BNL 7286

CONF-241-2

PROBLEMS OF PRESENT DAY GENETICS[1]

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[1]Research carried out at Brookhaven National Laboratory under the auspices of the U. S. Atomic Energy Commission.

At this, the XI International Congress of Genetics, the major portion of the program has been devoted to symposia, in which outstanding problems of modern genetics have been discussed. It required vision on the part of the Organizing Committee to conceive such a program for an international congress; and it required courage, a great deal of courage, to schedule 25 symposia on 6 meeting days. But the vision and courage have been amply rewarded. The symposia were outstanding, and have contributed toward making this Congress a strikingly successful gathering.

Current problems of genetics were well covered in the symposium meetings. More than one-fourth of the sessions (7 out of 25) dealt with areas of research that have become active only recently, for the most part since the Ninth Congress held in Stockholm in 1953; and the two sessions on molecular genetics were concerned with problems that have developed during the past five years, that is, since the time of the last Congress. Thus the program of this Congress truly reflects the present situation in what is known as classical genetics. It recognizes a breakthrough into new lines of research, and the intense activity characteristic of fields recently opened up.

In the sixty-odd years of its existence, classical genetics has passed through several distinct periods of heightened activity, each initiated by some important discovery followed by a stretch of settled exertion during which the details of problems generated by the new discovery were worked out. The first intensely active period began with the "rediscovery" of Mendel's published papers. The objective was to establish that the transmission of heritable characteristics, in both plants and animals, is accomplished by the same mechanism that is responsible for Mendelian segregation--in other words, to demonstrate the universal applicability of the basic laws of heredity. The second such period began about 1912, with the introduction of Drosophila as an

organism for genetic studies. Its most important accomplishment was the analysis of the physical basis of heredity, that is, the proof that genes are located in chromosomes and arranged there in a linear order. The discovery that genetic changes can be induced by ionizing radiations marked the beginning of a third period, during which our information about the genetic organization of chromosomes was greatly extended. This knowledge was further amplified during a fourth period, which was activated by the discovery of a close correspondence between the bands of the salivary-gland chromosomes of Drosophila and the positions of gene loci in these chromosomes. The fifth and sixth periods followed each other in close succession. One, initiated by the use of microorganisms in genetical research, provided evidence that the function of an enzyme is gene controlled, and that genes are complex in structure. The other began with the formulation of the well known WATSON-CRICK model of DNA, which opened the way for a flood of very prolific work in the new field of molecular genetics.

Although we are now mainly concerned with current problems, I feel that a few comparisons with the past will not be out of place, since there is a great similarity between present conditions and those that prevailed not so very many years ago. I have in mind the outlook of those workers who are active in molecular genetics. The research in that field is progressing exceedingly well, bringing forth rapidly spectacular results--a situation very like the one that characterized Drosophila research in the 1920's. Molecular geneticists, naturally enough, are full of confidence; they are self-assured, forceful, and exhilerated by the experience of riding the crest of a wave of success; they feel much the way Drosophila geneticists feld some three decades ago. Again duplicating an earlier pattern, they tend to look down on their less successful colleagues, and to regard themselves as a group especially chosen to solve all the vital biological

problems, not of genetics alone but of the origin of life itself. Just like Drosophila workers in the days when their research led the field, they have formed a closely knit group, within which information flows freely and a new vocabulary is being rapidly developed. The" outsiders", meanwhile, are beginning to experience a degree of discontent. They have reason to feel left out and disregarded.

But geneticists as a whole are a very special breed of scientists. Animosity is rare among them, and hardly ever violent. Therefore the present circumstances do not generate ill will or resentment. The "outsiders" are now biding their time, patiently awaiting their chance to come to the forefront. They are convinced that the present period is no different from others that have preceded it, and that the results of current research—like those of the past—are only stepping-stones on the long and winding path of progress. We now recognize that only the basic conclusions of earlier periods seem to be correct, for recent evidence has forced us to relinquish many prominent details of the models of genetic mechanisms painstakingly constructed not long ago. It seems reasonable to assume that although major accomplishments of the present period will survive the ravages of time, a considerable proportion of our apparently well substantiated conclusions will have to be modified or even discarded.

What is the status of basic genetics today? The symposia held at this Congress have provided a good survey of the situation. Probably the most important development of the past decade is the change that has occurred in our picture of the gene. We once visualized gene loci as indivisible units of the genetic system, whose sizes could be determined with the help of X-rays and whose numbers could be confidently estimated. We now

view the gene locus as a complex structure, extending over a section of chromosome, within which changes occurring at different positions that we call "sites" give rise to different alleles. These alleles can recombine, by a process analogous to crossing over, with alleles of a homologous locus of another chromosome. The number of sites genetically identified by BENZER (1961) in the rII region of phage T4 justifies the conclusion that a site corresponds to a nucleotide pair of the WATSON-CRICK DNA molecule. Therefore we can assume a very large potentiality for the occurrance of different alleles, perhaps at the rate of several hundred or thousands per gene locus, although there is no reason to assume that all changes occurring at different sites of a locus give rise to detectable mutants.

In our laboratory, and in the laboratory of Dr. P. E. Hartman at the Johns Hopkins University, studies have been made of recombination among alleles of 91 gene loci of Salmonella typhimurium. In all the experiments recombinants have been observed, showing clearly that all genes of that organism are complex in structure. Recent work with higher organisms has revealed the complex nature of genes in several fungi (particularly Neurospora, yeasts, and Aspergillus), in maize, and, as was pointed out by M. M. GREEN in the first symposium of this Congress, in Drosophila as well. Thus it seems reasonable to assume that complex gene structure is a general feature of living organisms and, moreover, that the organization of genetic material is basically similar throughout the living world. This material consists of a thread of nucleic acid: sometimes RNA, but in most of the organisms analyzed, DNA. The thread is composed, probably not exclusively, of a large number of regions (genes) that are in some way differentiated from one another. Each region controls a specific function. Perhaps in most cases, this control is effected through an enzyme, whose structure--and

consequently, in a large measure, character and specificity--are determined by the gene. Evidence is available indicating that the borderline between adjacent genes is sharp, with no overlapping.

The evidence about complex gene structure has been derived through a well-tested technique of classical genetics, that is, by studying the frequencies of recombination between alleles of gene loci. In phages, bacteria, and fungi, judging by the observed recombination patterns, the sites of changes responsible for alleles are generally distributed more or less at random within the gene loci. As far as I am aware, only one case of "clustering" of sites has been reported. In analyzing the genetic structure of locus cysC of S. typhimurium (DEMEREC, GILLESPIE and MIZOBUCHI 1963), we concluded on the basis of recombination frequencies that the sites are distributed at random in the right half of the locus but in the left half are arranged in three clusters; the frequency of recombination between two members of the same cluster is considerably lower than that between members of different clusters. Studies with Drosophila, have indicated that different gene loci may differ greatly with regard to the recombination patterns observed in crosses between their alleles. GREEN (1963) reports that in some loci (for example, lozenge) frequencies of recombination between alleles are high, whereas in others (e. g. rose) they are low, approximating those observed in microorganisms. His analysis of the Notch locus suggests that its alleles are organized as a continuum of adjacent sites. In the white locus, on the other hand, there seem to be only a few recombinational sites, with a number of independent alleles at each site, resulting in a pattern of discontinuous clusters.

We suggested that the divergence of locus cysC from the usual pattern of recombination in Salmonella loci could be explained by the presence between

clusters of "silent sections" of DNA, consisting of noncoded material, in which recombinations occur but the changes responsible for allelic mutants do not. In the region that contains a group of five cys loci (C, D, H, I, and J), several silent sections have been detected. Our analysis indicates that these sections, in addition to being genetically undifferentiated, are so constructed that any portion of them is homologous to any other portion and synapsis may occur between any of their parts. We suggested that the silent sections are comparable to the heterochromatic regions of chromosomes of higher organisms and are composed of polynucleotide sequences. The available analyses of complex structure in genes of different organisms indicate that we may expect to find a considerable variety of composition, and that the complexity is probably greater in higher organisms.

The theory of the gene evolved in work with Drosophila postulates a close interaction among the genes of a cell. It assumes that the whole complement of genes forms an interrelated system, in which the function of any one is essential for the normal functioning of the whole. Recent work with bacteria, with phage, and (as reported at this Congress by N. H. GILES) with Neurospora has disclosed another important aspect of functional interrelationship among genes. Our work with Salmonella has shown that genes are not distributed at random along the chromosome. Those that control related biochemical functions are frequently clustered together, and moreover their order within the cluster occasionally follows the sequence of the biochemical reactions they control. The mere existence of such arrangements shows that they must be beneficial, conferring an advantage on individuals and populations that exhibit them. Whether or not adjacent loci have a common origin is irrelevant to the question of their ultimate position, as long as there is a mechanism capable of separating them. With such a mechanism in operation, only selective advantage--during a long series

of evolutionary adjustments--could preserve a specific gene arrangement if the genes involved had a common origin, or bring about such an arrangement if they originated in different parts of a genome (DEMEREC and HARTMAN, 1959). The most complete gene cluster so far discovered in Salmonella is the histidine cluster, analyzed genetically by HARTMAN and biochemically by AMES (HARTMAN, 1956; AMES and HARTMAN, 1963). It consists of eight genes, seven of which are sequentially arranged.

In Drosophila and other higher organisms, such clustering has not been detected. Even in Neurospora it must be rare. Although several instances of close linkage between two similar genes have been recorded (BODMER and PARSONS, 1962), the first report about closely linked structural genes constituting a coordinated unit of function (three or four histidine genes) was given by GILES at the first symposium of this Congress (GILES, 1963). Thus it appears that an evolutionary break from nonrandom to random distribution of functionally related genes has occurred between bacteria and organisms lower than fungi.

The existence of a gene cluster indicates that the proximity of genes concerned with a reaction is either essential or advantageous for the occurrence of that reaction, in other words that a whole group of genes is directly involved in controlling the reaction. Scattering of genes, on the other hand, suggests that each gene functions independently to control a different step of the reaction. With regard to some clusters--for example, the Salmonella leucine cluster (MARGOLIN, 1963) and histidine cluster (AMES and HARTMAN, 1963)--it has been determined that one gene in each group acts as operator, regulating the function of the others, so that the whole cluster behaves as a unit, named by JACOB and MONOD (1961) an operon.

The most spectacular example of arrangement of genes in accordance

with their functions was discovered in phage T4 (EPSTEIN, BOLLE, STEINBERG, KELLENBERGER and EDGAR, SUSMAN, LEILAUSIS, 1963). These workers found that all the genes affecting DNA synthesis are separated from all those affecting maturation. Within the maturation section there is further grouping of genes concerned with head synthesis, with tail-fiber synthesis, and so on. Moreover, all these groups appear to be arranged in a sequence according to the time in the life cycle when the genes within them become active.

The molecular structure of genetic material is exceedingly simple. DNA, an aperiodic linear polymer, consists of two antiparallel phosphate-deoxyribose chains, coiled helically around a common axis. Attached to each deoxyribose is a purine base (adenine or guanine) or a pyrimidine base (thymine or cytosine). The two chains are linked by hydrogen bonds that are formed between two purine-pyrimidine bases, adenine-thymine (A-T) and guamine-cytosine (G-C). The genetic information is carried in the sequence of the four bases (A, T, G, and C), which form a four-letter alphabet in which are written words and sentences of genetic message. A sequence of several hundreds or thousands of these letters (bases) makes up a sentence directing a simple function; and such a sequence constitutes a gene.

During the past three years tremendous progress has been made toward deciphering the genetic code. As was brought out in the third symposium, it now appears probable that the code is simple and uniform, consisting of three-letter words. Genic mutation constitutes change, loss, or gain of one or more letters of a word. Studies of chemical mutagenesis, discussed in our eighth symposium, gives us confidence that we now have tools for identifying the molecular nature of the changes responsible for mutations.

Differentiation of genetic material is the last problem I wish to discuss here. Since the early days of genetics this problem has interested

many geneticists. When the gene was considered the ultimate unit of the genetic system, it was proposed that evolutionary increase in complexity of that system came about through an increase in number of genes by duplication, and subsequent differentiation among sister genes by changes occurring within them. This model is still valid. Today we can state with certainty that a bacterium has a larger number of genes than a phage particle, and we can postulate with more assurance than a decade ago that organisms high on the evolutionary scale have more genes than those low on the scale. It is not difficult to visualize a mechanism for achieving increased numbers of genes. Our understanding of the processes involved in differentiation between homologous genes may be furthered by evidence obtained in our studies with S. typhimurium x Escherichia coli hybrids. A fuller report was presented a few days ago at the Third Erwin Baur Symposium in Gatersleben (DEMEREC 1963).

S. typhimurium and E. coli can be crossed. Transduction studies with the hybrids produced by these crosses reveal that Salmonella phage P22 is able to transport fragments derived from either the Salmonella or the E. coli chromosome. However, integration of E. coli genic material into the Salmonella chromosome occurs with a very low frequency. The evidence obtained in our experiments indicate that a lack of homology at the subgenic level is responsible for the failure of integration. It suggests that the genes of S. typhimurium and E. coli are homologous with respect to function and position on the chromosome but not with respect to fine structure. This divergence in homology could originate from changes within coding triplets which, because of the degeneracy of the code, do not affect transcription; or from changes that modify the transcription of amino acids of the nonfunctional regions of the protein; or from a combination of both. According to recently proposed hypotheses the degeneracy of the amino acid code is so extensive that almost every amino acid can be coded by at least two different triplets

(WOESE, 1962). Thus two sentences could be composed which would transcribe exactly alike but differ in almost every consecutive word. If identity of words is essential for synapsis and consequent recombination, then two genes represented by two such sentences, although responsible for the same enzyme would not recombine with each other.

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