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ISOLATION AND GENETIC CHARACTERIZATION OF
RECOMBINATION DEFICIENT MUTANTS IN
SACCHAROMYCES CEREVISIAE

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ISOLATION AND GENETIC CHARACTERIZATION
OF RECOMBINATION DEFICIENT MUTANTS IN SACCHAROMYCES CEREVISIAE

ABSTRACT

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Recombination in Saccharomyces cerevisiae has been studied genetically by selecting mutants of disomic yeast deficient in X-ray and ultraviolet light (UV) induced mitotic heteroallelic reversion. Disomics were obtained by isolation techniques based on detection of heteroallelic regions in one chromosome. They were also recovered spontaneously. Of ten identified rec mutants, seven were studied in detail. The mutants exhibit depressed frequencies of X-ray and/or UV-induced reversion in the heteroallelic region arg4-2 +/+ arg4-17 on disomic chromosome VIII. Two of the mutants are X-ray sensitive and one is both X-ray and UV-sensitive. The remaining four strains exhibit wild type response to both radiations. Each of the seven mutations segregates as one gene and is recessive. Complementation and allelism tests were performed and a minimum of four genes, denoted rec1, rec2, rec3, rec4, were identified among the seven mutants.

Mutations in rec1 depress X-ray and UV-induced mitotic conversion about 20 times and do not affect either X-ray induced mitotic segregation of ade2 or meiotic intragenic or intergenic recombination. Mutations in rec2 cause X-ray sensitivity and depress X-ray induced conversion to about 20% of the normal value, but do not affect UV induction of reversion. Homozygous rec2/rec2 diploids yield only 1 to 2 percent sporulation and 10 percent spore viability. They also exhibit normal frequencies of X-ray induced mitotic crossing over of ade2. Mutations in rec3 completely suppress spontaneous and X-ray or

UV-induced mitotic conversion and also suppress sporulation. X-ray induced mitotic segregation of cyh2 occurs at normal frequencies. Diploids homozygous for rec4 do not exhibit spontaneous and X-ray or UV-induced mitotic conversion. They show normal frequencies for X-ray induced mitotic crossing over in ade8. Such diploids exhibit 60 percent sporulation and 90 percent spore viability. Intragenic meiotic recombination between alleles 2 and 17 in arg4 is reduced about 500 times. Meiotic crossing over appears normal for the regions between mating type and ade8 and their respective centromeres and also in the intervals between thr1 and CUP1 and the centromere of chromosome VIII.

By analogy with results in E. coli it is suggested that induced lesions in DNA stimulate recombination, while rec mutants are unable to complete the steps required for exchange. Wild type sensitivity to radiation induced damage in some mutants suggests that recombination in yeast may also proceed in pathways not involving repair of radiation damage. Common processes for mitosis and meiosis are suggested by the existence of mutants rec2, rec3, and rec4. Mutations in rec4 suggest common steps leading to single site conversion in mitosis and meiosis. The low sporulation and low spore viability associated with the rec2 mutations are suggested to be an indication of impairment in the mechanism for chromosomal disjunction.

I. INTRODUCTION

Linked genetic markers are often found in new arrays in the meiotic products of hybrids or among the descendants of mitotically dividing cells. The underlying process generating new combinations of genetic material is called recombination. The studies detailed in this work are concerned with the genetic control of recombination in yeast.

Recombination of genetic markers has a parallel in the physical exchange by breakage and reunion of chromosomal segments which has been observed in Drosophila (62), in bacteriophage (40), and in grasshopper (66). That this exchange is under genetic control has been well established in bacteriophage and E. coli. Exchange of DNA segments in bacteriophage is controlled by two different sets of genes. One set controls recombination between two viral DNA molecules, while the other participates in integrating the prophage into the host cell genome (19). In E. coli the recA, recB, and recC genes control exchange in transformation and in the integration of the Hfr chromosome into the F⁻ genome during conjugation (6).

The molecular mechanisms of recombination are not as amenable to study in eucaryotic organisms as in viruses and bacteria. However, synapsis of homologous chromosomes at the time of recombination has been demonstrated by cytological techniques. The synaptonemal complex, widely observed in plant and animal germinal cells, may provide the necessary structure for intimate association between biparental homologues to facilitate the occurrence of molecular steps essential to exchange (45). Evidence for the genetic control of the synaptonemal complex is provided by mutant c(3)G in Drosophila melanogaster, which

is deficient in meiotic recombination and also is deficient in formation of the synaptonemal complex (18). Mutations at this locus also result in a high rate of nondisjunction (58). Synaptonemal complexes may not be essential for genetic exchange, since they have not been detected in procaryotes or in somatic eucaryotic cells which also undergo recombination. However, such nonmeiotic recombination may occur by other mechanisms.

Important to an understanding of the molecular mechanisms of recombination is the finding that DNA duplication and genetic exchange occur at different times during the meiotic cycle. This favors breakage and reunion mechanisms over models which require chromosome replication for exchange (copy choice). In the ascomycete Neotiella rutilans the chromosomal replication required for meiotic division occurs before fusion of the parental nuclei (55). A similar finding has been reported for the green alga Chlamydomonas reinhardi (64). However, completion of exchange may require localized DNA synthesis to fill in degraded regions appearing during formation of recombinants or to allow the repair of mismatched bases in heteroduplex regions (26). This idea is supported by findings in lily and trillium of two periods of synthetic activity in which about 0.3% of the DNA complement is produced. These periods correspond to the time in early prophase, in which recombination occurs. One of these periods has been associated with DNA repair and the other with the formation of synaptonemal complexes (63).

Various models have been devised which incorporate molecular mechanisms to explain reciprocal and nonreciprocal exchange. A main feature in these models is the temporal sequence for occurrence of

breakage and exchange and localized DNA synthesis. In the models proposed by Holliday (22) and Whitehouse and Hastings (70), nonreciprocal recombinations are generated as a result of synthesis following breakage of DNA strands, for example by repair of mismatched bases in heteroduplex regions. In the scheme proposed by Stahl (60), nonreciprocal exchanges occur between corresponding duplicated regions in the homologous chromosomes. These exchanges are nonreciprocal in nature, since only one of the two resulting recombinants is preserved. In these three models, single or double strand breakages and local DNA synthesis for repair or duplication require enzymes to mediate these reactions. Such enzymes have been proposed to include endonucleases to introduce breakages in the DNA molecule. Exonucleases may then prepare the broken strands for exchange introducing further degradation to produce gaps. Homologous regions would unwind and complementary single strands come into close proximity allowing base pairing. The gaps left after reannealing are presumed to be filled by repair synthesis which would involve polymerases. For example, DNA polymerase has both an exonuclease and a repair polymerase activity (32). Hybrid regions would then be covalently linked by the action of polynucleotide ligases (25).

Genetic analysis and biochemical characterization of strains that carry defective recombination genes have revealed much about the molecular aspects of recombination. Recombination defective mutants in E. coli are both X-ray and UV-sensitive (27) (68). A defective exonuclease has been identified in recB and recC mutants, suggesting the participation of nucleases in recombination (2).

Induced recombination has been studied in excision defective E. coli strains (HCR⁻). These mutants accumulate potentially lethal

unrepaired pyrimidine dimers after UV exposure. They are rec⁺ and exhibit enhanced frequencies of induced exchange. This suggests that accumulated dimers provide a substrate for recombination (28).

The correlation between radiation sensitivity and recombination deficiency in bacteria prompted the use of radiation sensitivity in the search for recombination deficient mutants in eucaryotic cells. In Ustilago one mutation for UV sensitivity was found to determine enhanced frequencies of spontaneous mitotic conversion and crossing over. A second mutation for UV sensitivity was responsible for a depressed frequency of UV-induced conversion and mitotic recombination. In this mutant meiotic products have not been observed (24). Similarly, UV-sensitive mutants in Neurospora (5) and Aspergillus (33) exhibited abortive meiosis.

The yeast Saccharomyces cerevisiae provides a simple test organism for studies of recombination (43). It has stable haploid and diploid vegetative phases. Meiotic products can be easily recovered by suitable techniques (31). In addition, about 100 genetic markers have been located in 17 linkage groups (21). Furthermore, recombination in yeast has been extensively studied. Tetrad analysis of unselected samples has allowed the investigation of informational transfer between homologous chromosomes (13). As a result, it has been proposed that nonreciprocal events alone can account for all meiotic recombination in the yeast genome (12). Radiation induced intergenic mitotic recombination is detected mainly as a result of reciprocal exchanges (47) (44). Occurrence of recombination during the cell cycle has been studied in synchronous cultures of vegetative cells. It has been found that recombination can be induced throughout the cell cycle (9, 71).

However, most conversional events seem to occur independently of DNA replication in both mitosis and meiosis (71, 11).

UV-sensitive mutations in yeast do not affect the frequencies of meiotic or spontaneous mitotic recombination (59; S. Fogel and D.D. Hurst, personal communication). However, one X-ray sensitive mutant was found to be deficient in X-ray induced mitotic conversion (41). Other X-ray sensitive strains exhibited low sporulation (50), indicating that the products of these genes are also required in meiosis. In the present study, mutants of yeast deficient in induced mitotic conversion were isolated to explore the following questions:

1. Are there common steps in mitotic and meiotic recombination?
2. Is recombination a repair process?
3. Is the effect of rec genes specific for a region of the genome?

Although mitotic recombination can be detected in heteroallelic or heterozygous regions, the diploid state precludes detection of recessive mutations affecting recombination. Disomic strains provide an excellent test system for the recovery of mutants affecting mitotic recombination, since their genomes have both diploid and haploid regions. Disomics were obtained by isolation techniques based on detection of heteroallelic regions in one chromosome. They were also recovered incidental to a study of gene conversion in the arg4 region of chromosome VIII (13). Mutants were identified by their depressed frequency of induced mitotic conversion at a heteroallelic locus on the disomic chromosome. The mutants isolated were tested for radiation sensitivity and for their behavior in mitosis and meiosis. The results are discussed in relation to repair and recombination.

II. MATERIALS AND METHODS

A. Yeast strains

Heterothallic strains of Saccharomyces cerevisiae were obtained from the stocks of Dr. Robert K. Mortimer. Gene symbols are indicated by three lower or upper case letters for mutant and wild type genotypes respectively. Locus and allele numbers are printed on the same line (e.g. arg4-17), in accordance with the conventions proposed at the Osaka Yeast Genetics Conference (69).

Two strains, disomic for chromosome VIII, were used and their genotypes are given below:

Z140-51C

α arg4-2 + + CUP1 leu1 trp5 trp1 his5 ade2
+ arg4-17 thr1 cup1

Z140-51D

a arg4-2 + + CUP1 leu1 trp5 trp1 his5 ade2 met1
+ arg4-17 thr1 cup1

The origin and genetic characterization of these strains are described in the RESULTS. The alleles arg4-2 and arg4-17 are non-complementing nonsense mutants of the arg4 (argininosuccinase) locus. Also in heterozygous condition on chromosome VIII are thr1 and CUP1. These markers allow verification of the disomic configuration. Both strains require arginine but revert to arginine-independence at rates lower than the corresponding diploids.

For allele identification at the arg4 gene, the following tester strains from the stocks of Dr. Seymour Fogel were used.

Z149-20A α arg4-17 leu1-12 ade1-1

Z149-15D a arg4-17 leu1-12 ade1-1

Z136-1-7D α arg4-2 leu1-1 ade1-1 trp5-48

Z136-1-13C a arg4-2 leu1-1 ade1-1 trp5-48

For part of the genetic analysis, wild type strains X2180-1A (a mating type) and X2180-1B (α mating type) were used.

B. Media

YEPD: yeast extract 1%, peptone 2%, dextrose 2%, (2% agar for solid medium); (obtained from Difco laboratory).

Minimal: Difco yeast nitrogen base without amino acids 2/3%, dextrose 2%.

Synthetic complete: minimal medium supplemented with, adenine sulfate 20 mg., uracil 20 mg., 1-arginine 20 mg., 1-leucine 30 mg., 1-lysine 30 mg., 1-histidine 20 mg., 1-methionine 20 mg., 1-tryptophan 20 mg., 1-threonine 100 mg. (obtained from CALBIOCHEM) per 1000 ml.

Synthetic omission: synthetic complete lacking specific amino acids or bases.

Petite: glycerol 3%, dextrose 0.025%, yeast extract 1%, Bacto-peptone 2%.

Presporulation: glucose 0.1%, yeast extract 0.25%, potassium acetate 1% (38); or potassium acetate 3%, raffinose 0.002% (14).

Copper: for 1000 ml. of synthetic complete, 60 mg. of copper added as $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$.

Actidione: for 1000 ml. of synthetic complete, 1.4 mg. of actidione (obtained from CALBIOCHEM).

C. Radiation sources and determination of survival following irradiation

A beryllium-window tube (Machlett OEG 60) supplied X-rays at a dose rate of 250 rads/sec (50 KVp, 25 mA). Three 8-watt General Electric UV germicidal lamps (G875, 90% intensity at 2537 Å) were used for UV treatment. The flux at the distance used for irradiation was approximately $27 \text{ ergs/mm}^2/\text{sec}$ as measured with a photocell (No. 935, RCA).

Cells from strains whose response to X-rays or UV exposure were to be studied were grown in YEPD liquid culture. YEPD plates inoculated with appropriate numbers of washed cells were irradiated to yield approximately 200 colonies per plate. The colonies were counted three or four days after treatment.

D. Radiation-induced heteroallelic reversion

Heteroallelic diploids, treated with low doses of X-rays, show linear kinetics of recombinant induction. The slope of these induction curves in prototrophs/ 10^8 survivors/rad, provide a measure of the distance between alleles (36).

In a typical experiment, heteroallelic diploid or disomic strains were inoculated in liquid YEPD and incubated on a shaker at 30°C for 4 to 6 days. Harvested cells were washed twice in distilled water. About 10^7 cells were plated on the appropriated omission medium and exposed to X-rays and UV. Clones were counted after 4 or 5 days incubation at 30°C . An inactivation curve was always determined for the same culture.

E. Allele identification at the arg4 locus

The arg4 alleles present in a strain were identified as follows. Each strain was mated to two tester strains, one bearing arg4-2 and the other arg4-17. The resultant diploids or trisomics were selected, replica-plated to arginine-deficient medium, and then exposed to X-rays (10 Krads) to induce heteroallelic reversion (10). Only heteroallelic combinations (2 +/+ 17, 2 +/2 +/+ 17 or 2 +/+ 17/+ 17) are expected to yield revertants. A disomic strain in which one chromosome carries arg4-2 and the other carries arg4-17 will show heteroallelic reversion when irradiated directly or when crossed with either the arg4-2 or the arg4-17 tester. The recombination test was the basis on which the disomics were originally detected and it was routinely used to verify the disomic configuration.

F. Induction of recombination-deficient mutants

Cells from strains Z140-51C and Z140-51D were grown to stationary phase in liquid complete medium (YEPD). The cells were washed and appropriate dilutions were plated on solid complete medium (YEPD) and irradiated with UV (795 ergs/mm^2) to induce mutations. The colonies formed by surviving cells (average number of colonies per plate = 38) were replica-plated to synthetic medium lacking arginine. The replicas were irradiated with X-rays (2.5 Krads) to induce arginine revertants. The size of the replicas and the dose of X-rays were adjusted to yield about 20 revertants per replica. Clones with replica imprints that did not exhibit revertants were classified as presumptive recombination deficient mutants. They were further characterized to eliminate false cases and to determine the mutant phenotype.

G. Phenotype of mutants

Putative mutants were qualitatively characterized for their sensitivity to radiation-induced lethality by exposing rectangular replicas (about 2 cm²) on YEPD to X-rays (40 Krads) or UV (1620 ergs/mm²). After one or two days incubation, phenotypes were scored by comparing the irradiated plates with control unirradiated plates (46). Strains whose replicas grew significantly less than the control were classified qualitatively as X-ray or UV sensitive. Such mutants were then further characterized by determination of an inactivation curve. In addition one YEPD replica was incubated at 37°C. Strains that did not exhibit growth at this temperature were classified as temperature sensitive.

Similar replicas on arginine-omission medium were treated with X-rays (2.5 Krads) or UV (324 ergs/mm²) to study their reversion patterns. Lack of X-ray induced reversion on the imprints was considered as evidence for the presence of mutational changes that interfered with recombination. Lack of reversion following UV treatment was considered as evidence for mutations that interfered with recombination and with back mutation, since arg4-17 reverts to prototrophy with a high frequency following UV exposure (34).

The nonreverting strains were tested for their disomic condition and then characterized quantitatively for their kinetics of prototroph induction by X-rays and UV. Survival curves following X-ray or UV exposure were determined as described previously.

H. Tetrad and random spore analysis

To perform tetrad analysis, zygotes were recovered by micro-manipulation from a four hour old mixture of freshly grown haploid

cells of opposite mating type. The resulting diploids were grown overnight on presporulation medium (GNA) and then transferred to sporulation agar plates and incubated for three or four days. Sporulated cultures were treated, for 20 minutes, with a 1:20 dilution of glusulase (Endo Laboratory, Garden City, New York) to digest the ascus wall. The ascospores were then separated by microdissection (31).

In random spore analysis, sporulated cells were treated with glusulase and suspended in distilled water (about 10^6 cell/ml.). About 10 ml of the suspension were sonicated (100 watt Ultrasonic Disintegrator, Measuring and Scientific Equipment, Ltd.) for three minutes at 21 KC/sec (51), to disrupt the asci. Appropriate dilutions of the resulting suspension of single spores, unsporulated diploids, and cell debris were plated to yield about 100 colonies per plate. These colonies were then replica-plated to appropriate diagnostic media for scoring the segregation of markers present in the cross. The percentage of diploid clones could be estimated from the number of replicas that had the phenotype expected of the diploid. By scoring the appropriate recessive markers at least a fraction of the ascosporal clones can be positively identified.

I. Segregational analysis of genes that interfere with induced mitotic recombination

Spores from the tetrasomic diploid resulting from the cross of disomic Z140-51C with Z140-51D are expected to be disomic for chromosome VIII. If pairing and disjunction of the tetrasomic chromosome are assumed to be the same as for tetraploids (53), the expected ascus classes are distributed as follows:

Ascus type	Spore configuration	Frequency
I	2/17, 2/17, 2/17, 2/17	$P(4:0) = 2/3 - 2/3 x + 1/2 x^2$
II	2/2, 17/17, 2/17, 2/17	$P(2:2) = 4/3 x - x^2$
III	2/2, 2/2, 17/17, 17/17	$P(0:4) = 1/3 - 2/3 x + 1/2 x^2$

In these equations x represents the second division segregation frequency. $P(4:0)$ for example, is the probability for an ascus with four heteroallelic spores.

To test for induction of recombinants in heteroallelic spores, cultures were grown overnight on YEPD plates. These were replica-plated to arginineless medium and the replicas were treated with X-rays (2.5 Krads). Replicas from heteroallelic disomics exhibited about 20 revertants after four days incubation, whereas replicas of homoallelic disomes had an average of two revertants.

To study the segregation of genes that interfere with induced recombination, mutants were crossed with the parental strains of opposite mating type. In each cross, asci bearing four heteroallelic spores, as determined by allele identification tests, were examined. If all such asci yielded two ascospore clones with normal heteroallelic response and two with five or less revertants per replica, it was considered that a single gene responsible for a depressed frequency of mitotic intralocus recombination was segregating.

J. Complementation test

Complementation groups represented by the mutants deficient for recombination were determined by phenotypic and genetic analysis of diploids arising from inter-crosses among the mutants.

In the phenotypic analysis the diploids were replica-plated to

arginineless medium and the imprints were exposed to X-rays (10 Krads) to induce heteroallelic reversion. Diploid replicas that did not exhibit heteroallelic reversion were considered to originate from crosses in which the two haploids carried mutations in the same gene for recombination deficiency, i.e. they were classified as noncomplementing mutants. If the replicas exhibited revertants, the mutant pairs were classified as complementing.

By genetic analysis such diploids were studied for the segregation of genes for recombination deficiency. If all spores in a cross exhibited a depressed frequency of induced recombinants, the two mutations were scored as alleles; otherwise, the mutations were assigned to different genes.

III. RESULTS: GENETIC ANALYSIS OF DISOMIC STRAINS

A. Recovery of disomic strains

The two strains Z140-51C and Z140-51D, which are disomic for chromosome VIII, were recovered as spontaneous meiotic products, incidental to a study of gene conversion at the arg4 locus located on this chromosome (13). These disomics were used to recover recombination deficient mutants. DS-542-VII, disomic for chromosome VII, was also used for recovering recombination deficient mutants; however, these mutants will not be discussed in the present study.

DS-542-VII was isolated directly by selecting particular spores from a diploid, D1, constructed for this purpose. The genotype of D1 is:

<u>a</u>	<u>trp5-1</u>	<u>leu1-19</u>	<u>ade6-121</u>	<u>+</u>	<u>ural</u>
<u>α</u>	<u>+</u>	<u>leu1-5</u>	<u>ade6-23</u>	<u>can1</u>	<u>+</u>

The genes trp5, leu1, and ade6 are located on chromosome VII (42, 20). can1 is a recessive mutation that confers resistance to canavanine. It allows the selection of spores in the presence of unsporulated diploids. The complementing alleles leu1-5 and leu1-19 are located close to each other within the leu1 gene. They allow selection of rare meiotic segregants that have chromosome VII in diploid configuration. However, some leucine-independent segregants will also result from gene conversion. ade6-23 and ade6-121 are widely separated non-complementing alleles in the ade6 gene. Cells bearing these ade6 alleles in heteroallelic configuration exhibit a high frequency of spontaneous or induced reversion to adenine independence. This provided a means for confirming the disomic configuration.

To isolate disomic haploids, aliquots of spore suspension from D1

were plated on leucine-deficient synthetic medium that contained adenine, tryptophan, uracil and canavanine. This random spore procedure is similar to one described by Magni (37). Leucine-independent canavanine-resistant clones selected as possible disomics were further tested for X-ray induction of adenine prototrophs, mating response and uracil requirement. Those selected clones that exhibited a heteroallelic response on adenineless medium, uracil requirement and a mating response were considered as presumptive disomics for chromosome VII. The phenotype of diploids homozygous for can1 approximated that of the disomics, but such rare mitotic segregants could be identified because they did not exhibit a mating response and were uracil independent. Disomics for chromosomes other than VII and VIII have been isolated by selecting spores that carry one heteroallelic region (T. McKey, personal communication).

B. Genetic analysis of trisomic diploids

The genotypes of Z140-51C and Z140-51D, as presented in the Materials and Methods, were inferred from the marker segregation in ascus 51 from diploid Z140. Also, crosses of these strains with wild type yielded threonine-negative and copper-sensitive spores as expected from the proposed genotypes. Similarly, the configuration of markers on chromosome VII of strains DS-542-VII was confirmed by crossing this disomic to a wild type strain (65).

To confirm the genotype of Z140-51D, this strain was crossed to the arg4-17 tester. The proposed genotype for the resulting diploid (IL10) is:

<u>arg4-2</u>	+	<u>thr1</u>	<u>CUP1</u>							
+	<u>arg4-17</u>	+	<u>cup1</u>	<u>leu1</u>	<u>trp5</u>	<u>trp1</u>	<u>his5</u>	<u>ade2</u>	+	<u>met1</u> <u>a</u>
+	<u>arg4-17</u>	+	<u>cup1</u>	<u>leu1</u>	+	+	+	+	<u>ade1</u>	+

The expected frequencies for segregation of markers in the trisomic region can be derived by the procedure used to predict tetraploid segregations (53). For genes in duplex condition in a trisomic, for example thr1 and CUP1 in the above cross, the following frequencies of segregation ratios (growth:nongrowth) are expected.

$$P(4:0)=1/3 (2-x); P(3:1)=2/3 x; P(2:2)=1/3 (1-x) \quad (1)$$

Where $P(4:0)$, $P(3:1)$, $P(2:2)$ are probabilities for the corresponding segregation ratios and x is the second division segregation frequency. For thr1 and CUP1, the second division segregation frequencies are 0.45 and 0.79, respectively.

For markers in heteroallelic configuration, as at the arg4 locus, the expected frequencies of segregation ratios for heteroallelic (2/17) to homoallelic (2,17,17/17) spores can be shown to be

$$P(2:2)=1/3 (2-x); P(1:3)=2/3 x; P(0:4)=1/3 (1-x) \quad (2)$$

The second division segregation frequency for arg4 is 0.17.

For heterozygous genes in other chromosomes a 2:2 segregation ratio is expected. In Table 1 are presented the expected and observed values for the numbers of different ascus types from the cross IL10. The close agreement between these values for thr1, CUP1, and arg4 confirms the trisomic condition in the diploid and therefore the disomic condition for chromosome VIII in one of the parental strains. Other chromosomes appear to be represented only once. The segregation ratios for tryptophan and adenine, not included in Table 1, were consistent with the expected digenic segregation patterns.

TABLE 1. Distribution of Ascus Types in Strain IL10 (Z140-51D x Z149-20A)

Segregation Ratios*	<u>arg4</u>		<u>thr1</u>		<u>CUP1</u>		<u>met1</u>		<u>his5</u>		Mating Type	
	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.
4:0	0	0.00	6	6.24	2	4.92	1	0	0	0	0	0
3:1	0	0.00	4	3.60	5	6.12	0	0	1	0	0	0
2:2	5	7.30	2	2.16	5	0.96	11	12	11	12	12	12
1:3	3	1.32	0	0.00	0	0.00	0	0	0	0	0	0
0:4	4	3.36	0	0.00	0	0.00	0	0	0	0	0	0

* The segregation ratios represent the distribution of spores per ascus as follows:

thr1, met1, his5- Prototrophic to auxotrophic, CUP1-resistant to sensitive, mating type a: α , arg4- heteroallelic to homoallelic.

C. Tetrad analysis of tetrasomic diploids

The crosses between two disomic heteroallelic strains are expected to yield a majority of asci with four heteroallelic spores. Only such spores are suitable for diagnosing genes related to recombination deficiency. Tetrad analysis of the cross Z140-51C x Z140-51D permitted the examination of segregation of markers on the tetrasomic chromosome and the evaluation of the frequency of heteroallelic spores. The genotype of the tetrasomic region in the cross is:

+	arg4-17	thr1	CUP1
arg4-2	+	+	cup1
+	arg4-17	thr1	CUP1
arg4-2	+	+	cup1

The disomic spores from this cross are expected to be of three types with respect to their arg4 genotype; 2/2, 17/17, 2/17. The expected frequencies of ascus classes in this type of cross were presented in the Materials and Methods. The calculations are the same as those used to predict tetraploid segregations. They assume homologous bivalent pairing and random disjunction of the chromosomes (53). The observed and expected values for the numbers of asci with different segregation ratios are presented in the Table 2. These values agree quite well for segregation of markers in arg4. The 3:1 and 1:3 segregations might be a consequence of gene conversion. Ascus frequencies for thr1 and CUP1 also agree with the expected values. These results support the hypothesis that chromosome VIII is present in quadruplicate and assorts at meiosis with homologous bivalent pairing and random normal disjunction as in the corresponding tetraploid.

Markers met1 and mating type segregate in a 2:2 fashion as expected for heterozygous genes in a diploid.

Spore viability was 85% in this cross. The segregation data were scored in asci with four viable spores. No phenotypic selection is apparent in asci with 2 and 3 viable spores.

TABLE 2. Segregation Ratios for Markers in a Diploid Tetrasomic for Chromosome VIII (Z140-51C x Z140-51D)

Segregation Ratios*	<u>arg4</u>		<u>thr1</u>		<u>CUP1</u>		<u>met1</u>		Mating Type	
	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.
4:0	17	18.2	14	15	13	14.2	0	0	0	0
3:1	1	0.0	11	12.8	13	13.6	0	0	1	0
2:2	6	6.4	7	4.2	6	3.8	32	32	31	32
1:3	3	0.0	0	0.0	0	0.0	0	0	0	0
0:4	5	7.8	0	0.0	0	0.0	0	0	0	0

* See foot note to Table 1.

D. Phenotype of disomics

1. Stability

The stability of disomics during vegetative growth or after treatment with mutagens was tested to establish their suitability for genetic and biochemical studies. It was expected that nondisjunction of the extra chromosome might result in haploid cells. Disomy was established by testing for the presence of heteroallelic regions on the disomic chromosome. Nonreverting subclones were assumed to be haploids or less probably disomes that had become homoallelic for one of the two alleles.

DS-542-VII was tested for stability by scoring for leucine independence and for induction of adenine prototrophs. After liquid culture, cells were plated on YEPD. In addition, samples were treated with EMS or UV before plating. The resulting clones were replica-plated on leucine-omission and adenine-omission media. The imprints on adenine-omission medium were exposed to X-rays. About 93% of the clones were scored as disomes in the control untreated population by this test. A very similar value was obtained for clones from cells that were treated with EMS or UV. Other data on the stability of this disomic are published elsewhere (65).

Z140-51C and Z140-51D were tested for stability during liquid culture by plating cells on YEPD, replica-plating the resulting clones on arginine-omission media and treating the imprints with X-rays. A sample of 250 clones was examined for each strain. In both strains about 96% of the clones yielded revertants, indicating a disomic configuration for chromosome VIII. The two strains were stable after UV exposure. At a dose of 769 ergs/mm^2 , about 94% of the survivors

were identified as disomic by induced heteroallelic reversion.

2. Radiation sensitivity

The sensitivity to radiation-induced damage of disomics Z140-51C and Z140-51D was measured by determination of their survival curves. Figures 1 and 2 show the X-ray and UV inactivation curves for the two disomics and the parental diploid Z140. Both disomics exhibit a radiation response that is comparable to that of haploids, while Z140 has a typical diploid survival curve (67). The tails on the X-ray survival curves of the disomics indicate the presence of a radio-resistant moiety which has been identified as the budding cell fraction (3). The values predicted for this fraction from the intercept of the tail at zero dose are about the same as that estimated by microscopic count of budding cells in the culture.

Figure 1

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Figure 2

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3. Heteroallelic reversion

Induced heteroallelic reversion in disomics was used to characterize the strains with mutations that interfere with recombination. Results on induction of phototrophs by X-rays and UV are presented in figures 3 and 4. The lines represent the least square regression of the results. Differences between the slopes of the induction curves for the two disomics and the parental diploid may be explained at least in part by the finding of Friis and Roman, who showed that UV-induced intragenic recombination is depressed in yeast diploids homozygous for the mating type (16). The disomics are hemizygous for the mating type and they might be expected to exhibit such depressed intragenic recombination.

For routine scoring of the recombination genes, the dose used for induction of heteroallelic revertants was 2.5 Krads for the disomics and 10 Krads for the diploids. These doses yield the maximum number of prototrophs per plated cell. On the assumption that the locus is analogous to his1 with regard to mitotic conversion (71), X-ray induced heteroallelic revertants are expected to arise mainly from conversions of arg4-2 or arg4-17. In mitotic conversion to prototrophy the heteroallelic region arg4-2 +/+ arg4-17 becomes 2 +/+ + or + +/+ 17. Other mechanisms that could explain the appearance of prototrophs are back mutations and suppressors, but these events occur at much lower frequency and are expected to be of minor importance.

The mechanism of induction of prototrophs by UV has not been studied. UV induces back mutations with a high frequency in haploids bearing arg4-17 (50) (34). UV also induces suppressors that act on the ochre mutations arg4-2 and arg4-17 (17). Thus the UV-induced

arginine prototrophs are expected to result from conversions back-mutations or suppressors in arg4-2 +/+ arg4-17, although the frequency of conversion is expected to be an order of magnitude higher than the frequency of induced back-mutations and suppressors on the basis of the response of homoallelic diploids.

The induction curves for the disomic DS-452-VII also exhibit a linear relationship between the prototrophs per survivor and the X-ray dose. These data are presented elsewhere (65).

Figure 3

DBL 709 5920

Figure 4

DBL 709 5921

IV. RESULTS: ISOLATION AND GENETIC CHARACTERIZATION OF MUTANTS DEFICIENT IN RADIATION-INDUCED HETEROALLELIC REVERSION

A. Isolation of mutants

One approach toward resolving recombination into its component processes is the identification and analysis of mutants which alter the frequencies of genetic exchange. In a search for such mutants in yeast, clones were selected on the basis of depressed frequencies of induced mitotic heteroallelic reversion from strains that were disomic for chromosome VIII and heteroallelic at the arg4 locus, located on this chromosome. Plate 1 shows the reversion patterns of clone imprints that have been exposed to 2.5 Krads of X-radiation or 324 ergs/mm^2 of UV. Clones that corresponded to non-reverting imprints were selected as potential recombination-deficient mutants. Among the strains selected on this basis were some, which were subsequently eliminated, that failed to grow on synthetic complete medium. Presumably these strains carried mutations that imposed nutritional requirements not satisfied by this medium. In addition, haploidized strains, identified by their failure to exhibit a heteroallelic response when crossed with either the arg4-2 or arg4-17 testers, were discarded from the sample of potential recombination deficient mutants. However, the phenotype of some mutants could be due to a second block in the arginine biosynthetic pathway. This would prevent the expression of heteroallelic reversion at arg4 and hence mimic the recombination deficient phenotype. Such cases were detected by scoring the segregation of the arginine requirement in crosses between the potential rec mutants and wild type strains. The genotype at the arg4 locus in such crosses would be 2/17/+ and 2:2 segregations for arginine (nonrequiring:requiring)

XBB 709-4187

Plate 1

would be expected. An additional heterozygous block in the arginine biosynthetic pathway would lead to 2:2, 1:3 and 0:4 segregations. The results of such crosses representing 13 potential mutants derived from Z140-51C and Z140-51D are displayed in Table 3. On the basis of a digenic segregation pattern for the arginine requirement, mutants 2C11, 2C18, 2C21 were discarded. Mutants 1D8, 1D16, 2D1, 2D7, 2D11, 2C4, 2C7, 2C8, 2C16, 2C23 exhibited predominantly 2:2 segregations and thus were retained for further analysis. As expected the genes in heterozygous condition his5, ade2, and leu1, exhibited 2:2 segregations. In addition X-ray and UV sensitivity segregated in a 2:2 fashion in crosses involving strains 2C16 and 2C21, while X-ray sensitivity segregated 2:2 in crosses with strains 1D16 and 2C4.

The 10 remaining presumptive mutants were recovered from approximately 4000 clones which grew from survivors of UV exposure (15% survival). This frequency of rec mutations is within the range of frequencies found for recovery of nutritional mutants in yeast (35). This result suggests a relatively high mutation rate, which in turn may indicate the existence of a large number of genes involved in genetic exchange.

TABLE 3. Segregation of Arginine Requirement in Crosses Between Presumptive Recombination Deficient Mutants and Wild Type

Segregation ratios	Numbers of asci with different segregation ratios for crosses of mutants x wild type												
nonrequiring:requiring	1D8	1D16	2D1	2D7	2D11	2C4	2C7	2C11	2C8	2C16	2C18	2C21	2C23
2:2	10	10	7	7	6	6	5	3	7	4	0	1	6
1:3	0	0	0	1	1	1	0	3	0	0	5	5	1
0:4	0	0	0	0	0	0	0	0	0	0	0	0	0

B. Phenotypes of mutants

1. Response to radiation-induced damage

The qualitative test for radiation sensitivity in the genetic analysis indicated that strain 2C16 was both X-ray and UV sensitive, and strains 1D16 and 2C4 were only X-ray sensitive. Survival curves were determined for these strains to confirm the radiation sensitivity of these mutants.

From the X-ray inactivation curves (Figures 5 and 6) for strains 2C16, 2C4 and 1D16, dose modifying factors (DMF) at 10% survival of 1.9, 1.3 and 1.4, relative to corresponding parental strains, were calculated. These values are comparable to DMF values measured in haploid yeast bearing mutations in genes *xs1*, *xs2*, *xs3*, and *uxs1* (52).

At 60 Krads the difference in survival between 2140-51C and mutants 2C4 and 2C16 is less than tenfold, a factor not large enough to account for the clear results found in the qualitative test. Perhaps plating at high cell density (replica-plating) as done in the qualitative test leads to lower survival at higher doses.

The X-ray inactivation curves can be resolved into two components for both mutants and wild type strains. The tail in the curves has been found to correlate with the presence of a resistant budded cell fraction in the culture (3). The fraction of budding cells in the cultures of the ten presumptive mutants was determined by microscopic observation and was found to correspond approximately to the percentage of radiation resistant cells.

Figures 7 and 8 show the response of the recombination-deficient strains to UV irradiation. Mutants 2C16 and 2D7 do not exhibit a shoulder in the inactivation curves at low doses. The DMF for strains 2C16 and 2D7 are 5.8 and 3 respectively, at 10% survival.

Figure 5

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Figure 6

DBL 709 5915

Figure 7

DBL 709 5916

Figure 8

DBL 709 5917

2. Radiation-induced heteroallelic reversion

The disomic strains Z140-51C and Z140-51D exhibited a linear dose response for induction of arginine prototrophs after irradiation with X-rays or UV (Figures 3 and 4). The dose range 0-4 Krads was used to quantitatively characterize the effect of the recombination deficient mutations in disomics, since this dose range yields a linear dose-effect curve in heteroallelic diploids.

The results for X-ray induction of heteroallelic revertants in Z140-51C, Z140-51D and mutants derived from these strains are presented in figures 9 and 10 respectively. The lines represent the least square regressions of the results. A depression in the rate of prototroph induction is apparent in all the mutant strains (See Table 4). The slopes of the X-ray induction curves for arginine prototrophs, as percentage of control, range from zero to about 50%, suggesting that the mutants regulate different aspects of meiotic recombination. In strains 2C8 and 2D7 spontaneous and induced mitotic recombination are completely suppressed. The UV induction curves for arginine prototrophs in Z140-51C and Z140-51D and the corresponding derived mutants are presented in figures 11 and 12 respectively. Of the 10 presumptive mutants described in Table 3, strains 2D1, 2C7, 2C23 did not yield reproducible results in all the subsequent crosses and were not studied further.

Figure 9

DBL 709 5925

Figure 10

DBL 709 5918

Figure 11

DBL 709 5924

Figure 12

DBL 709 5919

TABLE 4. Slopes of the X-ray prototroph induction curves for recombination deficient mutants and parental wild type strains Z140-51C and Z140-51D.

Strain	Slope in prototrophs per 10^8 survivors per rad*	Slope as fraction of control**
Z140-51C	0.51 ± 0.09	1.00
2C4	0.07 ± 0.07	0.14 ± 0.60
2C8	0.00	0.00
2C16	0.244 ± 0.006	0.479 ± 0.003
Z140-51D	0.72 ± 0.02	1.00
1D8	-0.04 ± 0.12	-0.05 ± 0.36
1D16	0.13 ± 0.13	0.17 ± 0.13
2D7	0.00	0.00
2D11	0.03 ± 0.01	0.04 ± 0.0004

* Regression coefficient $\pm$ standard error of regression coefficient

** Standard errors for slope as fraction of control were calculated

from the formula
$$\sigma^2 = \frac{\sigma_1^2}{x_1} + \frac{\sigma_2^2}{x_2}$$

C. Genetic analysis of mutants deficient in mitotic heteroallelic reversion

1. Dominance

The dominance of the recombination deficient mutations was tested in crosses between the parental and the mutant strains. The resulting tetrasomic diploids would be heterozygous for the mutations conferring recombination deficiency. A dominant mutation is expected to exhibit reduced X-ray induction of prototrophs in the tetrasomic cross, while crosses heterozygous for a recessive mutation should exhibit a normal pattern of heteroallelic reversion. In all crosses approximately normal frequencies of X-ray and UV-induced reversion were manifested, as expected for recessive mutations. Diploids homozygous for the mutations present in the strains 1D8, 1D16, 2C4, 2D7, 2C8, 2D11, and 2C16 were constructed and found to be recombination deficient in mitotic cells; clearly, the mutations are recessive. In addition, strains homozygous for the mutation 1D16, 2C4, and 2C16 were X-ray sensitive.

2. Segregation of recombination deficiency mutations

Six of the remaining seven mutants, 2C4, 2C8, 2C16, 1D16, 2D7 and 2D11, were analyzed in crosses between the mutants and the disomic parental strains of opposite mating type. The products of these crosses should have been tetrasomic for chromosome VIII and were expected to yield a fraction of asci containing four heteroallelic spores (See Materials and Methods - I). The segregation of rec genes is readily scored in ascospore clones from such asci. 1D8 was crossed to a haploid strain. The results of tetrad analysis of crosses involving the mutants 2C8, 2D7 and 1D16 are presented in Table 5. These crosses

gave the expected tetrasomic ratios. However, in addition to the cross for 1D8, the crosses involving mutants 2C4, 2D11 exhibited segregations characteristic of trisomic strains (See Table 6). A 2:2 segregation for heteroallelic reversion:nonreversion in the asci with four heteroallelic spores, identified by allele test, is exhibited by the mutations in strains 2C8 (rec4), 2D7 (rec3), and 1D16 (rec2), indicating a single gene difference for recombination deficiency segregating in these strains. Segregation of the recombination deficiency phenotype present in strains 1D8 (rec1), 2C4 and 2D11 was scored in disomic spores from the corresponding trisomic crosses. It is expected that with segregation for a single recombination deficiency locus, about 50% of the heteroallelic spores would yield revertants. The percentage observed for 1D8 (rec1) is significantly greater than 50%. For 2C4 and 2D11 the percentages are not significantly different from 50%. Because spore viability was low (20%) in crosses with mutant 2C16, a similar genetic analysis was not possible.

To determine the number of different genes included in the seven strains studied, all possible pairwise crosses (reca/recb) were made. The resulting hybrids exhibited levels of X-ray induction of revertants comparable to the corresponding rec/+ or +/+ control crosses. Thus, seven classes of complementing mutants were defined (See Table 7). Genes rec1, rec2, rec3, and rec4, corresponding to strains 1D8, 1D16, 2D7 and 2C8, were also defined by genetic analysis of the tetrasomic hybrids (reca/recb) constructed by pairwise crossing among these four strains (See Materials and Methods - J). In these crosses spores with recombination ability similar to the parental wild type strains were recovered. Strains 2C4 and 2C11 did not yield tetrasomic hybrids in

crosses with other disomics, preventing the identification by genetic analysis of the genes associated with these mutations. Crosses involving strains 2C16 were not analyzed because they exhibited low spore viability. The properties of the seven complementary rec mutants are presented in Table 8.

TABLE 5. Segregation of genes for Recombination Deficiency in Tetrasomics Resulting from the Cross Between Disomic Mutants and Parental Strains Z140-51C or Z140-51D

Cross	Hybrid no.	No. of asci	% spore viability	Segregation ratios heteroallelic:homoallelic					Segregation ratios for X-ray induced heteroallelic reversion: non-reversion*		
				4:0	3:1	2:2	1:3	0:4	2:2	1:3	0:4
2D8 x Z140-51D	IL80	19	88.75	9	1	4	1	4	9	0	0
2D7 x Z140-51C	IL76	22	88.54	9	1	4	0	8	8	1	0
1D16 x Z140-51C	IL74	8	64.58	4	0	2	0	2	4	0	0

*Only asci with four heteroallelic spores were included.

TABLE 6. Segregation of Genes Leading to Recombination Deficiency in Trisomic Crosses Between Mutants and Parental Strains Z140-51C and Z140-51D.

Cross	Hybrid number	Number of asci	% spore viability	Segregation ratios for heteroallelic: homoallelic spores			Heteroallelic spores by random spore analysis	Number of reverting heteroallelic spores	Percent
				2:2	1:3	0:4			
1D8 x Z136-167D Z149-20A	IL3	26	97.73	13	5	10	31	21	67.74
	IL4								
2C4 x Z140-51D	IL78	16	94.05	7	6	3	21	14	66.76
2D11 x Z140-51C	IL77	8	76.25	4	2	2	22	15	66.18

TABLE 7. Induced Heteroallelic Reversion* in Diploids Carrying Pairwise Combinations of the rec Mutations.

Strains*	1D8	1D16	2D7	2C8	2C4	2D11	2C16
1D8	-	+	+	+	+	+	+
1D16		-	+	+	+	+	+
2D7			-	+	+	+	+
2C8				-	+	+	+
2C4					-	+	+
2D11						-	+
2C16							-

* + means a level of heteroallelic reversion comparable to wild type,

- means significant reduction in the frequency of induced heteroallelic reversion.

TABLE 8. Phenotypes* of Recombination Deficient Mutants

Strain	Gene	Radiation Sensitivity		Induction of Heteroallelic Reversion	
		X-ray	UV	X-ray	UV
1D8	rec1	+	+	-	-
1D16	rec2	-	+	-	+
2D7	rec3	+	+	-	-
2C8	rec4	+	+	-	-
2C4	-	-	+	-	-
2D11	-	+	+	-	-
2C16	-	-	-	-	-

* + indicates a similar phenotypic response in the mutant and in the parental wild type strain,

- indicates radiation sensitivity or a lack of induced heteroallelic reversion.

3. Correlation between recombination deficiency and radiation sensitivity

Radiation sensitive strains in Ustilago (23), E. coli (7) and yeast (41) are also recombination deficient and a single gene may determine both characters. Thus it was of interest to examine the correlation between recombination deficiency and radiation sensitivity in the X-ray sensitive mutants 2C4 and 1D16 (rec2) and the X-ray and UV-sensitive mutant 2C16. In 16 of 21 spores examined in the cross between 2C4 and 2140-51D the two traits segregated together. The cases where only one or the other character was expressed could represent misscoring. The same procedure was repeated for crosses involving mutant 1D16 (rec2). In 34 of 35 disomic heteroallelic spores examined, radiation sensitivity and recombination deficiency segregated together. These results indicate that only a single mutant locus, rec2, determined both radiation sensitivity and recombination deficiency in 1D16. Again low spore viability prevented a similar genetic analysis in crosses with mutant 2C16.

D. Effect of the rec mutations in mitotic segregation and in meiotic recombination

The effect of the rec genes on X-ray induced mitotic segregation was tested in diploids homozygous for the rec mutations and heterozygous in appropriate markers for scoring sectoring (44). The relevant genotypes of the diploids used in this experiment are:
rec1/rec1 ade2/+, rec2/rec2 ade2/+, rec3/rec3 cyh2/+, rec4/rec4 ade8/+.
 These four diploids exhibited frequencies of X-ray induced sectoring, comparable to the control hybrids, which were heterozygous (rec/+) (See Table 9). These results suggest that the rec genes do not affect

intergenic mitotic recombination.

Diploids homozygous for mutations rec1, rec2, rec3 and rec4 present in strains 1D8, 1D16, 2D7 and 2C8 were constructed to examine their effects on meiotic exchange at the intergenic and intragenic levels.

Thirty-five different crosses between spores bearing rec2 were analyzed. All these rec2/rec2 hybrids were X-ray sensitive and exhibited sporulation between 1 and 2 percent (S. Fogel, personal communication). However, spore viability was only 10%, or lower, preventing a genetic analysis of these strains.

Four different crosses, homozygous for rec3, were constructed. None of these crosses sporulated. Also these diploids did not exhibit X-ray or UV-induced heteroallelic reversion.

Meiotic conversion in the heteroallelic region arg4-2/arg4-17 was estimated by scoring the frequency of arginine prototrophs in random spore populations from diploids homozygous and heterozygous for rec1 and rec4. These results are presented in Table 10. In the same table are also included data on sporulation and spore viability for rec1, rec2, rec3, and rec4 bearing strains.

The percentage of arginine prototrophs in the diploids homozygous for rec1 is very similar to control values, indicating that this mutation does not affect meiotic conversion. Meiotic crossing over between the genes leu1 and trp5 in chromosome VII was also normal in this strain. The frequency of recombinant spores (about 16%) is close to that determined by Resnick, et al. (51).

The rec4 mutation apparently suppressed meiotic conversion of arg4-2/arg4-17. The frequency of arginine prototrophs in spores from

the diploid homozygous for rec4 was reduced by a factor of about 10^3 . Critical to the finding on suppression of meiotic conversion is the demonstration that the sporulated cultures were in fact heteroallelic for parental markers 4-2/4-17. This was subsequently proved by tetrad analysis in which spores from 98 dissected tetrads (spore viability 94%) were backcrossed to tester strains carrying arg4-2 and arg4-17 in a rec+ background (13).

Meiotic crossing over in chromosome VIII was tested in a rec4/rec4 diploid. This hybrid was tetrasomic for chromosome VIII with the configuration thr1 CUP1/+ +/thr1 CUP1/+ +. The expected frequencies of ascus classes for genes in duplex condition in a tetrasomic were calculated from the equations presented in the Materials and Methods (I). The expected and observed values for the segregations of these genes are given in Table 11. The second division segregation frequencies (x) for thr1 and CUP1 used in these calculations were 0.50 and 0.83 respectively (42, S. Fogel and R.K. Mortimer, personal communication). The close agreement between expected and observed values indicates that crossing over occurs at the expected frequencies, suggesting that rec4 does not affect crossing over in the regions between the centromere of chromosome VIII and thr1 or between thr1 and CUP1. Meiotic crossing over in the rec4/rec4 diploid was also tested for the regions between mat1 (mating type) and ade8 and their respective centromeres. The tetratype asci arise only from crossing over in these two regions. The observed ratios for parental-nonparental:tetratype asci (PD:NPD:T) were 17:8:48. These values indicate the occurrence of crossing over in one or both of these centromere-gene intervals. However, since neither of these genes is close to its centromere, a

considerable reduction in the number of exchanges between the centromere of chromosome 3 and mat1 or between the centromere of fragment 2 and ade8 would go undetected. However, a complete suppression of crossing over in these two intervals would eliminate the tetratype asci. Since approximately 2/3 of the asci were tetratype for these two genes, meiotic exchange must be occurring but whether it is at a frequency lower than the control cannot be ascertained. Other heterozygous markers must be incorporated in the rec4/rec4 crosses to determine fully the effect of this mutation on meiotic recombination.

TABLE 9. X-ray Induced Mitotic Segregation in Diploids Bearing rec Mutations

Diploid	0		4		3		12	
	percent* sectoring	percent survivors	percent sectoring	percent survivors	percent sectoring	percent survivors	percent sectoring	percent survivors
<u>rec1</u> <u>ade2</u> <u>rec1</u> +	0.05	100	2.50	97	2.70	85	3.60	81
<u>rec1</u> <u>ade2</u> + +	0.38	100	2.80	92	3.00	82	4.10	78
<u>rec2</u> <u>ade2</u> <u>rec2</u> +	0.00	100	1.10	78	2.30	52	3.40	30
<u>rec2</u> <u>ade2</u> + +	0.00	100	1.50	98	2.80	92	3.90	90
<u>rec3</u> <u>cyh2</u> <u>rec3</u> +	0.00	100	2.40	100	2.90	92	3.80	88
<u>rec3</u> <u>cyh2</u> + +	0.00	100	2.20	100	2.90	94	9.00	90
<u>rec4</u> <u>ade8</u> <u>rec4</u> +	1.00	100	3.90	96	4.00	93	5.30	90
<u>rec4</u> <u>ade8</u> + +	0.00	100	3.50	95	4.30	92	5.80	92

* A minimum of 162 colonies were examined for sectoring for each dosepoint and diploid.

TABLE 10. Meiotic Segregation in Heteroallelic Diploids (arg4-2/argr-17) Bearing rec Genes as Estimated by Random Spore Plating.

Cross	Genotype	% asci	% viable spores	Number of spores plated	Number of viable spores	Arginine prototrophs	% arginine prototrophs
IX1	<u>rec1/rec1</u>	30	85	2.35×10^5	2×10^5	380	0.19
IIA	<u>rec1/+</u>	30	87	1.38×10^5	1.2×10^5	238	0.20
Z140	+/+	50	96	2.46×10^4	2.36×10^4	45	0.19
IX3	<u>rec2/rec2</u>	1-2	10	-	-	-	-
II84	<u>rec3/rec3</u>	0	-	-	-	-	-
4491	<u>rec4/rec4</u>	60	90	1×10^5	0.9×10^5	1	0.001
4493	<u>rec4/+</u>	60	90	1.4×10^5	1.26×10^5	573	0.46
4496	+/+	90	90	1.9×10^5	1.71×10^5	578	0.34

TABLE 11. Segregation Ratios for Markers in a Diploid Tetrasomic for Chromosome VIII and Hemozygous rec4/rec4. (Strain 4495)

	I (4:0)*		II (3:1)		III (2:2)	
	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.
<u>thr1</u>	30	34	27	31	16	9
<u>CUP1</u>	35	32	28	31	10	9

* The segregation ratios represent the distribution of spores per ascus as follows:

thr1- prototrophic to auxotrophic, CUP1-resistant to sensitive.

V. DISCUSSION

Recombination in Saccharomyces cerevisiae has been studied by genetic analysis of mutants deficient in induced heteroallelic reversion. It was of interest in this study to search for possible common steps in mitotic and meiotic recombination in either intergenic or intragenic systems. It was also of relevance to study the relation between recombination and radiation damage repair.

In the present study seven classes of complementing mutants that were deficient in induced mitotic conversion were characterized genetically. Four of these, corresponding to strains 1D8, 1D16, 2D7, and 2C8, were shown by both complementation and recombination tests to define the genes rec1, rec2, rec3, and rec4. The three additional complementation classes, represented by strains 2C4, 2C11, and 2C16 (Table 8), were not shown to recombine independently of the other rec mutations, but probably represent three additional rec genes. Two of the seven mutants were X-ray sensitive, and one was both X-ray and UV sensitive, while four were wild type in their radiation response. Two, when homozygous, were sporulation deficient and one was deficient in meiotic gene conversion to prototrophy.

The X-ray sensitive phenotype in 1D16 (rec2) and 2C4, when these genes were heterozygous, exhibited 2:2 segregations that indicated single genes for X-ray sensitivity. Both mutants showed a positive correlation between radiation sensitivity and recombination deficiency among the segregants. At least six crosses involving mutant 2C16, which is both X-ray and UV-sensitive, yielded spores with low viability (about 20%).

Diploids homozygous for rec2 (1D16) are X-ray sensitive and have

depressed frequencies of X-ray induced heteroallelic reversion but normal frequencies of X-ray induced mitotic sectoring in ade2 heterozygotes (Table 9). These diploids show 1 to 2 percent sporulation and about 10 percent spore viability, similar to yeast sporulation mutants studied by Esposito and Esposito (8). Other X-ray sensitive mutants have also been reported to interfere with sporulation (50).

Among the rec mutants that were wild type in their radiation response, strains 1D8 (rec1) and 2D11 had detectable frequencies of spontaneous mitotic conversion, while mutants (2D7) rec3 and (2C8) rec4 yielded neither spontaneous nor radiation-induced recombinants. The occurrence of these two groups of mutants suggests two processes for initiating recombination, one responsible for spontaneous and the other for induced conversion. Diploids homozygous for the mutations rec1, rec3 and rec4 have depressed frequencies of X-ray and UV-induced heteroallelic reversion. However, the frequencies of X-ray induced mitotic sectoring in these strains were comparable to those seen with wild type diploids. This suggests different processes for intragenic and intergenic mitotic exchange. In rec1/rec1 diploids meiotic recombination and conversion occur at normal frequencies (Table 10). Diploids homozygous for rec3 do not sporulate. Similar mutants responsible for aberrant meiosis have also been reported in Neurospora (5), Aspergillus (33), and Ustilago (23), but in contrast to rec3 these strains are UV-sensitive. Diploids homozygous for rec4, and heteroallelic for non-complementing mutations at arg4 fail to yield prototrophic spores. Such prototrophs normally arise from 3:1 conversions of one of the two alleles. These rec4/rec4 diploids had a high frequency of sporulation and spore viability (about 90%) so it seems unlikely that the failure

to recover prototrophic recombinants was due to selection at this level. The effect of rec4 on meiotic conversion could be due to a small inversion in the arg4-2/arg4-17 region which would block exchange. According to this hypothesis rec4 would be expected to segregate with arg4. However, in crosses involving rec4, recombinants between these two loci were recovered, ruling out this possibility.

In the rec mutants steps in the process leading from the radiation damage to the formation of recombinants presumably are defective. A description of the various schemes for explaining this process is relevant to identify the nature of the rec mutations:

- a. Radiation induced lesions in the DNA are repaired and the repair leads to the production of recombinants (25).
- b. Radiation damage in the cells induces a paramitotic stage which favors recombination (29).
- c. Radiation inactivates a repressor for recombination (24).

The idea that radiation induced lesions serve as a substrate for recombination is suggested by results from experiments on UV induction of recombinants in E. coli. In the cycle of DNA replication following irradiation, gaps are left in the newly synthesized strand opposite unexcised pyrimidine dimers. The missing information in this strand is transferred from a complementary strand in a process that leads to the formation of recombinants (28). An alternate mechanism proposed to explain the production of recombinants is that unexcised dimers favor pairing between DNA molecules, promoting recombination (1).

A similar process for production of recombinants by UV may operate in yeast. This idea is suggested by the finding that the frequency of UV-induced intragenic recombination is depressed by photoreactivation

(48, 49), which promotes in situ repair of dimers (53). Thus, dimers may be a substrate for recombination in yeast.

X-ray induced lesions in the DNA could favor exchange, in a manner similar to the pyrimidine dimers. For example, reparable X-ray induced gaps may be a substrate for recombination (15), (30). It is suggested that one of the processes of repair of broken DNA strands leads to the formation of recombinants. According to this idea, recombination deficiency results from the inability of the mutants to complete the steps leading from the initial gap to the completion of exchange. Mutants 1D16 (rec2) and 2C4 may be deficient in a process of this kind, since they are both X-ray sensitive and recombination deficient. In this scheme it is assumed that X-ray sensitivity is the result of deficiency in the mechanism for repair of DNA damage.

Six out of seven mutants studied were deficient in both X-ray and UV induction of recombinants, indicating that these induction processes have steps in common. Strains bearing rec2 are deficient only in X-ray induction of recombinants, suggesting that the processes for X-ray and UV induction also have different steps. This is also suggested by the existence of mutants which are deficient only in UV induction of heteroallelic revertants (See Plate 1). Additional evidence for common steps between the processes participating in induction and repair of radiation-induced damage, is provided by mutations in strain 2C16, which confer sensitivity to X-ray and UV damage.

An alternate hypothesis is based on the proposal that generalized synapsis of homologous chromosomes (parameiosis) provides favorable conditions for exchange (29). According to this idea some rec mutants may be unable to enter the parameiotic state. If this stage is also

prerequisite for meiosis, then the mitotic rec mutants would also be deficient in meiosis, as was observed for rec2 and rec3.

Induction of recombinants as a result of inactivation of a repressor for exchange is a scheme that leads to many alternatives for explaining the origin of the deficiency in the rec mutants. However, increased frequencies for spontaneous mitotic crossing over, observed in Ustilago uvs strains, can be interpreted as resulting from a derepressed system for production of recombinants (24). No such case was observed among the yeast rec mutants.

The assumption of a general mechanism responsible for conversion, with or without associated crossing over, during mitosis and meiosis greatly simplifies the interpretation of data on genetic exchange in yeast. Different approaches have been proposed to define this mechanism for genetic exchange.

The models proposed by Holliday (22), Whitehouse and Hastings (70) and Stahl (60) explain meiotic recombination as a consequence of certain molecular events in the region of exchange. These models easily account for the observation that half of the conversions are associated with outside markers recombination (30). The occurrence of this nonparental array of outside markers depends on the nature of the second exchange in the region involved in recombination. These models for meiotic exchange might possibly be extended to include mitotic recombination since the same type of reciprocal and nonreciprocal exchanges are observed in mitosis and meiosis (54, 44). Furthermore, in discussing induced mitotic recombination, the same models can be invoked. According to the view previously suggested, radiation induced lesions only stimulate exchange, without altering the molecular steps involved in

recombination.

Another approach to explain recombination has been suggested by Simchen and Stamberg (57). These authors have proposed two genetic systems responsible for the control of recombination, identified as fine and coarse control of exchange. Mutations in genes participating in the coarse control of recombination severely depress the frequencies of exchange. Such effects are observed, for example, in rec mutants in E. coli or in c(3)G mutants in Drosophila. The genes participating in the fine control affect restricted regions of the genome. Mutations in these genes depress the frequencies of recombination by an order of magnitude as for example in mutants in Neurospora (4) and in Schizophilum (61).

The rec mutants described in the present study were selected for deficiency in X-ray or UV-induced heteroallelic reversion. Such reversion events have been shown to be due mostly to single site conversions to prototrophy in meiosis (13). These single site events represent about 10% of the conversions in the arg4-2/arg4-17 region. If conversion is a part of all the recombination events, one would expect the rec mutants to be deficient in various types of genetic exchange, such as mitotic crossing over and meiotic conversion and crossing over. Deficiency in recombination may also be reflected in abnormal sporulation.

X-ray induced mitotic segregation was found to be normal in diploids homozygous for rec1, rec2, rec3, and rec4, suggesting that these genes participate in the events involved in intragenic exchange but not in crossing over (Table 9). One speculation based on this observation is that, in yeast, the steps in the mechanisms for induced

conversion and induced crossing over are not all commonly shared. In Ustilago one uvs mutant is defective in both UV-induced intragenic and intergenic mitotic recombination, suggesting at least some common steps leading to these two events (24).

The relationship between meiotic conversion and crossing over can be evaluated from the results obtained with rec1 and rec4. In rec1/rec1 diploids, sporulation, spore viability, and meiotic conversion are comparable to wild type, suggesting that this mutation controls a step specific for mitotic recombination. On the other hand, rec4/rec4 diploids exhibit depressed frequencies of spontaneous and induced mitotic conversion and also meiotic conversion, suggesting common steps in mitotic and meiotic recombination at the intragenic level. However, meiotic crossing over is apparently normal in these diploids, which indicates some unique steps for intragenic and intergenic exchange.

The theoretical models for recombination (22,70,60) are mostly concerned with molecular mechanisms. According to these models, a change in the frequency of conversion may be expected to alter the frequencies of crossing over, since the same molecular events determine both processes. Thus the effect of rec4 restricted to intragenic exchanges, would not be predicted from the models.

According to the results found in unselected tetrads, half of the conversions are associated with crossing over (12) (30). From these results the authors concluded that all crossing over is associated with conversion, thus suggesting the possibility that the mechanisms for conversion are adequate to account for all crossing over. However, the results found in rec4 bearing strains, which exhibit defective conversion but apparently normal crossing over, indicate that intragenic

and intergenic exchange can be separated. This suggests that at least some of the events leading to meiotic conversion are distinct from those that lead to meiotic crossing over.

Similarly, two different systems for conversion and crossing over in mitosis are suggested by results on sector induction in yeast (54, 36). X-ray and UV-induced mitotic segregation have been found to result mainly from reciprocal exchanges between the centromere and the marker studied, while EMS induces principally conversion events without outside markers exchange. Thus, EMS appears to induce lesions which provide a substrate for the molecular events leading to conversion, while X-ray and UV trigger the mechanism leading to crossing over. In addition, none of the rec mutants, deficient in mitotic conversion, are deficient in X-ray induced mitotic sectoring, which also suggests two different systems for conversion and crossing over.

It is conceivable that rec4 mutations prevent the formation of arginine prototrophs by changing the size of the converted region around the markers 2 and 17 in arg4, which may result in an excess of coconversions and a depression in the frequency of single site conversions. This idea must be tested by doing tetrad analysis of unselected samples of rec4/rec4 diploids which are heteroallelic in arg4, to identify the conversional events altered by this mutation.

Since genetic exchange is an essential process in meiosis, it is expected that defective recombination might also be manifested as a defective meiosis, as in diploids rec2/rec2 and rec3/rec3 which are sporulation deficient. This character is not ascribable to trivial explanations such as respiratory deficiency and homozygosity for the mating type. Sporulation deficiency was correlated with homozygosity

of the rec genes in 35 crosses for rec2 and four crosses for rec3, while hybrids heterozygous for the same two genes always exhibited normal sporulation.

Diploids homozygous for rec3 could be defective in any step included in sporulation, i.e., meiosis and the formation of four haploid nuclei, or incorporation of the meiotic products into mature spores. However, the nature of the rec2 mutations can be speculated from the fact that they determine deficiency in ascospore formation (1 to 2 percent) which is associated with low spore viability (about 10%). A possible explanation for low spore viability is that the spores are aneuploids as a consequence of abnormal chromosomal assortment. This type of genetic imbalance explains the low spore viability, observed in the meiotic products of triploid strains (43). Correlation between recombination deficiency and aneuploidy occurs in c(3)G mutants in Drosophila (58). In this case, the genetic imbalance has been related to abnormal chromosomal assortment determined by deficiencies in synaptonemal complex formation (45). Thus, it is conceivable that rec2 determines a function similar to the c(3)G gene. In such case, it would be expected that the diploids rec2/rec2 would also exhibit an anomalous synaptonemal complex.

In understanding recombination as a process involving exchanges between molecules, it is necessary to identify the functions determined by the rec genes. In the models for exchange the action of enzymes involved in repair synthesis, nucleases, polymerases and ligases, has been postulated. The methods for characterization of these enzymes have been extensively reviewed by Howard-Flanders (25).

Examination of these enzymes in E. coli recB and recC strains has

led to interesting results (2). An exonuclease was shown to be lacking in these strains. The identification of the exonuclease was facilitated by the ATP dependence of the enzymatic activity. To apply the method to a study of the yeast mutants would require that all the nucleases be identified.

It would be important to identify the enzymatic activities related to recombination, for example, by doing assays for nuclease activity in meiosis, i.e. in sporulated cultures. These enzymatic activities could be also correlated with late periods of DNA synthesis, possibly related to recombination (63).

In the present study a method for the genetic study of intragenic recombination in mitosis has been proposed. This approach of using recombination deficient mutants has also permitted the study of meiotic recombination. An understanding of recombination in yeast must also include the study of genes participating in intergenic meiotic and mitotic recombination.

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