoculating loop after every step?

_____

_____

_____

[Stations 9 - 11:Ames Test Plates]

9. Does this plate illustrate that a mutagen was present? If so, was the mutagen very inhibiting, explain why or why not? _____

_____

_____

10. Does this plate illustrate that a mutagen was present? If so, was the mutagen very inhibiting, explain why or why not? _____

_____

11. Briefly describe the steps used in the Ames test lab. _____

_____

_____

_____

_____

_____

[Stations 12 - 15:*Drosophila* Examples]

12. Describe the fly represented here. _____
13. Are the recessive genes represented linked or not? _____
14. Describe the phenotype of this fly. _____
15. What experiment would this fly work good in, explain. _____

_____

Laboratory Exercise 1: *Drosophila* Culture and Mitosis

Setting up *Drosophila* Culture

Materials and Procedure:

- Microscope
- Fly strains
- Media in stoppered bottles
- Small soft brush
- Anesthetizing chamber
- Fly Nap ®
- Anesthetic wand
- Index Card

*These will be the same materials needed for each lab in which we work with *Drosophila.*

1. Prepare the Medium:

   A culture medium needs to be prepared by each student. Place a measuring cap full of the media formula in a plastic vial. Pour one measuring cap of water into the vial, make sure that the formula is dissolved. Then place several grains of yeast to the vial, this will serve as the food source for the flies. The medium will solidify quickly and will be ready for use. Place a foam stopper in the top of the vial, this will allow for gas exchange and keep out stray flies.

2. Anesthetizing the *Drosophila*:

   Transfer the flies from the stock vials into an empty clean vial, by inverting the vial and removing the stopper. *Drosophila* are negatively geotropic, that is they will tend to crawl up and away from the stopper. Once in the clean vial, dip an anesthetic wand into the Fly Nap ® and allow the excess liquid to drip back into the bottle. Place the wand into the anesthetizing vial. Within a few minutes, the flies will be anesthetized and ready for manipulation.

3. Distinguishing Sex:

   Pour the anesthetized flies onto an index card and place under a microscope. The files can easily be moved around using a soft brush. The first vial created needs to be virgin females. The flies in the stock vials were emptied this morning, so all the females present will be virgins. The dorsal surface has a different appearance in the males and females. The male has heavy pigmentation on the entire posterior part of the abdomen with two anterior pigmented bands, whereas the female has five bands of pigment along the entire abdomen. The posterior tip of the abdomen in females is also somewhat pointed, whereas in the males it is rounded. Separate at least 10 females and place into your personal medium-containing vial; be sure to include a label with your initials on the vial! You will be required to keep a stock vial of virgin females throughout the semester.
4. Complete handout #1 and place in a *Drosophila* journal that you create and keep up with the entire semester.

Mitosis in Onion Root Tips

Materials and Procedure:

- Microscope, microscope slides, and cover glasses
- 1 molar hydrochloric acid
- Razor blade
- Forceps
- Slide warmer
- Prepared slide of mitosis in onion root tips

1. Creating your own slide:

   First cut off the terminal 1 cm of the root tip from the onion bulb. Place 1 M HCl in the watch glass, be careful not get the acid on your skin or clothes. Place the terminal end of the tip you collected into the watch glass for approximately two minutes. This will make the root tip soft and easy to manipulate. Using forceps, remove the root tip from the acid and transfer it to a slide containing a single drop

of acetocramine stain. Take the razor blade and chop the root into small slices. Apply a clean cover glass tot he slide and gently heat it on a slide warmer, be sure not to boil! Invert the slide on a paper towel while pushing down firmly. This action flattens the cells and disperses them so they can be observed. Finally examine your prepared slide under the microscope.

Questions:

1. Can you see the various stages of mitosis, refer to handout #2 for a reference of the stages?
2. What stage are most of the cells in?
3. Can you see the chromosomes in any of the cells?

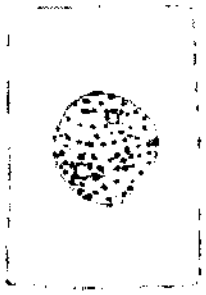
2. Using prepared slides:

Obtain a prepared slide of an onion root tip arrested in mitosis. Observe the slide under low and high powers.

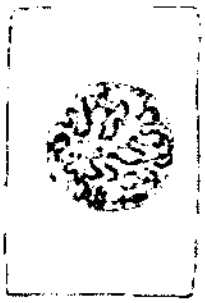
Questions:

1. How does this slide differ from the one you created?
2. Can you see the spindle fibers in the cells?
3. Are you able to identify each stage of mitosis within your slide?

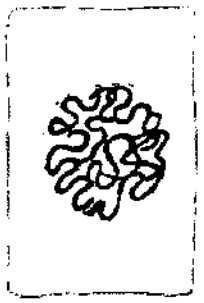
Worksheet 1



Interphase



Early Prophase



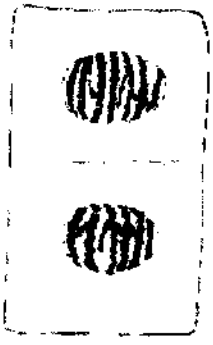
Middle Prophase

Late Prophase

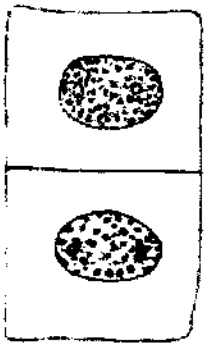
Metaphase

Anaphase

Early Telophase



Late Telophase



Daughter Cells

Worksheet 2

Drosophila Culture

Name:

Lab Section:

Date:

1. What happens when you hold the vial upside down and why?

2. Why is it important to limit the amount of time the anesthetic wand is in the vial with the flies?

3. How time elapses between the time you get the files in the anesthetizer vial and they are completely immobilized?

4. Describe the difference in abdomen appearance between males and females.

5. How many flies were you able to retrieve from the stock vial?

6. How many virgin females were you able to place in your personal vial?

7. Approximately how long did it take for the females to wake from the anesthetic?

Laboratory Exercise 2: Meiosis

Meiosis in Ascaris Oogenesis

Materials and Procedure:

- Microscope
- Slides of *Ascaris* oogenesis

1. *Ascaris* oogenesis:

   Ascaris has a low number of chromosomes, which makes it an easy organism for studying meiosis. This species has two pairs of chromosomes in its diploid cells. The slide will contain different sections of the uterus of *Ascaris*. Each section will contain cells in a different stage of meiosis. First place the slide under the microscope and look at it under low power and gradually increase the power. Spend some time looking at each section, and become familiar with what each stage looks like.

2. What you need to identify:

   Be sure that you are able to see a primary oocyte, chromatids, spindle, first polar body, secondary oocyte, male and female pronuclei, and a mature ovum. In order to remember what these look like, draw a picture of each in your lab notebook. Look at several different slides so that you can get an idea of how the cells look.

Crosses to Setup

Mating in *Drosophila*

Materials and Procedure:

- Wild type males from stock vial
- Virgin females from person vial

1. Checking your vial:

Check your personal vial and make sure that only virgin females were collected last week. If there are larvae present on the sides of the vial, then a male is present in your vial. If this occurs, you need to repeat last week's experiment and come in later this week to set up the cross we are making today. Anesthetize your virgin females, count out five of them, place five of these back in your vial, and place the rest in a new vial marked virgins, be sure to include your initials. This will make it easier when you are trying to place the males in the vial with the females. Be sure to only place five males and five females back into your vial.

2. Retrieving males:

   From the stock vial, anesthetize the flies in the same manner as last week. Place flies on index card and separate males and females. Place the females back into the stock vial and place the males in your personal vial. In five days, come into the lab and check to see if you have larvae present on the walls of your vial. If you do, anesthetize the flies present in your vial and place them in the fly morgue. If you do not have larvae present, check your vial the next day, or until you do. This will allow you to only count the progeny next week.

Laboratory Exercise 3: Probability and Chi^2

Tossing Coins

Materials and Procedure:

- Four coins

1. Tossing two coins:

   Toss two coins until you obtain two heads. Record the number of tosses that is required for you to get two heads on handout 3. By using the law of coincident happenings, find the mathematical expectation of tosses required to get the two heads. This law states that the chances of obtaining two things simultaneously is the product of obtaining either separately. Next, toss one coin until you get a head, you do not have to record the number of tosses needed for this. Then, toss the second coin until it also is a head be sure to record the number of tosses it requires to get a head on the second coin. Place your data on the board and compare it with the rest of the class's data.

2. Tossing four coins:

   Toss four coins one after another and record the data on handout 3. Write your data on the board and compare it with the rest of the class's data. Plot the results on the handout. Determine the probability for each of the head-tail combinations through the use of the binomial method. Allow p to represent the chance for a head on one toss and q the chance for a tail. Use n to represent the total number of coins in each toss. Now, plot the expected number of each combination based on the number of students in the class; plot the data on the same graph used to show the obtained results. In order to determine if the obtained show a significant deviation from the expected, use the chi-square test. Since there are five possible cases, use four degrees of freedom. Compare the result with the numbers on Table 3.1 in the lab book.

Drawing Beans

Materials and Procedure:

- Shoe box
- 50 black and 50 white beans

1. First, place all beans in the shoebox and shake. Allow the black beans to represent genes for normal pigmentation, while the white beans represent the genes for albinism. Assume that you and your mate have normal pigmentation, but both of you carry the gene for albinism. The two allelic genes will be present in equal numbers, as are the beans. Draw a bean from the box to represent the gene present in the sperm, then draw a second bean from the box to represent the gene present in the egg. Record the type of first child you will have. Place the beans back in the box, and draw two more pairs out, resulting in three "children". Determine the distribution of phenotype that would be present in these children. Now, find the probability that you will obtain the combination that you actually get, and tabulate the results of the class and graph them on your worksheet.

Sex Ratio in *Drosophila*

Materials and Procedure:

- Flies from cross prepared last week

1. Examining The Progeny:
   Anesthetize the resulting flies from last week's cross. Once they are immobile, place them on an index card and view them under the microscope. Tabulate the number of each sex; females tend to emerge earlier, so there may be a larger number of females. Determine the expected ratio for the two sexes, assuming they are produced in equal numbers. Record the number of males and females on your worksheet.

2. Significant Deviation:

   Use standard error to determine if you have a significant deviation from the expected 1:1 ratio. Use *p* and *q* to represent the chance for males and females, and use *n* to represent the total number of flies. The formula is the square root of *p* X *q* divided by *n*. If the result is a deviation of more than twice the standard error, then there is a significant deviation.

Crosses to Setup

Monohybrid Cross with *Drosophila*

Materials and Procedure:

- Wild type virgin females
- Ebony-body colored males

1. Collect Virgin Females:

   Perform the same methods as before and collect five virgin females. All of your females are wild type for body color, since they came from the stock vial. Mix a solution of medium and place in a new clean vial. Place your five wild type females in this vial.

2. Collect Males:

   From the stock vial labeled ebony, follow the same methods as before and collect five males. Place these five males in the vial with the five wild type females. Ebony is a good recessive trait to use for a monohybrid cross, the cross that we will be looking at next week. When an individual is homozygous recessive for this trait, they will appear to have a darker body and a shield shaped pattern on the dorsal surface of the body.

3. Labeling The Vial:

   Be sure to include your initials on this vial. You also need to mark the date and cross on the label. A plus sign is often used to indicate wild type, whereas the letters of the recessive trait, in lower case, are used to represent the recessive allele. Your label should look like this:

+/+ females X **e/e** males

Date Initials

4. Removing Parents:

   Return to lab in four or five days and make sure that successful mating has occurred. You will know this because there will be larvae on the sides of your vial. Anesthetize the flies and remove them from the vial. Place the flies in the morgue. This ensures that you do not count the parents as progeny for next week's lab.

Dihybrid Cross with *Drosophila*

Materials and Procedure:

- Vestigial-ebony males
- Wild type females

1. Collecting parents for cross:

   Anesthetize and remove five males from the vestigial-ebony stock vial. These males are homozygous recessive for these traits. Look in the lab book to determine which chromosome these genes are located. Become familiar with the mutant traits by spending time looking at them under the microscope. Next, place them in a new medium-containing vial. Then, remove five wild type female flies from your personal stock vial and place them with the mutant males. Be sure to label this vial in the same manner as before. It will be required to return to the lab in two days and remove the parents from this cross.

Worksheet 3

Probability

Name:

Lab Section:

Date:

I. Tossing a coin:

1. Circle the result of the first coin toss: Head Tail
2. Class results: Heads____ Tails_____
3. Expected number of heads (1/2 of total coins tossed)___
4. Deviation of obtained heads from expected heads (ie. +2.5, -3,0)___
5. Tabulate the results of ten tosses by the entire class, include the results obtained as the first ten crosses:

											Total	Average
Heads	___	___	___	___	___	___	___	___	___	___	___	___
Tails	___	___	___	___	___	___	___	___	___	___	___	___

6. Deviation of average from mathematical expectation ___
7. Is the average deviation of ten tosses greater or less than the deviation obtained from the first toss? _____
8. Give possible explanations as to why this deviation is greater or less.

_____
_____
_____
_____

II. Tossing Two coins:

1. How many times did you have to toss two coins to get two heads? ___
2. What is the expected number of tosses required to get two heads? ___
3. Show the calculation for the expected number.

_____

4. Give the total number of tosses required by the class to get two heads. ___

5. What is the average number of tosses required by the class to get two heads? ___

6. Which is closer to the expectation, your toss or the class average? ___

Why? _____

_____

_____

7. How many times did you toss the second coin in order to get a head? ___

8. What is the expected number of tosses, show calculation.

_____

9. What was the average number of second tosses by the class to get a head? ___

10. Is this average different from the average needed to get two heads when two coins were tossed? If so, why?

_____

_____

_____

III. Tossing Four Coins:

1. Calculate the various proportions expected of the different combinations of head and tails when four coins are tossed.

2. What are the results of your toss? Heads ___ Tails ___

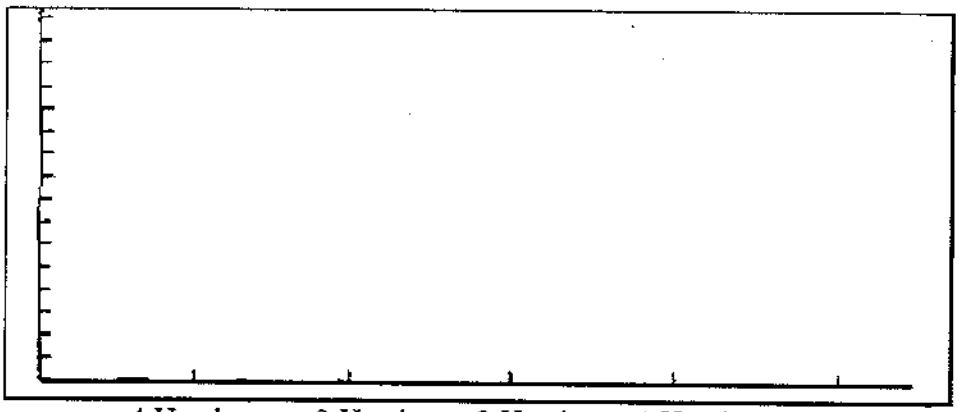
3. Tabulate the number of persons in the class who obtain each of the combinaitons of heads and tails. Calculate the expected number of each.

	No. Times Obtained	No. Times Expected
Four heads	_____	_____
Three heads, one tail	_____	_____
Two heads, two tails	_____	_____
One head, three tails	_____	_____

Four tails _____ _____

Total _____ _____

5. Plot the obtained results and the expected results on the graph, using different colors to represent each. Fill in the numbers on the vertical line so that the largest number will reach almost to the top.



6. Use chi-square to determine if your obtained results show a significant deviation from the expected.

	Observed Frequency X	Expected Frequency m	Deviation Squared $(X-m)^2$	Chi-square $\frac{(X-m)^2}{m}$
4 Heads				
3 Heads				
2 Heads				
1 Head				
0 Heads				
Total Σ				

X^2 = > P >

Degrees of Freedom: ___

7. Does the Chi-square result and the P value indicate that anything other than chance accounted for the deviation from mathematical expectation? Tell why.

_____

_____

IV. Drawing Beans:

1. What is the phenotype for the your first three children?

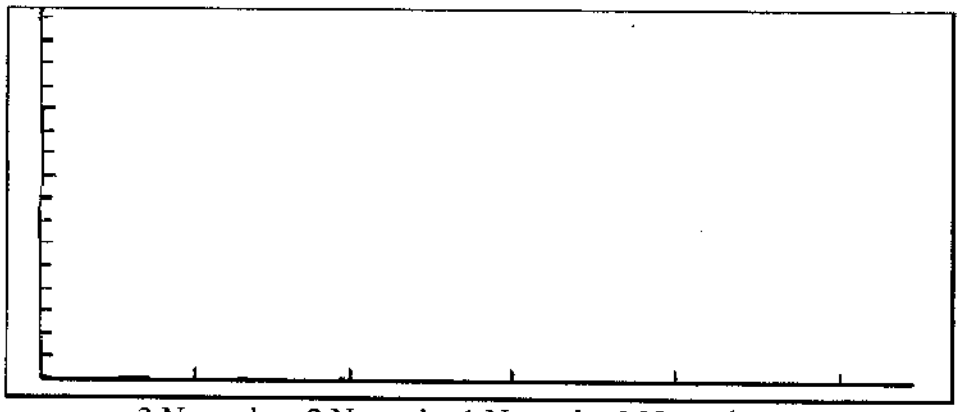
_____	_____	_____	_____Normal:_____Albino
1st Child	2nd Child	3rd Child	Ratio

2. Use $(p+q)^n$ to get the expected proportion of each of the four possible combinations of children.

3. Give class results below along with the expected calculated above:

	All Normal	2 Normal 1 Albino	1 Normal 2 Albinos	All Albinos
Obtained:	_____	_____	_____	_____
Expected:	_____	_____	_____	_____

4. Plot the obtained combinations and the expected combinations on the graph, using different colors to represent each. Put numbers on the vertical line so that the top of the curve reaches almost the top of the graph.



3 Normal 2 Normal 1 Normal 0 Normal

Albino 2 Albino 3 Albino

V. Sex Ratios in *Drosophila*:

1. Tabulate the number of males and females:

Males _____ Females _____ Total _____ Percent males _____

2. Calculate the standard error to see if your obtained results differ from the 1:1 expected ratio.

Total offspring (n) _____ Expected males (p) _____ Expected females (q) _____

Obtained males: _____ Obtained females: _____

Deviation of obtained from expected (d): _____

3. Do these results indicate that anything other than chance has influenced the deviation you obtain from a 1:1 ratio? Tell why.

_____

_____

_____

Laboratory Exercise 4: Maize and Monohybrid Cross

Monohybrid Cross with *Drosophila*

Materials and Procedure:

- F_1 progeny from monohybrid cross

1. Examining The Progeny:

   By this time, the F_1 generation of flies should be emerging. Anesthetize these flies and examine them under a microscope. Separate the ebony flies from the wild type flies and record the numbers of each. Then, separate the flies by sex and record the number of males and females.

2. Preparing The Next Monohybrid Cross:

   Select five to ten pairs of health looking F_1 flies and put them in a new clean vial. This is known as a brother-sister mating. Be sure to label the vial with your initials and the cross (HINT: it will not be the same as the last cross!). You will need to return to the lab in two days and check the vial for larvae. If larvae are present, remove all the flies and discard them in the morgue. Return two days later and check for flies, if flies are present, remove flies and count the different sexes and body colors. Discard those flies in the morgue. The remaining larvae should emerge by next week's lab and then they can be counted as well.

3. Answer the questions about this experiment on worksheet 4.

Examining Corn from a Monohybrid Cross

Materials and Procedure:

- Ear of corn

1. Counting Kernels:

   Select an ear of corn. These ears were selected from parents that were heterozygous for the recessive gene for white endosperm (w). The dominant

allele results in a purple (W) kernel. Count every kernel present on the ear and record the number of purple and the number of white. It is easiest to do this row by row so that you do not recount any kernels.

2. Answer the questions on handout 4.

Crosses to Setup

Dihybrid Cross with *Drosophila*

Materials and Procedure:

- F_1 flies from dihybrid cross

1. Examining The Progeny:
   Anesthetize the F_1 flies and examine them under the microscope. Record their characteristics on worksheet five.
2. Setting up Inter se Cross:
   Select ten to fifteen healthy pairs and transfer them into a new vial. This will create the inter se, or brother-sister, cross that you will examine next week.
3. Setting up a Backcross:
   Return to the lab tomorrow and remove all flies. Examine them under a microscope and separate them by sex. These flies should be virgin females from the original cross. Place at least five females in a new medium-containing vial with five males from the homozygous mutant stock vial. This will create a testcross, also known as a backcross; be sure to label this vial as well.

Worksheet 4

The Monohybrid Cross

Name:

Lab Section:

Date:

1. *Drosophila*

   What is the name and symbol used for the mutant that was used in your cross?

   _____

   Tell how the flies with the mutant phenotype differ from the wild type.

   _____

   What did your label look like?

   _____

   _____

   _____

   When were the parents removed from the cross? _____

   Does the mutant trait appear in any of the offspring? _____

   If so, explain. _____

   When were the first generation flies placed back into the vial for mating? _____

   When where those flies removed from the vial? _____

Phenotype of F_2

Date Counted	No. Wild Type	No. Mutants	Total Flies
_____	_____	_____	_____
_____	_____	_____	_____
Total	_____	_____	_____

Ratio of wild type: mutants expected: 3:1

Ratio of wild type: mutants observed: :1

Draw Punnet squares for each of the generations involved in this monohybrid cross:

P_1

F_1

F_2

By using chi-square analysis, determine if the results you obtained from the brother-sister cross differed significantly from the expected ratio of 3:1.

F_2 Results	Observed Numbers X	Expected Numbers m	Deviation Squared $(X-m)^2$	Chi-square $\frac{(X-m)^2}{m}$
Wild Type				
Mutants				

Degrees of Freedom =

Total Σ =

χ^2 = $\%$ > P > $\%$

By the value of the chi-square, do you think that your results deviated significantly from the expected? If so, what factors other than chance do you think might explain this great of a deviation?

_____

_____

_____

2. Corn

Tabulate the nature of the seeds on the ear.

Obtained on one row	purple _____	white _____	Ratio ___ : ___
Expected	purple _____	white _____	Ratio ___ : ___
Obtained on all rows	purple _____	white _____	Ratio ___ : ___
Expected	purple _____	white _____	Ratio ___ : ___

Does the ratio from all rows come closer to the expected than that obtained on one row? Explain.

_____

_____

Use Chi-square analysis to determine how frequently you might expect deviations of this magnitude from the mathematical expectations for all rows.

	Observed Frequency X	Expected Frequency m	Deviation Squared $(X-m)^2$	Chi-square $\frac{(X-m)^2}{m}$
Purple				
White				

Degrees of Freedom =

Total Σ =

χ^2 = % > P > %

Upper limits of the chi-square at 5% level of significance 3.841

Do these results indicate significant deviation? _____

Laboratory Exercise 5: Independent Assortment in a Dihybrid Cross

Dihybrid Cross with *Drosophila*

Materials and Procedure:

- F_2 flies from dihybrid cross

1. Examining The Progeny:

   By this time, there should be flies present in both the inter se cross and the backcross. Tabulate the characteristics of the F_2 flies and record the results on worksheet 5.
2. Be sure to use chi-square to determine if your results lie within the realm of chance variation.

Examining Corn from a Dihybrid Cross

Materials and Procedure:

- Ear of corn

1. Determining Phenotype:

   The ear of corn was created by a dihybrid cross. The cross was between a dominant purple-starchy plant and a recessive white-sweet plant. The color of the kernels is easy to distinguish, and the sweet trait is identified by the kernel being shriveled. The starch grains on the other hand, will not become shriveled. Count row by row and tabulate the number of combinations found on the ear of corn.
2. Record the results on worksheet 5; then use chi-square analysis to determine if the results lie within possibilities for chance variation.

Examining the Monohybrid Cross From Last Week

Materials and Procedure:

- F_2 flies from monohybrid cross

1. Examining The Progeny:

   Anesthetize and tabulate the characteristics of the F_2 flies from the cross created last week. The sex of the flies does not have to be recorded, since the cross was made with an autosomal gene. Record the characteristics on worksheet 4.

2. Disposing of The Flies:

   Following your tabulation of the characteristics, dispose of the flies in the morgue.

Crosses to Setup

Linkage Cross

Materials and Procedure:

- White-eyed males and virgin females
- Wild type males and virgin females

1. Collecting Females:

   Genes on the X chromosome are also called X-linked genes because their distribution is affected by sex. An example of an easily recognized X-linked gene in *Drosophila* is white eyes (*w*). Anesthetize and collect five virgin white-eyed females from the stock vial provided. Place these females in a new medium-containing vial. Then collect five wild type males from the stock vial provided, and place them with the white-eyed females. It will be required to return to lab and remove the parental generation of flies in approximately two days.

2. Inverse Cross:

   The next cross to set up will consist of flies with the same genes, however, this time the males will be white-eyed and the females will be wild type. Collect the white-eyed males from the stock vial provided and collect the wild type females from your personal stock vial. It will be required to return to lab and remove the parental generation of flies in approximately two days.

Location Determination Cross

Materials and Procedure:

- Sepia-eye/ebony-body colored males
- Wild type virgin females

1. Setting up Linked Gene Cross:

   Linked genes do not exhibit independent assortment, unless they are involved in crossover events. First, anesthetize and examine five males that are homozygous recessive for sepia-eye color (se) and ebony-body color (e). Retrieve these males from the stock vial provided. These genes are located far apart from each other, but on the same chromosome; therefore, they will exhibit a large number of crossovers. Place these five males in a new medium-containing vial, along with five wild type females; do not forget to label the vial. It will be required to return to the lab and remove the parental flies in two days.

Three-Point Cross

Materials and Procedure:

- White-eye (w)/miniature wing (m)/forked bristle (f) male flies
- Wild type virgin females

1. Setting up Cross:

   Collect five males homozygous for white eyes, miniature wings, and forked bristles. Spend some time examining them under the microscope to become familiar with the phenotype. Place these five males in a new medium-containing vial; be sure to label the vial. Place five wild type virgin females from your stock vial with these males. Remove the parental generation two days following the initiation of the cross.

Worksheet 5

Independent Assortment in a Dihybrid Cross

Name:

Lab Section:

Date:

I. *Drosophila*

1. Give the name, gene symbol, and the phenotypic expression of the two mutant genes used for this cross.

   Mutant 1 _____ _____ _____

   Mutant 2 _____ _____ _____

2. Date the cross was made. _____
3. What was the phenotype of the offspring? _____
4. Date when inter se cross was made. _____
5. What was the phenotype of the F_2 generation?

Date	Wild Type	_____	_____	_____	_____
____	_____	_____	_____	_____	_____
____	_____	_____	_____	_____	_____
Total	_____	_____	_____	_____	_____

Total flies in all four categories: _____ Ratio expected: _____

Number of flies expected in each category: _____ _____ _____ _____

6. Use chi-square to determine goodness of fit for your results, show P value.

7. Does the P value indicate that anything other than chance is operating in giving the observed numbers in the F_2 generations? If so, give an explanation.

_____

_____

8. Give a complete diagram of both generations, including genotype and phenotype of offspring from cross.

9. Date when testcross was made. _____

10. Phenotype of Offspring from Testcross:

Date	Wild Type	_____	_____	_____	_____
____	_____	_____	_____	_____	_____
____	_____	_____	_____	_____	_____
Total	_____	_____	_____	_____	_____

Total flies in all four categories: _____ Ratio expected: _____

Number of flies expected in each category: _____ _____ _____ _____

12. Use chi-square to determine goodness of fit for your results, show P value.

12. Does the P value indicate that anything other than chance is operating in giving the observed numbers in the F_2 generations? If so, give an explanation.

_____

_____

II. Corn

1. Tabulate the seed traits found in the ear of corn you examined.

	Purple Starchy	Purple Sweet	White Starchy	White Sweet	Total
Obtained	_____	_____	_____	_____	_____
Expected	_____	_____	_____	_____	_____

2. Use chi-square to determine if deviations are due to anything but chance.

3. Does the P value indicate anything other than chance occurring? If so, explain.

_____

_____

Laboratory Exercise 6: Inheritance Related to Sex

X-linked Cross in *Drosophila*

Materials and Procedure:

- F_1 flies from X-liked cross

1. Examining The Progeny:

   Anesthetize and examine the resulting progeny from last week's lab. Tabulate the characteristics of the F_1 flies from both crosses made. Record this information on worksheet 6.

2. Setting up Inter se cross:

   From these F_1 flies select five healthy males and five healthy females. Place these flies in a new medium-containing vial; be sure to label this cross. It will be required to return to lab in two days in order to remove the parental generation. We will examine the F_2 generation next week.

X-linked Human Characteristics

Materials and Procedure:

- Color charts

1. Red-green Color-blindness:

   Red-green color-blindness is exhibits X-linked inheritance. This disorder is easily recognized by examining color charts. Look at the color charts provided and determine if you have any difficulties distinguishing the two colors. Tabulate the results for all males and females in the class, and record the results on worksheet 6.

2. Hemophilia:

   There is a human pedigree located in worksheet 6. Fill in the genotype for each person in the pedigree. If both genes are not known, you can represent the

unknown gene with a dash. For example, a normal female that might or might not carry the gene would be represented as H/-, whereas a male with hemophilia is represented as h/Y.

Barr Bodies in Human Cells

Materials and Procedure:

- Albuminized slides
- 5 N hydrochloric acid
- 1% thionine stain
- Mouthwash
- Toothpick
- Microscope

1. Collecting Cells:

   First, rinse the mouth with the mouthwash provided. Wash hands and scrape, lightly, the inside of cheek with a toothpick. Place the collected cells on an albuminized slide along with a single drop of water. Be sure that there are no large masses on the slide, if there are, break them up with the toothpick.
2. Adding Acid and Stain:

   Allow the slide to dry, this should take about five to ten minutes. Add a drop of 5 N HCl and leave it on there for 10 seconds only. Gently remove the acid with water. With a paper towel, draw off the remaining water from the slide, and then add two drops of the thionine stain. Leave the stain on for ten minutes. Rinse this stain off with water in the same manner as the acid, be sure to draw up any excess water from the slide. Place the cover glass on the slide very carefully, without allowing air bubbles to form underneath the cover slip.
3. Examining The Cells:

   Place the slide under the microscope and focus. Using high power, examine the cells and look for sex chromatin bodies. These will be slightly concave, stained dark blue/black, and lying next to the nuclear membrane. After examining your

slide, borrow a slide from the opposite sex so that you can examine cells from both males and females, and record the differences on worksheet 6.

Crosses to Setup

Location of Genes on a Chromosome

Materials and Procedure:

- F_1 flies from location determination cross
- Sepia-eye/ebony colored males

1. Examining The Progeny:
   After retrieving the vial with the homozygous recessive male and wild type female cross from last week, anesthetize the flies and examine them. Record the results on worksheet 7.
2. Set up Backcross:
   Separate five healthy females from the F_1 generation and place them in a new medium-containing vial. Anesthetize five males from the stock vial with the homozygous recessive for sepia-eye color and ebony-body color, and place these males in the vial with the females. Label the vial. It will be required to return to lab and remove the parental generation in two days. We will examine the resulting progeny of this cross next week.

Three-point Test

Materials and Procedure:

- F_1 flies from three-point cross

1. Examining the Progeny:
   Anesthetize and examine the F_1 flies from the three-point cross. Record the phenotype of the F_1 flies on worksheet 7.
2. Set up Inter se Cross:

Remove five males and five females from the F_1 generation. Place these flies in a new medium-containing vial. Return to the lab and remove these parents in two days.

Worksheet 6

Inheritance Related to Sex

Name:

Lab Section:

Date

I. X-linked Genes in *Drosophila*

1. What X-linked mutant did you use for your cross? _____
2. What is the symbol for this gene? _____
3. What is the phenotype for flies with this mutant gene as compared to wild type flies? _____

_____

4. Mutant females crossed to wild type males:

Date cross was made. _____ Date parents were removed. _____

Phenotype of F_1 offspring:

Males _____ Females _____

Date inter se cross was made. _____ Date parents were removed. _____

Phenotype of F_2 offspring:

Date	Wild Type Males	Mutant Males	Wild Type Females	Mutant Females
____	_____	_____	_____	_____
____	_____	_____	_____	_____
Total	_____	_____	_____	_____

Total Males _____ Total Females _____

Expected:	Wild Type	Mutant
Males	_____	_____
Females	_____	_____

5. Use chi-square to determine if your results differ significantly from the expected. Only use the results from males for this analysis.

6. Did you get a significant deviation from the expected? If so, why? _____

_____

7. Diagram both generations of this cross, showing genotype and phenotype of the offspring.

8. Wild type females crossed to mutant males:

Date cross was made. _____ Date parents were removed. _____

Phenotype of F_1 offspring:

Males _____ Females _____

Date inter se cross was made. _____ Date parents were removed. _____

Phenotype of F_2 offspring:

Date	Wild Type Males	Mutant Males	Wild Type Females	Mutant Females
_____	_____	_____	_____	_____
_____	_____	_____	_____	_____
Total	_____	_____	_____	_____

Total Males _____ Total Females _____

Expected:	Wild Type	Mutant
Males	_____	_____
Females	_____	_____

9. Diagram both generations, showing genotype and phenotype of offspring.

II. Human X-linked Characteristics

1. Give the results of the color-blind test on yourself:

   Sex: _____ Color-blind: ____ Not color-blind: _____

2. Class results:

   Color-blind males: _____ Number of males in class: ____

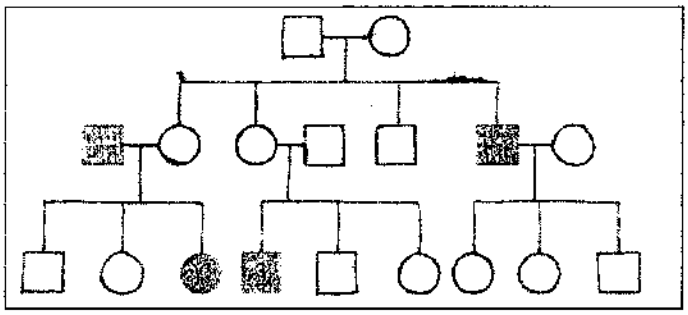
   Color-blind females: _____ Number of females in class: _____

   Percent color-blind males: _____% Percent color-blind females: _____%

3. Determine how many females in the class could have color-blind sons, show work.

4. About how many males in the class could have color-blind sons, show work.

III. Pedigree for Hemophilia:



Place the genotype of each individual below the corresponding circle or square. Shaded circles and squares indicate a hemophilia phenotype. Use the symbol H for normal and h for hemophilia.

Laboratory Exercise 7: Linked Genes and Location of Genes on a Chromosome

Linked Genes in *Drosophila*

Materials and Procedure:

- F_2 flies from location determination cross

1. Examining The Progeny:
   Anesthetize and examine the F_2 progeny. Tabulate the characteristics on worksheet 7.
2. Compare The Results:
   Compare your results to what would have been expected if the genes had been on different chromosomes. Determine the percentage of crossovers you have obtained. Analyze results with chi-square analysis. Record results on worksheet 7.

Three-Point Test

Materials and Procedure:

- F_2 flies from the inter se three-point cross

1. Examining The Progeny:
   Anesthetize and examine the F_2 flies. Record the results on worksheet 7 for the male offspring only. The females will all be wild type.
2. Determining Sequence of Genes:
   First, consider double crossovers. These can be recognized easily because they will represent the smallest group of recombinations. The gene that is expressed singly among the double crossovers is located between the other two. For single

crossovers, the two genes on the end will by represented singly, while the gene in the middle will always be with one of the other two.

3. Determining the Distance:

   After the sequence has been established, determine the distance between the genes. Add up the entire crossover events that have occurred between two of the genes, determine the percentage of crossovers that these represent, and the result equals the crossover units. Repeat the last step for all the genes to determine the distance between each one. Record all information on worksheet 7.

Crossing-over in *Sordaria*

Materials and Procedure:

- Petri dish containing sterile medium
- Wild type culture of *Sordaria*
- Inoculating loop
- Bunsen burner

1. Labeling Petri Dish:

   On the bottom of your petri dish, which contains sterile medium, place masking tape in the form of a "T." In two diagonally separated sections, place a "+" with a black marker. In the other two diagonally separated sections, place a "-." Also, be sure to include your initials and the date on the bottom of the petri dish.

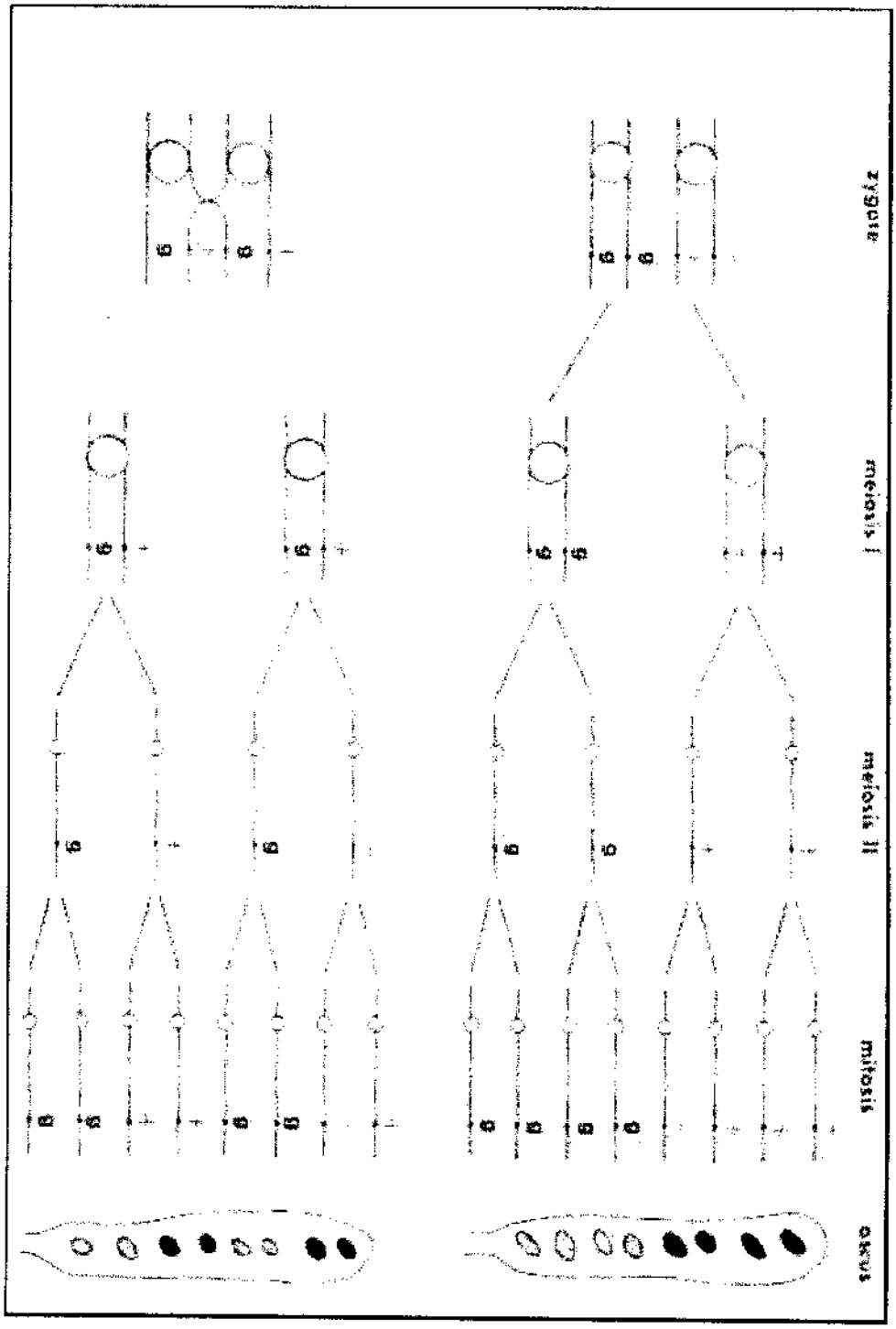
2. Setting up the Cross:

   First, sterilize the inoculating loop by holding the loop in the flame of the Bunsen burner until it is red hot. Let it cool down for about fifteen seconds, and then lift the lid of the culture dish contain the (+) strain of *Sordaria* and remove a small bit of the medium containing the hyphae. Place this sample in your personal petri dish containing the sterile medium. Place it about 2 cm from the edge at the 12 o'clock position, where you have a "+" sign labeled on the dish, and then close the lid. Repeat the steps for a second inoculation at the 6 o'clock position. Repeat the steps again, except this time use the culture medium with the (-) strain of *Sordaria*. Flame the loop once more before returning it to the table.

3. Incubate:

   Place your Petri dish in the incubator that is set at 25° C. By next week, the two mating strains will have covered much of the medium in the dish, and where the two strains have come in contact, dark perithecia will have formed.

Worksheet 7



Worksheet 8

Linked Genes and Location of Genes on a Chromosome

Name:

Lab Section:

Date:

I. Linked Genes in *Drosophila*

1. What are the names, symbols, and phenotypes for the two mutant genes that you are using in this cross? _____

   _____

2. Date cross was made. _____
3. Date parents were removed. _____
4. Appearance of F_1 generation. _____
5. Date F_1 females were backcrossed to double recessive males. _____
6. Date parents were removed. _____
7. Phenotype of offspring:

Date Counted	Wild ++	se e	se +	+ e	Total
_____	_____	_____	_____	_____	_____
_____	_____	_____	_____	_____	_____
Total	_____	_____	_____	_____	_____

8. Do the results indicate any difference in viability of one trait as compared to the other? If so, which has the lower viability? _____
9. Tabulate the results, and determine the number of crossovers obtained and the percentage of crossovers.

   Total flies _____

   Noncrossovers (++ and se e) _____

   Crossovers (se + and + e) _____

   Percentage of crossovers _____

10. Using the results, how many units apart are the two mutant genes? _____
11. What is the distance between these genes according to the map in your book?

12. Use chi-square analysis to determine if your results could be only chance variation or if double crossovers have made a significant difference in the results obtained.

12. Diagram this cross, show linkage by placing the two genes on one line; indicate which of the gametes carry crossovers.

13. Would the results have differed if the cross had been between F_1 males and females that were homozygous for the double recessive? If so, explain, how and why.

_____
_____
_____
_____

II. Chromosome Mapping with Three-Point Test

1. What is the name, symbol, and phenotype for each sex-linked mutation you used in this cross? _____
_____

2. Date cross was made. _____

3. Date parents were removed. _____

4. Appearance of F_1. _____

5. Date inter se cross was made. _____

6. Phenotype of Male Offspring

Date	+++	wmf	wm+	++f	+mf	w+f	+m+	Total
___	___	___	___	___	___	___	___	___

7. From these results, what is the sequence of the genes on the chromosome? How did you reach this conclusion? _____

_____

_____

8. According to your results:

What is the distance between the white and miniature? _____

Between the miniature and the forked? _____

Between the white and the forked? _____

9. Allow the line below to represent the X chromosome, with white located at 1.5. Show the location of miniature and forked according to your results.

1.5

w _____

(1.5)

In parenthesis below the letters, show the location of the genes according to a chromosome map in your text. Give an explanation for deviations from your results. _____

_____

III. Crossing-over in *Sordaria*

1. Explain the procedure and any difficulties in making the culture. _____

_____

_____

_____

2. How many days passed before observed perithecia? _____

3. Approximately how many asci are found in each cluster? _____

4. Do any asci have all eight ascospores of one kind? If so, give a possible explanation of how this could happen. _____

_____

5. Draw a cluster of asci containing ascospores.

6. Tabulate the number of ascospore combinations in asci:

Noncrossovers	Crossovers
++++gggg _____	++gg++gg _____
gggg++++ _____	gg++gg++ _____
	++gggg++ _____
	gg++++gg _____
Total _____	Total _____

7. What was the percent of crossovers from your observations? _____%

8. What was the percent of crossovers from the class's observations? _____%

9. What is the map distance between the centromere and the gene for ascospore color?

_____

10. Why do some crossovers give the combination of ++gg++gg and others give the combination of ++gg++?

_____

_____

_____

Laboratory Exercise 8: Restriction Digestion and Electrophoretic Separation

Cutting with Restriction Endonucleases

Materials and Procedure:

- Digestion buffers (10X), running buffer (TBE1X), and gel casting buffer (10X)
- Loading dye (10X)
- Ethidium bromide solution, 10,000X
- DNA sample
- Marker DNA
- Distilled water
- Agarose
- Restriction endonucleases
- Tape
- Microfuge tubes
- Water baths
- Gel apparatus, gel plate, power supply
- Centrifuge
- UV transilluminator

1. Restriction Digestion:

   First, label one microfuge tube for each enzyme. Be sure to record everything down on worksheet 9. To each of the tubes, add 18 µl of water, 1 µl of buffer, 1 µg of DNA, and finally, two units of the enzyme. Mix the tube gently by flicking it with your finger. Place each of the tubes in a test tube rack, and place the rack in the 37° C water bath. Allow these to digest for one hour.

2. Gel Assembly and Casting:

   While you are waiting for your enzyme to digest the DNA, begin assembling the gel electrophoresis apparatus. The tray is already assembled for you, and the gel

is already mixed and sitting in the 50° C water bath. Pour the gel solution into the gel tray up to the fill mark. Allow the gel to cool and harden. Once hard, insert the gel into electrophoresis apparatus, put on some gloves, and then remove the comb from the gel and the tape from the tray. Place the gel tray in the apparatus so that the open ends face the electrode chambers, be sure that the wells, which were created by the comb, are nearest to the (-) end. Add 1X running buffer until it completely covers the gel by about 5mm.

3. Sample Preparation and Electrophoresis:

   When the digestion is complete, dry the tubes off and centrifuge them briefly to gather all the drops at the bottom. Open the tube and add 1:10 volume of the 10X loading buffer. Recap the tubes and add the marker tube containing predigested DNA fragments in loading buffer, to the rack. Place the rack in the 50° C water bath for five minutes. Using a micropipetter, take up 15 µl of the sample. Insert the tip of the pipette into the well and slowly dispense the liquid, avoiding any air bubbles. When the last sample is added, recover the apparatus, connect the leads, and turn on the power supply to 70 volts for one hour. Record the time and voltage when the electrophoresis step begins. The dye in the gel should move towards the (+) side of the apparatus. When the run is over, turn the power supply off, disconnect the leads, and remove the gel tray. Place a notch in the upper left-hand corner to preserve orientation, and then place the tray on the UV transilluminator. Be sure to wear goggles before turning the UV light on. A picture will be taken of your gel, and will be given to you at the next lab period for analysis.

Crossing-over in *Sordaria*

Materials and Procedure:

- *Sordaria* culture
- Microscope
- Inoculating loop
- Bunsen burner

- Microscope slide
- Cover glass

1. Examining the Cross:

   Remove your Petri dish from the incubator, while the gel electrophoresis is running. Use a flamed inoculating loop to remove a perithecium to a drop of water on a microscope slide and add a cover glass. Press gently with the eraser of a pencil to break open the perithecium and expose the asci. Examine the asci under the microscope.

2. Looking at the Arrangements of Ascospores:

   Tabulate the numbers of different arrangements, and determine the percentage of ascospores that are crossovers. If the arrangement is anything but four black and then four gray, a crossover has occurred. Divide the percentage by two in order to convert it to map distances, since only two of the four chromatids present during the first meiosis are involved in crossing-over, and no linkage changes will be observed in gametes coming from the other two. Refer to worksheet 8 for an example of crossing over.

Worksheet 9

Restriction Digestion, Electrophoretic Separation, and Mapping

Name:

Lab Section:

Date:

I. Restriction Digestion

1. For the restriction endonuclease *Eco*RI, how frequently would you expect to find recognition sites in a random sequence of DNA? _____

2. How about for the restriction endonucleases *Hpa*II and *Hae*II? _____

3. Of the ones listed, which enzyme is most tolerant of different buffer conditions? _____

4. List at least two hazards within this experiment. _____

II. Sample Preparation and Electrophoresis

2. In the space provided, attach the photograph of your gel.

2. What were the running conditions? $\frac{\text{\_\_\_\_\_}}{\text{Voltage}} \text{ X } \frac{\text{\_\_\_\_\_}}{\text{Time}} = \text{\_\_\_\_\_}$

III. Sizing the Fragments

1. List the enzymes used for each digestion, and list the migration distance for each fragment above the dashed line. Then, use the graph you plotted for the marker fragments and determine the size of each fragment and place that below the dashed line.

Lane	*Enzyme*	*Band*	*Migration Distance (cm)* ---------- *Size (determined from plot)*
1			
2			
3			
4			
5			
6			
7			
8			
9			
10			
11			
12			

2. Using the semilog graph paper provide, plot migration and length of the known samples and the fragment bands in your restriction digests.

IV. Restriction Site Mapping

1. On page 154 in the lab book, prepare a map in the space as described in the lab book.

Laboratory Exercise 9: Mapping By Restriction Sites

Continuation of Laboratory Exercise 8

Methods and Procedure:

- Photograph of gel
- Worksheet 9
- Semilog graphing paper

1. Sizing the Fragments:

   When you receive your photograph, label each lane on the photograph and measure the distance each band has migrated. Record the distances migrated on worksheet 8, listing the distances migrated above the dashed line. Next, plot these distances on the semilog paper provided on the worksheet, and determine the size of each fragment. Be sure to plot the known fragment markers first. Locate each fragment on the map, and draw a straight line to connecting the points. Take the distance migrated for each unknown, determine where it crosses the line you have drawn, and read the size from the vertical axis. When all the fragments have been sized, add them up; the total for each lane should be the same.

2. Restriction Site Mapping:

   If the DNA you start with is circular, then there are as many sights as there are fragments, whereas if your DNA is linear, then the number of sights is equal to the number of fragments plus one. Look in the lab text for an example of a map, and then map your own in the space provided in worksheet 9.

Laboratory Exercise 10: The Ames Test

Qualitative Test

Materials and Procedure:

- Culture of *Salmonella typhimurium*, *his*, *uvrB*, and *rfa*
- LB agar plates
- Minimal agar plates
- Soft agar
- Histidine biotin solution
- Pipettes, sterile filter paper, forceps, and rubber gloves
- Alcohol, sterile distilled water, and compounds to test
- Bunsen burner

1. Creating Plate:

   This test determines if a substance is mutagenic or not. Label each plate with your name, date, and the compound under test. Also, place an X in the exact center of the plate, this will show you where to place your compound. Prepare 1 LB plate, 1 minimal plate, at least three known mutagens, at least three unknown synthetic compounds. The unknown compounds can be things such as cosmetics, food additives, or even cleaning products. Use one tube of agar for each plate; add 0.1 ml of the *Salmonella* culture and 0.2 ml of histidine-biotin solution to each tube of agar. Mix well and pour in plate at a tilted angle. Place the plates upright so that they will cool and harden. Place the forceps in the alcohol and then pass them through a flame to sterilize. Take the forceps and transfer one filter to the center of each minimal agar plate; then place a few drops of sterile water on the filter in the control plates, and a few drops of each test compound on the filter in the appropriate plate.
2. Examining Plate:

After 48 hours, return to the lab and examine the plates. The LB control plate should have allowed for a rich growth of the mutant bacteria and a thick lawn should be evident. Count the number of colonies on the minimal control plate, and not the random distribution. Note the number of distribution of the colonies on the plates containing the known mutagens, and record your answers on worksheet 10. Then look at the plate with the test compounds. Record the number and distribution of colonies on worksheet 10.

Quantitative Test

Materials and Procedure:

- The same materials will be used for this test

1. Diluting the Samples:

   This test determines the concentration of the mutagen needed in order to inhibit growth. Select one mutagen, from the list of known mutagens, and prepare dilutions that give final concentrations of 0.1 μg/ml, 0.5 μg/ml, 1 μg/ml, 2 μg/ml, 4 μg/ml, and 10 μg/ml.

2. Preparing the Plate:

   Take one minimal agar plate and one soft agar plate for each of the concentrations you have created. Be sure to label each plate and each tube. Add 0.1 ml of the *Salmonella* culture and 0.1 mil of sterile distilled water to the control tube. Then add 0.1 ml of each dilution of mutagen to 3 ml of soft agar, creating a 1:30 dilution factor. When the agar has hardened, invert the plates and incubate them in the 37° C incubator for 48 hours.

3. Examining the Plates:

   Remove your plates from the incubator and count the number of colonies present. Record all information on worksheet 10, and then plot the curve of the mutagen added to the soft agar tube versus the number of induced mutant colonies.

Drosophila Project

Procedure:

1. Starting Cross:

   Begin your *Drosophila* project today. Select any fly with a recessive phenotype. Select a number of males and female exhibiting the recessive phenotype. Place them in separate medium-containing vial. Then, select male and virgin female wild type flies and place them in the corresponding vial, so that you will have wild type males mating with the recessive females and recessive males mating with the wild type females. Be sure to label your vial!

2. Researching Gene:

   Research the gene(s) that you have selected for your recessive flies, and determine which chromosome it/they are located on.

3. Removing Parents:

   Return to the lab in two days, if larvae are present on the walls, remove and discard parents in the morgue.

Worksheet 10

The Ames Test, Detecting Mutagens

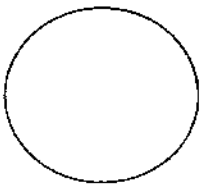
Name:

Lab Section:

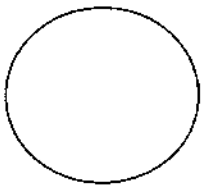
Date:

I. Qualitative Test

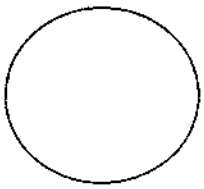
1. In the spaces below, sketch the distribution of *his*$^+$ colonies on the control plate, a positive test plate of a known mutagen, and any positive test plates of new mutagens you have detected.



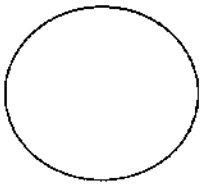
Control



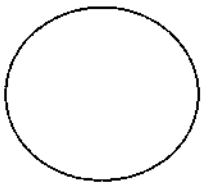
_____
(Compound)



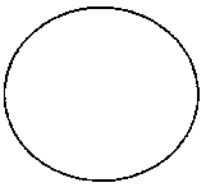
_____
(Compound)



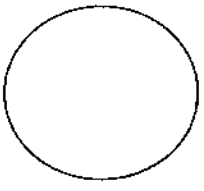
_____
(Compound)



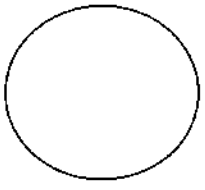
_____
(Compound)



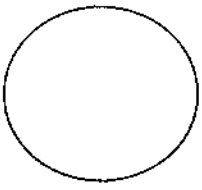
_____
(Compound)



_____
(Compound)



_____
(Compound)



_____
(Compound)

2. In the chart below, list the compound, concentration (if known), and the results of the test:

Plate #	***Compound Under Test***	***Results***
1.		
2.		
3.		
4.		
5.		
6.		
7.		
8.		
9.		
10.		
11.		
12.		
13.		
14.		
15.		

3. Which compound was the most active mutagen in this test?_____
4. Which household compounds tested positive in the test? _____
5. Which food compounds tested positive?_____
6. What type of mutation caused the bacterium to become *his*$^-$? _____

_____

7. What type of mutation was necessary to cause reversion of this strain to *his*$^+$?

_____

_____

8. Could a substance be strongly mutagenic and not test positive in the Ames test if only one *Salmonella* strain is used? If yes, explain. _____

_____

9. What advantages does the Ames test offer? _____

_____

_____

_____

10. List three limitations of this test in regard to human health. _____

_____

_____

_____

II. Quantitative Test

1. Compound under test: _____

Plate #	***Concentration in Tube (actual)***	***Number of Colonies***
1.	0 (control) ( )	
2.	0.1 µg/ml ( )	
3.	0.5 µg/ml ( )	
4.	1 µg/ml ( )	
5.	2 µg/ml ( )	
6.	4 µg/ ml ( )	
7.	10 µg/ml ( )	

2. Dose Response Graph:

Use the graph paper that is in the lab book first. It has grid lines to make the graph easier to plot. Then transfer that graph onto the graph below, this is what needs to be turned in. Do not forget to label graph!

Laboratory Exercise 11: Phage Recombination

Recombinational Difference Between Two Different *rII* Mutations

Materials and Procedure:

- LB agar plates
- LB media
- Soft agar, 3 ml
- *E. coli B, E. coli K12*, and mixed phage T4 *rII* mutants
- 2 mM KCN
- Dilution tubes
- Erlenmeyar flask, 125 ml with foam plug
- Pipettes and micropipettes
- Water bath, shaker bath, and incubator

1. Infection:

   Retrieve a tube containing two mutant phage; record the numbers on worksheet 11. Place 0.5 ml of the phage mixture and 0.5 ml of *E. coli B* in the KCN tube, mix together gently, and incubate for eight minutes. Check to be sure that the growth flask is prepared during this time. Following incubation, transfer 0.1 ml from the KCN tube into the growth flask, cover, and place in the 37° C shaker, allow it to shake for 60 minutes. During this time, titer both the initial and the unadsorbed phage.

2. Titer of Phage:

   Perform a serial dilution in the A tubes. Remember to use clean pipettes every time! Place 0.1 ml of phage stock in tube A-1 and mix, then add 0.1 ml of tube A-1 to A-2 and mix, then add 1 ml of A-2 to A-3 and mix. Swirl the stock tube of *E. coli B* and add 0.1 ml of bacteria to soft agar tubes 1, 2, 3, 4, and 5.

   To tube 1, add 0.1 ml media. To tube 2, add 0.1 ml from A-2.

   To tube 3, add 0.1 ml from A-3. To tube 4, add 0.2 ml from A-3.

To tube 5, add 0.4 ml from A-3.

Mix the contents of each of the tubes, and then spread the mixture evenly on each plate.

3. Titer of Unadsorbed Phage:

   First transfer 0.1 ml from the KCN tube to tube B-1, and then add two drops of chloroform to the tube. Mix this well, and let it stand for ten minutes. This will lyse the bacteria, and any phage that might have adsorbed to the bacteria will render noninfectious. Chloroform will not, on the other hand, harm the free, intact, unadsorbed phage. Then perform a serial dilution in the B tubes. Transfer 0.1 ml of tube B-1 into tube B-2 and mix; then add 1 ml of tube B-2 to B-3 and mix. Swirl the stock of *E. coli B* and add 0.1 ml of bacteria to soft agar tubes 1, 2, 3, and 4.

   To tube 6, add 0.1 ml from A-2. To tube 7, add 0.1 ml from A-3.

   To tube 8, add 0.2 ml from A-3. To tube 9, add 0.4 ml from A-3.

   Mix contents well and then spread the contents of each tube evenly over the appropriate plate.

4. Titer of Total Phage After Infection:

   Following the 60 minutes of shaking at 37° C, lyse the bacteria in the growth tube by adding two drops of chloroform and allowing it to sit for ten minutes. Titer the phage in the growth flask by transferring 0.1 ml from the flask into tube C-1. Transfer 0.1 ml of tube C-1 into C-2 and mix, and then add 1 ml of tube C-2 to C-3 and mix. Both *rII* mutants and recombinant wild type phage will grow on *E. coli B*, so plating will give the total number of phage produced by the infection. Swirl the stock *E. coli B* and add 0.1 ml of bacteria to soft agar tubes 10, 11, and 12.

   To tube 10, add 0.1 ml from C-3. To tube 11, add 0.2 ml from C-3.

   To tube 12, add 0.4 ml from C-3.

   Mix contents and spread evenly over the appropriate plates.

5. Titer of Recombinant Phage After Infection:

   The recombinant phage that are wild type for *rII*, will grow on *E. coli K(-)*, but the *rII* mutants will not, so this plating will give the number of wild type

recombinants. Swirl the stock tube of *E. coli* and add 0.1 ml of bacteria to soft agar tubes 13, 14, 15, and 16.

To tube 13, add 0.1 ml from C-2. To tube 14, add 0.2 ml from C-2.

To tube 15, add 0.3 ml from C-2. To tube 16, add 0.4 ml from C-2.

Mix the contents and spread evenly over the appropriate plates.

After the agar has hardened in all the plates, invert them and place them in the 37° C incubator overnight. The following day, recover the plates and count the plaques on each plate. Compare the physical appearance of plaques on the *E. coli B* and *E. coli K* plates. Record all data on worksheet 11. After counting the plaques, store the plates in the refrigerator for later reference.

Drosophila Project

Procedure:

1. F_1 generation:

   Anesthetize and examine the F_1 generation from your personal cross. Select a number of males and females, and put each sex into a new medium-containing vial. Place wild type flies of the opposite sex into the respective vials. Return in two days, if larvae are present, remove the parents and begin a new vial, so that you have more F_2 generation flies for your report.

2. Inter se Cross:

   Collect a number of males and females from your F_1 generation and place them into at least two vials, in order to determine what occurs with a brother-sister mating.

Worksheet 11

Phage Recombination

Name:

Lab Section:

Date:

I. Concentrations

1. Calculate the concentrations as phage per ml of solution.

Tube # ***Dilution Factor*** ***Plaques*** ***Phage per ml***

Total Phage in mixed stock:

1			Control
2			
3			
4			
5			

Unadsorbed Phage:

6			
7			
8			
9			

Total phage produced by infection:

10			
11			
12			

Recombinant wild type phage:

13			
14			
15			
16			

2. Mutants examined: _____ X _____
3. Recombination frequency: _____
4. In the following spaces, list the mutants you examined and the ones your classmates examined. From the recombination frequencies, prepare a map showing the various mutations.

Mutant		Mutant	Recombination Frequency
_____	X	_____	_____
_____	X	_____	_____
_____	X	_____	_____
_____	X	_____	_____
_____	X	_____	_____
_____	X	_____	_____
_____	X	_____	_____

5. In the space below, prepare a map illustrating the relative positions of the mutations.

II. *rII* Locus

1. What is the ratio of phage to bacteria in the initial infection?

_____

2. Why are there more phage than bacteria in the infection?

_____

_____

3. What percentage of phage were adsorbed? _____

4. What is the probability of a bacterium being infected by only one type of phage? What is the probability of a bacterium being infected by an equal number of both types? _____

5. What is peculiar about the life cycle of T4, that allows it to serve as a recombination tool? _____

6. Why was KCN used during the first step? _____

7. Compare the total number of phage adsorbed (per ml) with the total number of phage produced after infection? _____

8. Why are the wild type phage doubled for the calculation of recombination frequency? _____

Laboratory Exercise 12: Human Genetics

Monogenetic Human Characteristics

Materials and Procedure:

- Worksheet

1. Analysis of Inherited Characteristics:

   Review the list of characteristics that are described on worksheet 12. Examine yourself for these characteristics and record your observations on worksheet 13. Now, choose two of the characteristics and determine the chance of each happening separately and then the chance of them happening together (product rule). Count the number of people showing both characteristics and compare this with the mathematical expectation. Use chi-square to determine if the variation is within the bounds of chance deviation.

2. Hardy-Weinberg Law:

   The Hardy-Weinberg Law can be used to determine the appropriate number of students in the class who carry certain recessive genes in the heterozygous state. For example, consider attached earlobes, suppose there are 5 students out of 30 with attached earlobes. All five of these students must be homozygous recessive (ee) for gene, which is about 16% of the total class. To determine how many students out of the rest of the class are carriers, allow p to represent the dominant gene and q to represent the recessive gene. The three groups can then be represented as follows: $p^2 + 2pq + q^2$. Now, determine the values for p and q. Since 16% of the students represent homozygous recessive, you known that q^2 is the square root of 0.16, which is 0.40 or 40%. To determine the value of p, subtract the 40% from 100%, this gives you 60% or 0.60 as the value for p. Since the value for number of students homozygous for free earlobes is p^2, you square 60% and obtain 36%. With a class of 30 students, this would constitute about 11 people, leaving 14 that must be heterozygous and expressing the free earlobe trait. Check this number by multiplying 2 X 0.6 X 0.4, or $2pq$, and you get 48%, which

is about 14 people. Now, choose one of the recessive characteristics already studied and determine the distribution of homozygous and heterozygous students who express the dominant allele.

Drosophila Project

Procedure:

1. You should continue working on your project. By this time, you should have at least counted some of your F_1 generation. Begin examining your F_2 generation today. Another cross should be made in order to produce an F_3 generation. Be sure to record all your data on your project sheets.

Worksheet 12

Monogenetic Human Characteristics

I. Attached Earlobes

Most people have earlobes that hang free, but when a person is homozygous recessive for a certain gene (e), the earlobes are attached directly to the side of the head. Look at yourself in a mirror or with the help of a classmate, and determine your phenotype for the characteristic. Record your observation on worksheet 13.

II. Widow's Peak

When the hairline on an individual drops downward and forms a distinct point in the center of the forehead, the person is known to have a widow's peak. This results from the action of a dominant gene (W). Determine and record your phenotype for this characteristic.

III. Tongue Rolling

When a dominant gene (R) is present, the individual is able to roll the tongue into a distinct U-shape when the tongue is extended from the mouth. Individuals, who do not posses this gene, are unable to roll their tongue, instead they can only produce a slight downward curve of the tongue when it is extended from the mouth. Try to roll your tongue, and record your results.

IV. Bent Little Finger

The last joint of the little finger can bend inward toward the fourth finger, when a dominant gene (B) is present. Lay both hands flat on the table, relax the muscles, and note whether or not you have a bent or straight little finger.

V. Hitchhiker's Thumb

The hitchhiker's thumb, known as "distal hyperextensibility of the thumb," can be determined by bending the distal joint of the thumb back as far as possible. There is some degree of continuous variation, but some individuals are able to bend their thumbs back to an almost 90 degree angle. This characteristic is thought to be caused by a recessive gene (h). There is a five- percent penetrance with this characteristic, which means that about one person in twenty who carries the gene

will not express the characteristic. Look at your own thumb and determine and record your phenotype.

VI. Long Palmar Muscle

A long palmar muscle, which is caused by a recessive gene (l), can be detected by examining the tendons that run over the inside of the wrists. Clench your fist tightly and flex your hand. Feel the tendons, if there are three, you have the long palmar muscle; if there are only two, then you do not have this muscle. Be sure to examine both wrists, if it is present in even one of your wrists, you have the recessive gene. Determine and record your phenotype for the presence or absence of this trait.

VII. Pigmented Iris of the Eyes

When there is no pigment in the front part of the eye and a blue layer at the back of the iris shows through, a recessive gene (p) is present in the homozygous state. This gives blue eyes. When the dominant gene (P) is present, pigment is deposited in the front of layer of the iris, which masks the blue to a varying degree. Determine whether or not you have this pigment and record your results on worksheet 13.

VIII. PTC Tasting

Some people are able to detect a bitter taste from a chemical, phenylthiocarbamide (PTC), while others cannot. A dominant gene (T) seems to confer the ability to taste these chemicals at a low concentration. To test your tasting ability, place a piece of the PTC paper on your tongue and allow it to remain there for ten seconds. If you are a taster, you will know it. Record your results on the worksheet.

IX. Mid-digital Hair

Some people have hair growth on their middle joint of one or more fingers, while others do not. The complete absence of hair is due to a recessive gene (m), whereas its presence is due to the dominant gene (M). Use a hand lens to determine whether or not you have hair present on the middle joint of any of your fingers, and record your results.

X. Second Finger Shorter than the Fourth

This characteristic appears to be sex-influenced. Use the symbol (S^S) for the short second finger and the symbol (S^L) for the longer second finger. Tabulate these results according to sex, since the frequency should vary among the sexes.

XI. Interlocking Fingers

Some people will place their left thumb over their right thumb, when the fingers are interlocked. Studies have suggested that the placing of the left thumb over the right is due to a dominant gene (F), while the opposite is due to the recessive gene (f). Determine and record your phenotype.

Worksheet 13

Human Genetics

Name:

Lab Section:

Date:

I. Some Monogenetic Human Characteristics

1. Tabulate your phenotype for each of the human characteristics studied, also give your possible genotype and the total number of students in the class that show each characteristic.

	Check Your Phenotype	Give Your Genotype	Number of Each In the Class
Attached Earlobes			
Free Earlobes			
Widow's Peak			
No Widow's Peak			
Can Roll Tongue			
Cannot Roll Tongue			
Bent Little Finger			
Straight			
Hitchhiker's Thumb			
No Hitchhiker's Thumb			
Long Palmar Muscle			
No Long Palmar Muscle			
Pigmented Iris			
No Pigmented Iris (blue)			
PTC Taster			
Nontaster of PTC			
No Mid-digital Hair			
Mid-digital Hair			

2nd _____ _____

3rd _____ _____ _____

4th _____ _____

5th _____ _____

Second Finger Shorter Than the Fourth-

Male _____ _____

Female _____ _____

Second Finger Not Shorter Than the Fourth- _____

Male _____ _____

Female _____ _____

Interlocking Fingers:

Left Thumb on Top _____ _____ _____

Right Thumb on Top _____ _____ _____

II. Analysis of Inherited Characteristics

1.List the two characteristics that have been chosen and determine the cahnce of each showing in any person picked at random.

Characteristic Number 1. _____

Number of students showing it. _____

Total number of students. _____

Percentage showing it. _____

Characteristic Number 2. _____

Number of students showing it. _____

Percentage showing it. _____

2. Consider the percentage showing a characteristic as the chance of any one person showing it, and calculate the chance of any one person showing both characteristics.

3. According to the figure obtained from question 2, how many in the class would be expected to show both these characteristics, show how you obtained results.

4. How many in the class actually show these two characteristics? _____
5. What is the deviation? _____
6. Does the deviation appear to be significant in light of the number of persons involved in this study? _____
7. Use chi-square to determine if the variation is sufficient to be significant.

8. What is the recessive expression of the characteristic chosen to demonstrate the H-W law? _____
9. How many persons in the class show this recessive trait? _____
10. How many express the dominant allele? _____
11. What percentage of the class show the recessive? _____
12. What percentage would be expected to be heterozygous? _____
13. How many in would be heterozygous? Show work below.

Laboratory Exercise 13: Isolation of Eukaryotic DNA from Tissue

Isolation of DNA

Materials and Procedure:

- Plastic capped tubes
- Crushed ice
- Parafilm
- Scalpel
- Pipettes
- Lysis buffer, DNA buffer TE
- Absolute ethanol
- Liver tissue
- Dry ice
- Mortar and pestle

1. Guanidine HCL Extraction:

   Retrieve a 0.5-gram sample of tissue fragments, which are wrapped in foil and frozen in the dry ice. Weigh the tissue fragment, while it is still wrapped. Unwrap the tissue and place it in the mortar that contains a little dry ice. Using a frozen pestle, crush the tissue to a fine powder, and then pour it into a plastic tube containing 10 ml of lysis buffer. Cap this tube, submerge the powder, and mix by rocking gently at room temperature for one hour. Then, add two volumes of (20 ml) ethanol at room temperature to a 50-ml plastic tube, use a wide-mouth pipette and underlay the DNA solution carefully. Next, using a sealed disposable pipette, slowly stir the interface. The DNA will precipitate out as a string fiber, spool the DNA onto a pipette, and gently pat off the ethanol on the side of the tube and allow the ethanol to dry. Examine the DNA fragment while you are spooling it. Notice how long it is and what it looks like, this is the objective for the whole lab, for you to get an idea of what DNA looks like. Place the DNA into a fresh tube

with 1 ml TE, cover and mix gently. Allow the DNA to remain for at least one hour in the refrigerator at 4° C.

2. Weighing the Sample:

   Remove the sample from the refrigerator and weigh it. After you have finished weighing your sample, place it back in the refrigerator for later use by your instructor. Then, answer the questions on worksheet 14.

Drosophila Project

While you are waiting on this lab to complete, work on your project. Make sure that you have enough flies to generate your report. This will be the last lab that you will be able to work on this project!

Worksheet 14

Isolation of Eukaryotic DNA from Tissue

Name:

Lab Section:

Date:

I. Guanidine HCl Extraction

1. Source of tissue: _____
2. Weight: Total: _____ (- wrap): _____ sample = _____
3. DNA yield: _____
4. Describe what the DNA looked like as you spooled it onto the pipette?

5. How many grams of DNA are in mammalian cell, on average? _____
6. How many cells do humans have? _____
7. How many grams of DNA are in the body, on average? _____
8. Where do you think that the most damage occurred to the DNA in your experiment?

_____
_____
_____

9. Where do you think the greatest loss of DNA occurred in your procedure?

_____
_____
_____