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Fungal Recombination

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INTRODUCTION

Despite many years of intensive genetic analysis, the molecular mechanisms of the recombination events that have been studied in the fungi remain unknown. Numerous models have been proposed to explain the genetic data, and many of these models remain controversial. In this review, we emphasize recent molecular and genetic data that address possible recombination mechanisms. We review representative examples of experiments which provide necessary background information, but several reviews of fungal recombination which provide a thorough description of classical genetic results have recently been published (34, 130, 141).

We begin with a detailed review of meiotic and mitotic recombination. We then discuss the roles of recombination in meiosis and in vegetative growth and contrast the nature of meiotic and mitotic recombination. We review plasmid-chromosome recombination and several site-specific recombination events in yeast cells and describe attempts to define recombination in enzymological terms. Finally, we describe the Holliday, Meselson-Radding, and double-strand break repair models for recombination and compare and contrast the ability of these models to account for the properties of fungal recombination.

MEIOTIC RECOMBINATION

Gene Conversion and Postmeiotic Segregation

Meiotic recombination events have been studied by analysis of the segregation patterns of genes in meiosis. Much of this work has been carried out with *Saccharomyces cerevisiae*, with its advantages for genetic analysis and, more recently, molecular studies. Elegant genetic studies on fungi such as *Ascobolus* and *Sordaria* species have also contributed greatly to our understanding of meiotic recombination. These fungi have the advantage that large numbers of meiotic events can be scored by direct visual analysis of

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their eight-spored asci, as opposed to the slow and laborious tetrad analysis of yeast cells.

In yeast cells four spores are produced after meiosis, each spore containing one of the four chromatids present after premeiotic DNA replication in the diploid cell. In some ascomycetes eight spores are produced; after meiosis each chromosome is replicated once before formation of the spores. Therefore, each spore contains the genetic information present on one DNA strand of one of the four DNA duplexes in the meiotic cell. Although yeasts produce only four spores, the first replication of the spore DNA and subsequent division produces one cell with the genetic information present on one DNA strand and another containing genetic information present on the other strand. These cells grow into a colony that is sectored for any genetic difference between the two strands. Because yeasts can be readily monitored for sectored spore colonies, we will refer to yeasts as if they contained eight spores to more easily correlate data from yeasts with data from the other fungi.

By looking at the genetic composition of the spores of eight-spored fungi or by analyzing spore colonies for sectored phenotypes in yeasts, one can determine the genetic constitution of each DNA strand of each chromatid present after meiosis. A diploid cell heterozygous for a marker, A, will usually produce four A spores and four a spores, designated normal 4:4 segregation (Fig. 1A). Analysis of segregation patterns after meiosis reveals that occasionally a heterozygous marker segregates aberrantly (for review see reference 141). Such segregations fall into three classes. (i) The first class is $6^+:2^-$ or $2^+:6^-$ segregation. The information present on one chromatid is lost and replaced with the corresponding information from another chromatid. Such a nonreciprocal transfer of information is termed gene conversion (Fig. 1B). (ii) The second class is $5^+:3^-$ or $3^+:5^-$ segregation. The information present on a single strand of one DNA duplex is replaced by information from one strand of another chromatid. This event produces a DNA duplex in which the two strands contain different information for the segregating marker, thus the term heteroduplex DNA. Heteroduplex DNA is not detected genetically until an additional round of DNA replication produces two duplexes, each expressing the information contained on one of the strands of the heteroduplex DNA. Such segregations are therefore designated as postmeiotic segregations (Fig. 1C). (iii) An aberrant 4:4 segregation is a double postmeiotic segregation due to heteroduplex DNA present on two chromatids. The four wild-type and four mutant spores are genetically visible after the first postmeiotic division. Heteroduplex DNA that is present on two chromatids is called symmetric heteroduplex, in contrast to asymmetric heteroduplex DNA, present on only one chromatid.

For clarity, we will refer to 6:2 segregation as gene conversion and to 5:3 and aberrant 4:4 segregation as postmeiotic segregation. In this way, we hope to avoid the confusion found in the literature, where 6:2 and 5:3 segregations are sometimes referred to together as gene conversion.

A gene conversion event, a $6^+:2^-$ or $2^+:6^-$ segregation, is formally the transfer of two strands of information from one chromosome to another. In most recombination models it has been assumed to occur by transfer of one strand of information to generate a heteroduplex DNA intermediate. Repair of mismatches in the heteroduplex could then result in either gene conversion or restoration of the genotype of each chromatid. Alternative models that do not invoke mismatches in heteroduplex DNA have also been proposed and are discussed below.

Gene conversion and postmeiotic segregation have been extensively characterized. Although gene conversion and postmeiotic segregation are nonreciprocal recombination events, they are often associated with reciprocal exchange of flanking markers (for review see reference 141). Closely linked markers are often gene-converted together, a phenomenon called co-conversion (31). Gene conversion is recombinational, not mutational; it does not generate new alleles (10, 32, 114). The frequency of aberrant segregations varies with different genes from <1% to 20% (for review see reference 141). Different sites within a gene may show different frequencies of aberrant segregation and may vary in their spectrum of aberrant segregations (34, 72, 73).

In the following sections, we present a detailed discussion of each of these properties of gene conversion and postmeiotic segregation.

Heteroduplex DNA and Meiotic Recombination

Postmeiotic segregation is thought to occur as a direct consequence of the formation of heteroduplex DNA. Here

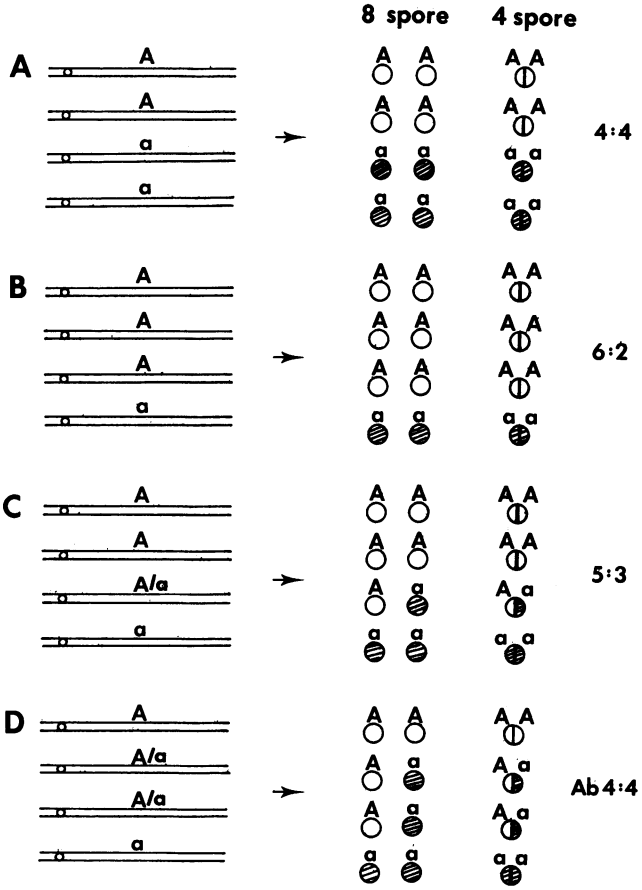


FIG. 1. Segregation patterns for the lower fungi. (A) Normally a heterozygous marker, A, will segregate 2A:2a or 4A:4a. (B) Occasionally the marker segregates 3A:1a or 6A:2a, a gene conversion event. (C) A postmeiotic segregation event, 5A:3a, is detected after one round of postmeiotic DNA synthesis and produces a sectored spore colony in the fungi with only four spores. (D) If heteroduplex DNA is present on two chromatids and no mismatch correction occurs, an aberrant 4A:4a segregation results. In the four-spored fungi an aberrant 4:4 segregation gives two-sectored spore colonies.

we review the conclusions derived from classical genetic analysis concerning the nature of the heteroduplex DNA formed during meiotic recombination. Several experiments indicate that such heteroduplex DNA is often formed on only one chromatid; i.e., it can be asymmetric. Aberrant 4:4 segregations are diagnostic of the presence of symmetrical heteroduplex DNA. These are found to be quite rare in *Saccharomyces cerevisiae* (35) compared with the frequencies predicted from the appearance of 5:3 segregations.

Another argument in favor of asymmetric heteroduplex is that symmetric heteroduplex results in two classes of 5:3 segregation events, called normal and aberrant and described in Fig. 2. At *buff* in *Sordaria brevicollis* (123), *w17* in *Ascobolus immersus* (128), and *SUP6* in *S. cerevisiae* (17) aberrant 5:3 segregations represent <5% of the 5:3 events or are not even detected. However, at the *grey* locus of *Sordaria fimicola*, where symmetric heteroduplex is seen, two-thirds of 5:3 segregations are normal whereas one-third are aberrant 5:3 segregations (60).

Finally, if symmetric heteroduplex is formed, the originally wild-type chromatid is corrected to mutant while the originally mutant chromatid is corrected to wild type, and no associated crossover occurs, the result is an apparent double crossover involving two chromatids (Fig. 3). In the absence of this effect, double crossovers involving two, three, or four chromatids are predicted to occur in a 1:2:1 ratio. No excess

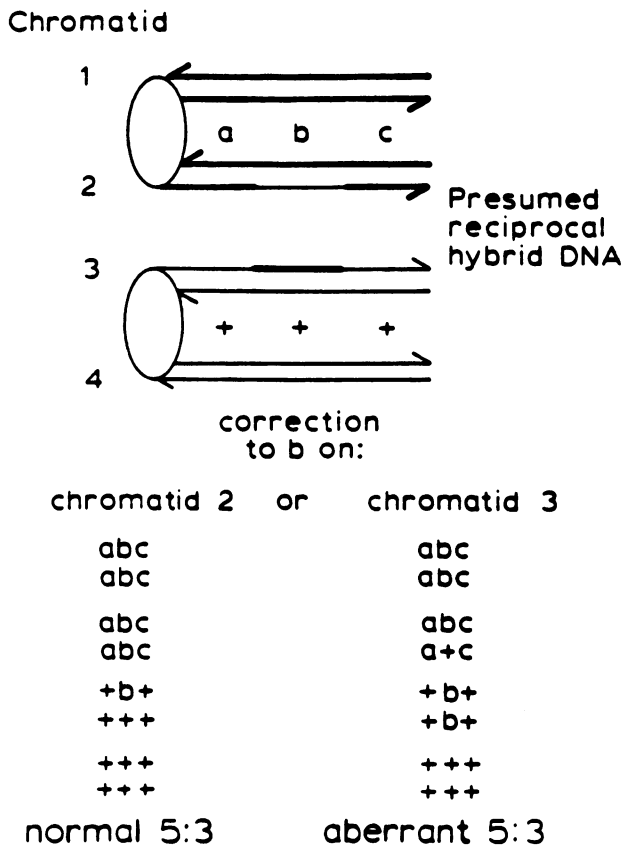


FIG. 2. The two kinds of non-crossover asci predicted to result from symmetric heteroduplex DNA. The flanking markers (a and c) are used to distinguish crossover from non-crossover asci. Symmetric heteroduplex covers the central marker, b. Correction to b on chromatid 2 results in normal 5:3 segregation; correction to b on chromatid 3 leads to aberrant 5:3 segregation.

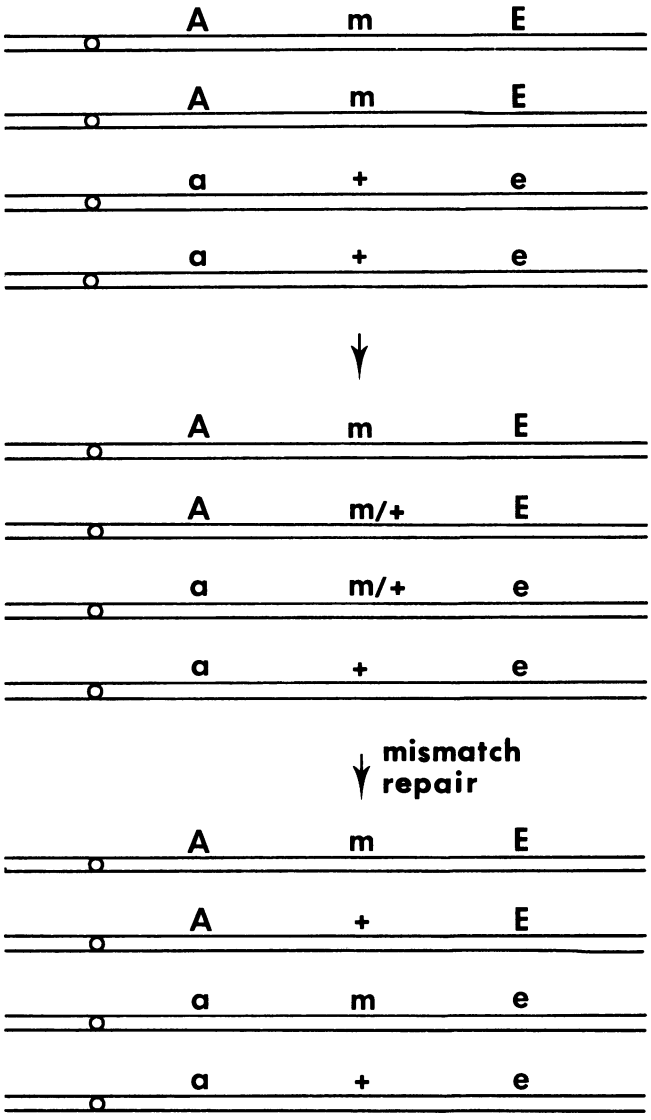


FIG. 3. Apparent two-strand double crossover. If symmetric heteroduplex DNA is formed at site m and the A, E chromatid is corrected to wild type, while the a, e chromatid is corrected to mutant, an apparent two-strand double crossover between flanking markers A and E results.

of two-strand double crossovers within short intervals is observed in *S. cerevisiae* (35).

By these three criteria, little if any symmetric heteroduplex DNA is formed in *S. cerevisiae* for all loci tested. The *buff* locus of *Sordaria brevicollis* and the *w17* locus of *A. immersus* also have little symmetric heteroduplex DNA. In contrast, symmetric heteroduplex DNA is common in the *b2* locus of *A. immersus* and the *grey* locus of *Sordaria fimicola*. Any unified model for fungal gene conversion will thus have to account for the existence of both asymmetric and symmetric heteroduplex DNA.

Co-Conversion

Analysis of crosses with strains containing two or more markers within one gene has shown that, when one allele segregates aberrantly, adjacent alleles in the same gene frequently also show an aberrant segregation pattern. These observations show that the events that lead to gene conver-

sion and postmeiotic segregation involve a region of DNA, and not just a point within a gene. For example, crosses of *Neurospora crassa* strains with three heterozygous alleles in the *pan-2* locus demonstrated frequent coincident conversion of two or even all three alleles. In addition, simultaneous postmeiotic segregation of two of the sites was also observed (10). Such events must result from formation of heteroduplex DNA over a region including both alleles.

Fogel and Mortimer (31) examined conversion events at heteroalleles within the *arg4* locus of *S. cerevisiae*. They observed co-conversion of two sites within the gene. Moreover, in comparing the frequency of two-site versus one-site conversion events to map position of the alleles (as determined by the frequency of X-ray-induced recombinants), it was observed that the frequency of co-conversion decreases with increasing distance between the alleles. These workers later excluded the possibility that apparent co-conversion events were actually due to independent conversion events at the two alleles, because they were unable to obtain classes of coincident conversion tetrads predicted to arise by independent events (33). Deletion mutations also show co-conversion (28).

The demonstration that adjacent alleles are co-converted at frequencies dependent on their separation suggests that gene conversion occurs over a region that is hundreds of nucleotides long. Longer regions of gene conversion may occur, as suggested by the observation of DiCaprio and Hastings (17) of co-conversion of as many as four genes across a region of 1 centiMorgans (cM). However, the genetic distance in this case may be inflated relative to the physical distance due to the presence of strong initiation sites. The data will be easier to interpret when the physical distances between alleles have been mapped on cloned DNA fragments.

Adjacent alleles can affect each other's patterns of aberrant segregation. In crosses heterozygous for two alleles of the *b2* locus of *A. immersus* the frequency of total aberrant segregation for each allele was not altered by the presence of the second allele, but the spectrum of aberrant segregation was changed. A mutation which normally showed predominantly 5:3 aberrant segregation showed a decrease in 5:3 segregation accompanied by a corresponding increase in 6:2 segregation if a mutation showing 6:2 segregation was placed adjacent to it (74). However, alleles that normally show 6:2 aberrant segregation could not be induced to give 5:3 segregation by any adjacent allele. Similarly, introduction of adjacent site heterozygosity on either side of the *arg4-16* allele of *S. cerevisiae* (an allele normally showing a high frequency of postmeiotic segregation) depressed the level of postmeiotic segregation observed at *arg4-16* (35). The effects of adjacent site alleles have been interpreted in terms of mismatch repair tracts within regions of heteroduplex DNA (see section on models).

Allele-Specific Segregation Patterns

An important question in addressing the mechanism of gene conversion is whether the types of aberrant segregation observed are a function of the nature of the mutant allele or of its position within the gene. The relationship between segregation pattern and type of allele has been extensively studied in *A. immersus* (72, 73). Mutations affecting ascospore color were induced by three types of mutagenesis: by a frameshift mutagen (ICR_{170}), by nitrosoguanidine, or by ethyl methanesulfonate (EMS) treatment. The aberrant segregation patterns of 62 mutant alleles were analyzed. Most alleles could be grouped into three classes: (i) those giving an excess of $6^+:2^-$ over $2^+:6^-$ segregation and showing rare postmeiotic segregation (class A); (ii) those showing excess $2^+:6^-$ over $6^+:2^-$ segregations and rare postmeiotic segregation (class B); and (iii) those mutations giving many postmeiotic segregation events and an excess of $6^+:2^-$ over $2^+:6^-$ segregations (class C) (72). All except three of the ICR-induced mutations were of class B, the majority of the nitrosoguanidine mutations were class C, and the EMS mutations were mostly class C, although some were class A or B. Spontaneous mutants behaved like the EMS mutants.

The physical nature of each mutation was determined by reversion analysis (73). Revertants of the class C mutations were all extragenic, allele-specific suppressors. This observation, coupled with the knowledge that EMS and nitrosoguanidine induce base substitutions in other organisms, led Leblon to conclude that class C mutants, showing high frequencies of postmeiotic segregation, were base substitutions. In contrast, the ICR-induced mutations and the class A EMS-induced mutations could be reverted by intragenic second-site mutations. One ICR-induced class B mutation was studied and found to be readily reverted by alkylating agents or EMS, but not by ICR. In contrast, an EMS-induced class A mutation could be reverted by ICR. Analysis of the second-site mutations showed that the suppressor of the class B mutation was a class A mutant, whereas the suppressor of the class A mutation was a class B mutant. Thus, it was concluded that ICR-induced class B mutations are single base insertions, and the EMS-induced class A mutations are single base deletions, or else that the converse is true. Intragenic suppression can be achieved if the two types of mutations are close together in the gene, but one mutation cannot suppress another mutation of the same class. Although the mutations have not been analyzed molecularly, the fact that ICR induces base pair additions in *S. cerevisiae* (18) suggests that the *A. immersus* class B mutations are additions.

The *A. immersus* data were interpreted in terms of gene conversion occurring by the repair of mismatches in heteroduplex DNA. Since base addition or deletion mutants rarely give postmeiotic segregations, mismatches involving such mutations must be efficiently repaired. The apparent directionality of conversion (single base addition mutations converted to mutant, and single base deletions converted to wild type) was assumed to result from a mismatch correction mechanism in which a DNA strand opposite an unpaired loop was corrected to the information in the loop. Base substitutions are rarely corrected and must be inefficiently recognized by the correction mechanism. The situation with large deletions is more complex. Most large deletions show parity; that is, they are converted to wild type or to mutant at approximately equal frequency. However, some deletions show strong disparity and are preferentially converted to wild type (J. L. Rossignol, A. Nicolas, H. Hamza, and A. Kalogeropoulos, *in* L. Brooks, ed., *The Recombination of Genetic Material*, in press; J. L. Rossignol, A. Nicolas, H. Hamza, and T. Langin, Cold Spring Harbor Symp. Quant. Biol., in press).

Segregation patterns of mutations in *S. cerevisiae* differ dramatically from those of *A. immersus*. Almost all mutations, including base substitutions, are efficiently corrected and show low frequencies of postmeiotic segregation events (35). Fogel et al. (30, 35) have analyzed gene conversion patterns in unselected tetrads for 30 sites induced by UV or EMS. Seventeen of the 30 are known base substitutions. A frameshift mutant has also been studied (33). All show

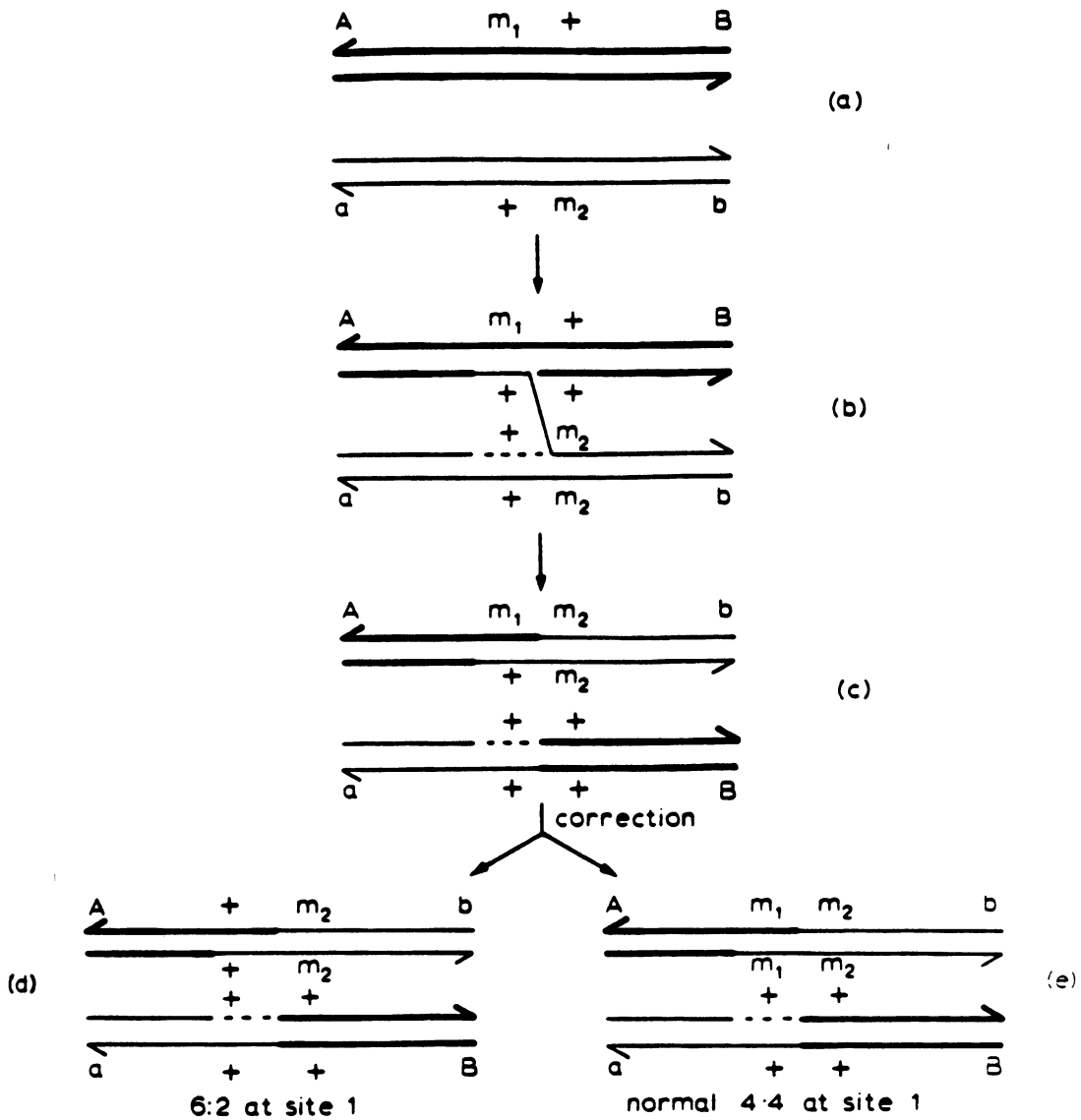


FIG. 4. Conversion-restoration experiment. (a) The close markers m_1 and m_2 are flanked by the distant markers A and B. (b) Asymmetric heteroduplex DNA covers only one of the close markers, such that the recombination event is resolved between the markers to yield a crossover (c). Mismatch repair can lead to conversion (d) or restoration (e).

approximate parity in conversion frequencies of $6^+:2^-$ to $2^+:6^-$. The ratio of conversion to wild type versus conversion to mutant ranges from 0.38 to 2.12, compared with the range of 0.01 to 100 observed in *A. immersus*. Large deletions in yeasts are observed to convert to both mutant and wild type (28, 71), and postmeiotic segregation events have not been detected for these deletions (35).

An elaborate series of experiments has been performed by Hastings and colleagues in *S. cerevisiae* (46, 125) and in *A. immersus* (45) to test the hypothesis that mismatch repair can correct either towards the information on the invading strand (to give a conversion event) or towards the information on the recipient strand (to give a restoration event). In these studies it is assumed that all aberrant segregation arises from a heteroduplex DNA intermediate. In *S. cerevisiae* the analysis was done on the *his1* gene, using pairs or *his1* alleles, and closely linked flanking markers. The *his1* gene shows polarity (see below), so the *his1* allele closest to the presumed initiation site was known. Tetrads were identified in which this allele had segregated 6:2, with an associated crossover (Fig. 4). If these 6:2 segregation events result from mismatch repair, and if mismatch repair occurs in either direction, then equal numbers of conversion events and normal $4^+:4^-$ segregation events should occur at this site. Restoration to 4:4 segregation would result in tetrads with an apparent crossover between the two *his1* alleles; such tetrads are less frequent than predicted. The excess of conversion events over restoration events ranges from 3- to 12-fold for five *his1* alleles tested. Therefore, if gene conversion is occurring by repair of heteroduplex DNA, mismatch repair occurs in favor of the invading strand. However, an alternative explanation is that most conversion in yeast cells is not the result of mismatch correction of heteroduplex DNA (see section on models). Either explanation requires that the parity in $6^+:2^-$ and $2^+:6^-$ seen in *S. cerevisiae* result from equal frequencies of initiation of recombination events on the mutant and wild-type chromatids. A similar analysis was performed at the *b2* locus of *A. immersus* (45). In

contrast to yeasts, both restoration and conversion occur at equal frequencies; mismatch repair in *A. immersus* uses information on either strand as the template for correction.

Polarity

Frequencies of gene conversion tend to reflect the position of an allele within a gene. For many genes in which a large number of alleles have been examined, frequencies tend to be higher for alleles at one end of the gene and to decrease towards the other end of the gene (for review see reference 141). This phenomenon has been termed polarity and is postulated to reflect the occurrence of fixed sites of initiation of recombination (50). The probability of an allele being involved in a recombination event is thought to decline as a function of its distance from the initiation site.

The conversion frequencies of alleles of the *arg4* gene of *S. cerevisiae* reflect their position within the gene, rather than the nature of the mutation (30). For example, both *arg4-17* and *arg4-4* are ochre nonsense mutations, but they differ fourfold in their frequency of conversion.

The most detailed studies on the nature and mechanism of polarity have been done on the *b2* locus of *A. immersus*. In their initial characterization, Paquette and Rossignol (99) analyzed the conversion properties of 15 class C mutations (those showing mainly 5:3 aberrant segregation) with map positions spanning the *b2* locus. The total frequency of all aberrant segregation events was highest for alleles on one side of the gene (designated the left side) and decreased towards the other side. Thus the locus shows polarity, with the left side defined as the high conversion side. Mutants were classified by their frequency of mismatch correction, derived from the ratio of 5:3/6:2 segregation for each allele. For alleles with similar 5:3/6:2 ratios the frequency of aberrant 4:4 segregations increased strikingly for alleles towards the right (low conversion) side of the gene. (*A. immersus* does not produce ordered octads, but aberrant 4:4 asci can be detected by including an additional marker affecting spore shape in the cross.) Thus the *b2* locus shows polarity both in overall conversion frequencies and in the relative frequency of aberrant 4:4 to 5:3 segregations. This suggests that the low conversion end of the gene contains symmetric heteroduplex DNA more frequently than the high conversion end does.

The frequency and pattern of aberrant segregation were also examined for 6 class B (single base insertion) and 22 class A (single base deletion) mutations in *b2* (119, 120). The total frequency of aberrant segregation for these mutants also decreased across the gene, from a high value at the left end to a low value at the right end. Moreover, the disparity of conversion between $2^+:6^-$ and $6^+:2^-$ for class B (or between $6^+:2^-$ and $2^+:6^-$ for class A) increased from left to right and then reached a plateau. This result supports the idea that asymmetric heteroduplex DNA is found predominantly at the high conversion end of the gene, whereas symmetric heteroduplex is found more often at the low conversion end. This follows from the assumptions that disparity results from the operation of the mismatch correction system on individual mismatched bases and that the two chromatids have the same probability of being involved in heteroduplex DNA. Then, for asymmetric heteroduplex DNA, the disparity ratio is simply a measure of the bias in the direction of correction of the mismatch. However, for symmetric heteroduplex DNA, in which both chromatids contain heteroduplex DNA, the bias in correction will occur on two chromatids. Consequently, the disparity ratio will approximate the square of the correction bias and will therefore be higher for symmetric than for asymmetric heteroduplex DNA.

Crosses in which one chromosome contained two mutations, one from each end of the *b2* gene, and the other chromosome was wild type demonstrated a physical linkage of asymmetric and symmetric heteroduplex DNA (118). The two alleles both showed high postmeiotic segregation; aberrant segregations at one or both of the two alleles could be detected by altered spore color. The genetic constitution of each spore in octads in which postmeiotic segregation occurred was then determined by backcrosses. Aberrant segregation of the left allele (the allele at the high conversion end of the gene) was most often a single event, not accompanied by aberrant segregation of the allele at the low conversion end of the gene. On the other hand, aberrant segregation of the allele at the right, low conversion end was frequently accompanied by aberrant segregation of the other allele. Of the aberrant 4:4 events detected, the majority were at the right allele, and 50% of these occurred with a simultaneous 5:3 postmeiotic segregation at the left allele. From the low correction frequencies of both alleles, both should give frequent aberrant 4:4 segregation if they are covered by symmetric heteroduplex DNA. The association of 5:3 segregation of the left allele with 4:4 segregation of the right allele therefore suggests that, when asymmetric heteroduplex DNA is formed on the left side of the *b2* gene, it can be followed by symmetric heteroduplex DNA on the right side of the gene.

These conclusions predict the existence of a region in the *b2* locus in which a switch is made from asymmetric to symmetric heteroduplex DNA. This prediction has been verified by experiments using the G234 deletion (44). G234, located in the middle of the *b2* gene, has no effect on spore color. However, when heterozygous, it causes a decrease by one-third of total segregation events for alleles to its right. The decrease is strikingly specific for aberrant 4:4 segregation events, which are depressed by 90% in the presence of the heterozygous deletion. The disparity between $2^+:6^-$ and $6^+:2^-$ displayed by alleles on the right side is also decreased. The mutation exerts a polar effect; no alteration in the frequency or patterns of aberrant segregation for alleles on the left is observed. The effect of the G234 deletion cannot be explained by an increase in mismatch correction to its right, because no decrease in 5:3 segregation or increase in 6:2 segregation is observed in this region. G234 appears to block the propagation of symmetric heteroduplex DNA past itself, when it is heterozygous. In the *b2* locus, asymmetric DNA is apparently formed at the high conversion end, a transition to symmetric DNA occurs in the G234 region, and that symmetric heteroduplex is propagated rightward toward the low conversion end of the gene. This propagation of the symmetric heteroduplex DNA would be expected to be blocked by a large heterology.

For most genes, conversion frequencies decrease from one end of the gene to the other. However, in the *lysF* gene of *Aspergillus nidulans* (100) alleles at the two ends of the gene show high conversion frequencies, whereas alleles in the middle give low frequencies, producing a U-shaped conversion frequency pattern rather than a linear decrease. This can be explained by the existence of an initiation site at each end of the gene.

Murray (90) demonstrated that polarity is determined by a chromosomal region as opposed to being imposed by the centromere or chromosome ends. She used a strain of *Neurospora crassa* in which *met-6* and its flanking markers

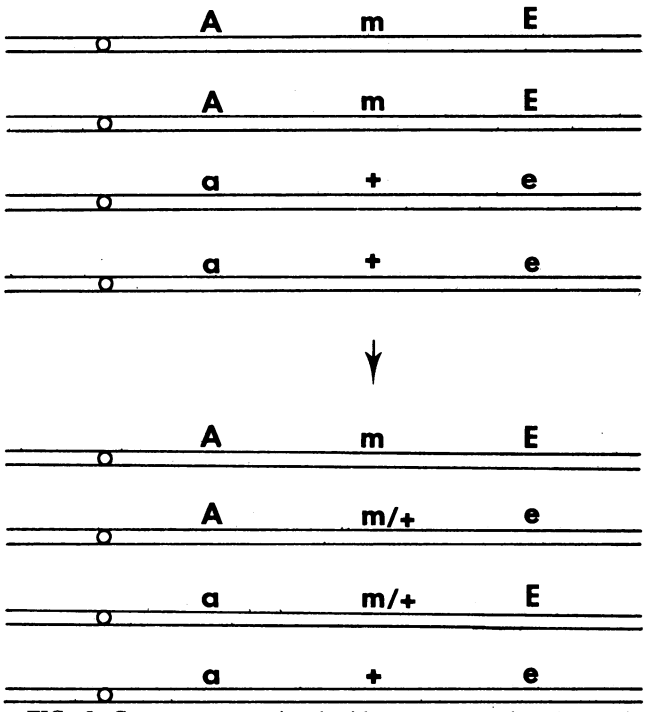


FIG. 5. Crossovers associated with gene conversion. In an aberrant 4:4 ascus it is possible to determine which two chromatids were involved in the recombination event. Thus it can be demonstrated that associated crossovers occur between the same two chromatids that undergo the aberrant segregation event.

were transferred in an inverted orientation from linkage group I to linkage group V. Analysis of the conversion properties for *met-6* alleles in the homozygous inversion strain showed that the alleles portrayed the same polarity pattern with respect to the flanking markers, even though the orientation of the gene relative to the centromere and telomeres was reversed. It would be interesting to know the size of the region transferred in the translocation. Similar experiments, in which regions of DNA are transferred by recombinant DNA techniques, may provide a means for localizing recombination initiation sites.

Crossing Over and Aberrant Segregation

Aberrant segregation events are associated with a high frequency of reciprocal exchange (crossing over) of flanking markers (Fig. 5). Analysis of associated reciprocal exchanges in asci showing aberrant 4:4 segregation allowed Kitani and co-workers (for review see reference 141) to show that crossovers flanking the *grey* locus in *Sordaria fimicola* occurred on the same two chromatids showing aberrant segregation. This physical association suggests that aberrant segregation and crossing over result from a common initial event.

The frequency of crossing over associated with aberrant segregation varies widely (for review see reference 141). At the *grey* locus of *Sordaria fimicola* 40% of aberrant segregations have an associated crossover (59); approximately the same percentage is observed at the *w17* locus of *A. immersus* (129), the *his1* locus of *S. cerevisiae* (55, 125), and the *SUP6* gene of *S. cerevisiae* (17, 55). Crossovers associated with conversions of different alleles of *arg4* ranged in frequency from 18 to 66% (35). The *buff* locus in *Sordaria brevicollis* (123) and the *met-7* gene in *N. crassa* (91) show low frequencies of associated crossover, 20 and 15%, respectively.

Crossover frequencies associated with aberrant segregation often vary with the type of segregation event. In the studies of Sang and Whitehouse (123), postmeiotic segregation events at the *buff* locus were associated with a lower frequency of crossing over than were gene conversion events. Crossover frequencies associated with aberrant segregation of three alleles of the *b8* gene of *A. immersus* varied significantly with the type of segregation event (95). Aberrant 4:4 segregations gave 50% associated crossovers, 5:3 postmeiotic segregations gave only 19%, and 6:2 gene conversions showed intermediate frequencies. The significance of this variation is not yet understood, and it does not occur at all loci. Postmeiotic segregations and gene conversions have the same frequencies of associated crossovers at *SUP6* (17).

Analysis of the position of a crossover relative to an aberrant segregation event in a locus with polarity provides additional information on the relationship between aberrant segregation and crossing over. The position of a crossover in a gene can be determined by examining tetrads with 5:3 segregation events (Fig. 6). Such an analysis for *arg4-16* in *S. cerevisiae* showed 44 crossovers on the high conversion side and 20 crossovers on the low conversion side of the gene (35). Crosses with two markers in the *grey* locus of *Sordaria fimicola* (for review see reference 141) were examined for asci in which one allele showed a 5:3 segregation and the other showed a normal 4:4 segregation. Crossovers could be classified as proximal, medial, or distal with respect to the centromere and the two markers within the *grey* locus (Fig. 7). If heteroduplex is propagated from an external initiation site, into the locus, and a crossover occurs at the endpoint of the heteroduplex tract, then single postmeiotic segregation events at either the proximal or distal allele would be expected to be associated with medial crossovers. However, the majority of proximal postmeiotic segregation events were associated with proximal crossovers and the

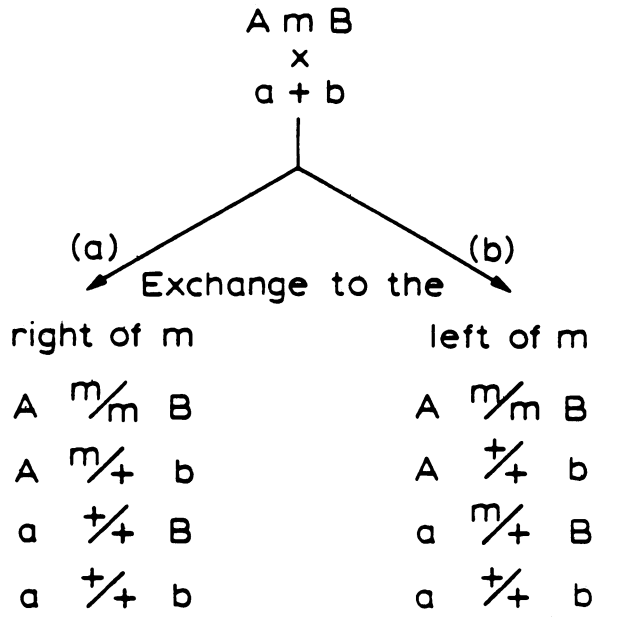


FIG. 6. Crossover position. Analysis of postmeiotic segregation events allows the position of associated crossovers to be determined relative to outside markers (A and B).

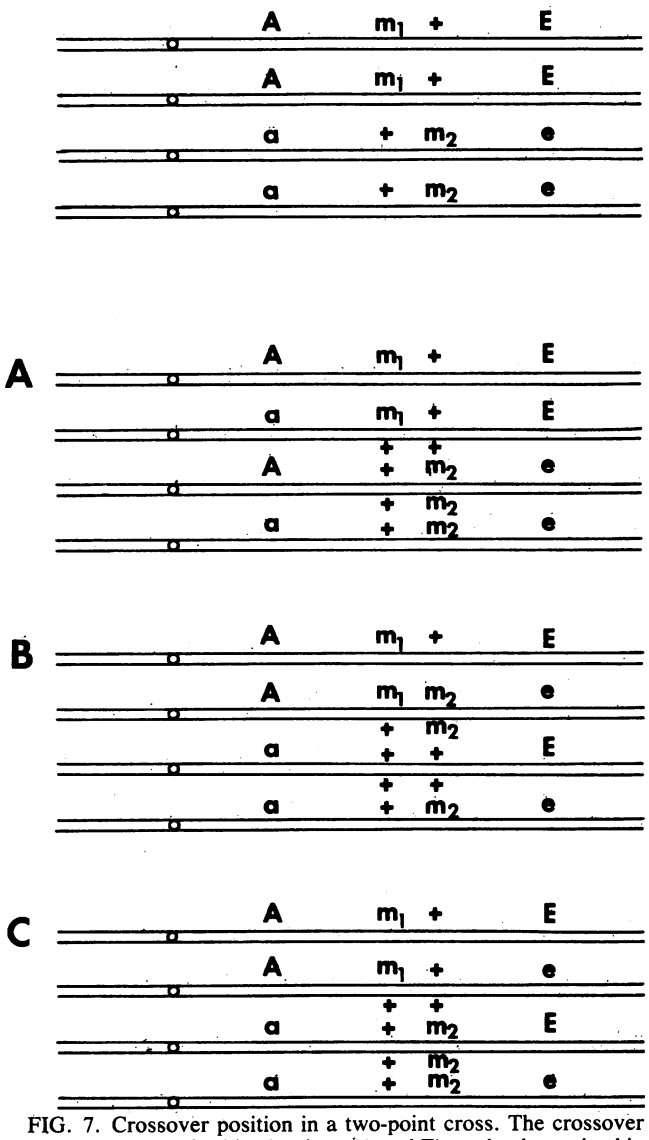


FIG. 7. Crossover position in a two-point cross. The crossover position relative to flanking markers (A and E) can be determined in a two-point cross in which one allele (m_1) shows a 5:3 segregation and the other allele (m_2) segregates 4:4. (A) The crossover is proximal to m_1. (B) The crossover is medial, occurring between the two alleles. (C) A distal crossover, between the unconverted allele m_2 and the flanking marker E. Where symbols appear both above and below a chromatid, the symbols refer to the two strands of the chromatid.

majority of distal postmeiotic segregations were associated with distal crossovers. In 100 crossover asci analyzed at *buff*, none gave medial crossovers (124, 137).

An even more striking observation on the position of associated crossovers is that crossovers are often separated from a site showing aberrant segregation by an allele with normal 4:4 segregation (Fig. 7C). At the *his1* locus of *S. cerevisiae* 25% of crossovers are at this position (125), as are 30% in the *grey* locus of *Sordaria fimicola* (141) and 50% in the *buff* locus of *Sordaria brevicollis* (124). These are not the result of incidental crossovers and cannot be explained by restorative correction of heteroduplex at the second site. If asymmetric heteroduplex is formed at both sites, then after correction of one of the two mismatches both (A + m2/+ E) and (A m1/+ + E) genotypes should be obtained. However, only one or the other would be obtained if asymmetric heteroduplex is present at only one of the alleles. Such an analysis for *buff* (shown to have symmetrical heteroduplex only rarely) shows that heteroduplex is extended to the second site in only 10% of the events (124, 137). Thus, models for gene conversion must not only account for the occurrence of associated crossovers but also explain their position both in a polar gene and with respect to unconverted alleles.

The G234 deletion in the *b2* gene of *Ascobolus* sp. blocks the progression of symmetric heteroduplex DNA when it is heterozygous, presumably because a Holliday junction cannot branch migrate past a large heterology (44). An increased number of crossovers are observed on the high conversion side of the heterozygous deletion, as expected from the resolution of these Holliday junctions (Rossignol et al., Cold Spring Harbor Symp. Quant. Biol., in press). Remarkably, few of these crossovers are associated with aberrant segregation events at the high conversion end of the *b2* locus (Rossignol et al., Cold Spring Harbor Symp. Quant. Biol., in press). This suggests that some of the crossovers induced by the deletion do not arise by a mechanism involving long tracks of heteroduplex DNA.

UV irradiation of *rad1* cells entering meiosis leads to a two- to threefold decrease in gene conversion, but essentially eliminates crossing over (107, 109). Meiosis itself is not inhibited, but the products are mostly inviable, probably because of nondisjunction resulting from the low levels of crossing over. The mechanism by which UV induced lesions in DNA block crossing over is not known.

Crossovers in *S. cerevisiae* show chiasma interference (89). Crosses were performed in which conversion events at *arg4* could be monitored for both an associated crossover and a crossover in an adjacent interval. Control crosses without conversion show chiasma interference: double crossovers occurred at lower frequencies than predicted from the frequencies of single crossovers. However, the distribution of double crossovers exhibited no chromatid interference. Asci with conversion events at *arg4* that were exchanged for flanking markers also showed depression of crossovers in adjacent intervals. This reduction was considerably larger than for asci with conversion events at *arg4* that retained the parental configuration of the flanking markers. Thus, conversion events with an associated crossover show interference, but conversion events without an associated crossover do not.

Intrachromosomal meiotic gene conversion events in *S. cerevisiae* do not appear to be associated with crossing over. Intrachromosomal gene conversion was first observed between duplications generated by the integration of a circular plasmid into a homologous chromosomal site (56, 64). In recent experiments (63) gene conversion events between two inverted copies of *his3* were analyzed. Gene conversion between the *his3* repeats accompanied by a crossover would result in an inversion of the intervening pBR322 DNA. No inversions were detected out of 6 conversion events seen in dissected tetrads or in 36 conversion events detected from random spore analysis. The possibility of mechanistic differences between inter- and intrachromosomal gene conversion remains to be clarified.

Recombination Initiation Sites

The discovery of polarity led to the hypothesis that recombination events are initiated at specific sites on the DNA. Recent work has focused on two questions: (i) do the

initiating lesions occur on the strand that is the donor or the recipient of genetic information? and (ii) do the initiating lesions occur at specific initiation sites or merely in the vicinity of such sites? Mutations which appear to generate recombination initiation sites provide opportunities for testing specific predictions concerning the mechanism of initiation. Such mutations have been described in *S. pombe*, *Sordaria brevicolis*, and *N. crassa*.

The M26 mutation of *S. pombe* lies within the *ade6* locus (42). Although induced by X rays, it can be extragenically suppressed and is most likely a nonsense mutation. The M26 mutation converts at 13-fold-higher frequencies than adjacent *ade6* mutations. The mutation shows extreme disparity, $6^+:2^-$ segregations being observed 12 times more often than $2^+:6^-$; postmeiotic segregation events are rare. In crosses with adjacent *ade6* mutations M26 caused double- and triple-site co-conversions in a polarized manner. M26 pulls adjacent sites into its conversion pattern; this effect is seen for *ade6* alleles on both sides of M26. Moreover, the frequency of co-conversion is distance dependent; one close allele co-converted with M26 in 100% of the events analyzed, whereas a more distant mutant was converted in only 60% of the recombinant asci.

A similar mutation has been characterized in *Sordaria brevicollis* (75). YS17, an ICR_{170}-induced mutation in the *buff* locus, converts at 10-fold-higher frequencies than other *buff* mutations; 98% of conversion events are $6^+:2^-$. The presence of YS17 overrides the normal polarity properties of the *buff* gene. YS17 also increases the frequency of aberrant segregation of adjacent *buff* alleles. Analysis of adjacent postmeiotic segregation events showed that they occurred on the same chromatids that were convertant for YS17. When crossed to a wild-type *buff* gene, YS17 conversions show 13% associated crossovers, whereas when crossed to another mutant, 40% of the conversion events are associated with crossovers. The reason for this difference is unclear. As was observed previously in the *buff* locus, one-third of the associated crossovers are separated from YS17 by the other *buff* mutant, which shows normal 4:4 segregation.

A naturally occurring mutation, *rec1*, is observed to depress the frequency of YS17 conversion to that of other *buff* mutants (76). The gene is recessive, unlinked to *buff*, and is postulated to encode an endonuclease that acts on YS17 to initiate recombination. An important result is that in the presence of *rec1* the pattern as well as the frequency of aberrant segregation at YS17 are altered. The frequency of aberrant segregations decreases from 8.6 to 0.14%, but the proportion of aberrant asci showing postmeiotic segregations for YS17 increases from 0.3 to 15%. Therefore, the striking segregation pattern of YS17 in $rec1^+$ strains is a reflection of the nature of the recombination initiation event, rather than of the mutation itself. Both YS17 and M26 are preferentially gene converted to wild type and thus act as recipients of two strands of genetic information.

In *N. crassa*, several mutations have been isolated which repress recombination in a dominant manner at specific sites in the genome (see below). The $rec2^+$ gene decreases recombination levels in the *his3* interval (1). A recognition site (*cog*) was discovered distal to *his3* (1). In $rec2^-$ derepressed cells, the dominant cog^+ allele causes six- to eightfold higher recombination frequencies at *his3* (13). cog^+ behaves analogously to M26 and YS17 in being the recipient rather than the donor of genetic information. Analysis of cog^+-induced recombination in strains containing a translocation of the distal segment of *his3* and the *cog* site has provided much information on the mechanism of cog^+ initiation (Fig. 8). When a *his3* allele on the same side the breakpoint as *cog* was crossed to the *his3* translocation strain, cog^+ exerted a normal stimulation of recombination when homozygous. However, a depressed level was observed if the strain was heterozygous for cog/cog^+, with the normal chromosome being *cog*. This is consistent with initiation at cog^+ but not at *cog*. Strikingly, if the *his3* allele is proximal, cog^+ still stimulates recombination, but the production of $his3^+$ recombinants requires that cog^+ be on the normal chromosome. Therefore, in the recombination event mediated by cog^+, the information transferred is able to skip across a translocation breakpoint. This observation excludes a mechanism in which heteroduplex DNA is initiated at cog^+; possible mechanisms to explain the observation are explored in the section on models.

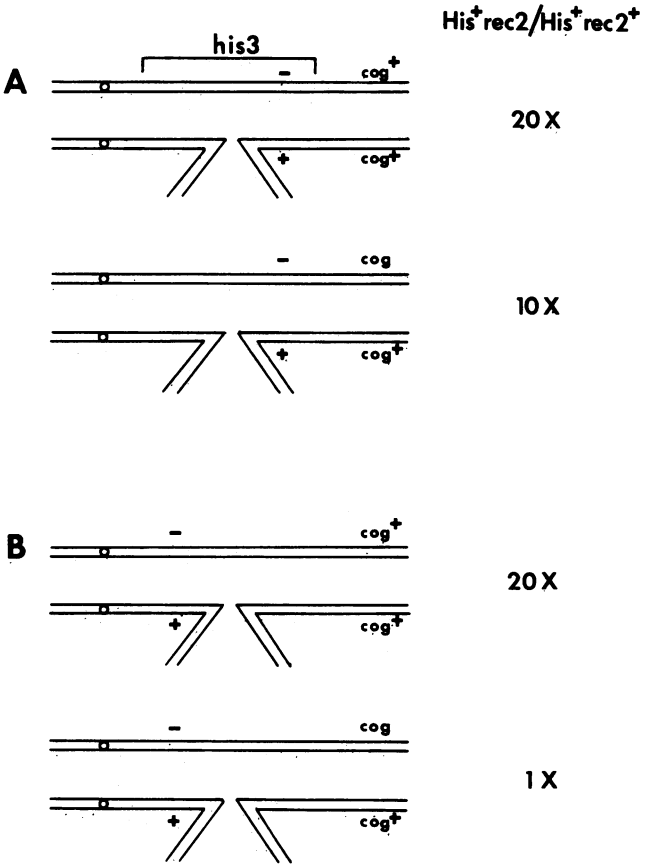


FIG. 8. cog^+-stimulated recombination across a translocation breakpoint. An *N. crassa* strain containing a translocation of the *his3* gene was tested for cog^+ stimulation of HIS^+ recombinants in a *rec2* versus $rec2^+$ background. (A) Both cog^+ and the *his3* mutation are on the same side of the translocation breakpoint; a 20-fold increase in recombination frequency is observed. cog^+ gives a higher stimulation of HIS^+ prototrophs when homozygous than when heterozygous. (B) cog^+ and the mutation are on opposite sides of the translocation breakpoint. cog^+ is able to increase recombination, but only if it is on the normal chromosome.

Mutations Affecting Meiotic Recombination

Three classes of meiotic recombination mutants have been characterized in *S. cerevisiae*: (i) those isolated in screens for mutations affecting intragenic gene conversion; (ii) mutations initially characterized as radiation sensitive and

subsequently shown to affect recombination; and (iii) mutations isolated for their effect on sporulation which are also deficient in recombination.

Two screens for recombination mutants have been performed in *S. cerevisiae*; both utilized heteroallelic disomes in an otherwise haploid strain. This permitted detection of recessive mutations. Roth and Fogel (121) used a chromosome III disome that was heterozygous for the *MAT* locus, and therefore able to undergo meiotic DNA synthesis and recombination, and was heteroallelic for *leu2*. They characterized three EMS-induced mutations, *con1*, *-2*, and *-3*, that were specifically deficient in meiotic gene conversion, showing normal levels of premeiotic DNA synthesis. A haploid strain disomic for chromosome VIII and containing *arg4* heteroalleles was used to screen for UV-induced mutations that blocked X-ray- or UV-induced mitotic gene conversion (111). Four complementation groups were defined, *rec1*, *-2*, *-3*, and *-4*. *Rec2* and *-3* both reduce sporulation; *rec2* was subsequently shown to be allelic to *rad52*. *rec4* affects both mitotic and meiotic gene conversion but is specific for *arg4* (110). *rec4* acts to increase co-conversion between *arg4* alleles and thereby decreases the frequency of $ARG4^+$ recombinants. The mutation has no effect on overall conversion levels (cited in reference 21).

Williamson and Fogel (33; personal communication) have recently isolated four recessive mutations, *cor1-4*, postulated to be defective in the correction of mismatched bases in heteroduplex DNA. These mutants were initially detected by their hyper-rec phenotype for intragenic meiotic recombination and they are also mitotic mutators. The *cor* mutations cause an increase in the frequency of 5:3 postmeiotic segregations coupled with a decrease in 6:2 segregations.

Mutations conferring sensitivity to ionizing radiation affect sporulation or spore viability, but UV-sensitive mutations do not. An extensive analysis of the role of four X-ray-sensitive mutations, *rad6-1*, *rad50-1*, *rad52-1*, and *rad57-1*, was carried out by Game et al. (38). Strains homozygous for the *rad* mutation, heteroallelic at *his1*, and heterozygous for *can1* were exposed to sporulation conditions and monitored for DNA synthesis, intragenic gene conversion, and haploid spore formation. All of the mutants were capable of DNA synthesis but all were blocked in gene conversion. *rad6* did not produce spores; the others produced inviable spores. Prakash et al. (104) and Malone and Esposito (77) demonstrated that *rad52-1* is blocked in meiotic gene-centromere recombination in addition to heteroallelic gene conversion. *rad52* mutants accumulate single-strand breaks in their DNA as meiosis proceeds (108). Recent evidence suggests that these single-strand interruptions are not at random sites, an intriguing observation if these breaks are indeed shown to be recombination related (M. Resnick, T. Chow, J. Nitiss, and J. Game, Cold Spring Harbor Symp. Quant. Biol., in press).

Temperature-sensitive mutations affecting sporulation were obtained by mutagenizing spores of a homothallic strain and screening diploid survivors for inability to sporulate (20). Three, *spo7*, *-8*, and *-11*, were observed to be deficient in meiotic recombination (for review, see reference 24). However, the recombination-minus phenotype of the first two is most likely a secondary effect resulting from the ability of these mutations to carry out premeiotic DNA synthesis (see below). *spo11* is deficient in gene conversion and intergenic recombination. Using a strain containing a chromosome III disome heterozygous for *MAT*, *spo13* (see below), and a *URA3* insert at *rDNA*, Esposito et al. (personal communication) have been able to measure meiotic sister chromatid exchange. This is monitored by analyzing sectored colonies in which one sector is ura^-. Such sectors have been previously shown (101, 136) to arise by unequal sister chromatid crossovers in the *rDNA* repeat, producing a duplication of the selected marker on one sister and a deletion on the other. This system has been used to show that *spo11* is 10-fold decreased in meiotic sister exchange, whereas *rad50-1* has no effect on sister crossovers (R. Esposito, personal communication). Synaptonemal complexes are missing in *rad50-1* cells (B. Byers, personal communication).

Several recombination mutants that occur as natural variants have been described in *N. crassa* and are denoted *rec1*, *-2*, and *-3* (12, 57, 127). The dominant alleles of these genes repress recombination at specific sites in the genome by an order of magnitude. Significantly, all three repressors alter the polarity patterns of the affected gene, as if they blocked a recombination initiation site. This conclusion is strengthened by the definition of the cog^+ site adjacent to *his3* through which $rec2^+$ appears to act. The polarity change induced by the repressor is explained by the use of an initiation site on the other side of the gene. A region linked to *nit2*, a gene repressed by *rec1*, has been found to exist as three different alleles in three different strains (11). These *ss* alleles suppress recombination when heterozygous and act multiplicatively with *rec1*. Similar modifiers that repress recombination when heterozygous have been found linked to four spore color mutants in *A. immersus* (39). Linked to the *w1* locus of *A. immersus* are three conversion control factors (*ccf2P*, *ccf2K*, and *ccf2-91*) that control conversion frequencies and patterns at *w1* and may constitute a recombination initiation site (70). Two unlinked genes, *ccf3E* and *ccf4r*, appear to be able to enhance or repress conversion frequencies at *w1* (47).

Role and Timing of Recombination in Meiosis

The observation that recombination mutants (e.g., *rad50*) lead to production of inviable meiotic products establishes the essential role of recombination in meiosis. Analysis of the timing and relation of meiotic events in *S. cerevisiae* has been largely via "return to growth" experiments. Cells exposed to sporulation conditions for varying lengths of time are returned to vegetative growth and monitored for DNA content, recombination, and haploidization. After prolonged exposure to sporulation media, diploid cells are committed to completion of meiosis and form spores before resuming growth in vegetative media. However, at earlier times meiotic processes can be examined. Such experiments have demonstrated that the commitment to meiotic recombination occurs coincident with or shortly after the commitment to DNA synthesis (23). The commitment to recombination is prior to the commitment to the meiosis I reductional division. Recombination is thought to require DNA synthesis because all mutations blocking DNA synthesis block recombination (24). Although meiotic recombination may be required for proper reductional division, they are separable events. The commitment to recombination does not commit a cell to a reductional division. In the return to growth experiments meiotic levels of recombination can be obtained without reductional division. Furthermore, the *spo12* and *spo13* mutations that bypass the reductional division (see below) exhibit normal levels of recombination.

Analysis of recombination mutants has been facilitated by the use of *spo12* and *spo13*. These genes, isolated from

naturally occurring strains, bypass the reductional meiosis I division, producing two diploid spores (61, 62). Studies with *spo13* have demonstrated that recombination functions are required for successful reductional segregation or that recombination that is initiated but not completed produces a lethal intermediate during the reductional division. This conclusion is based on the observation that double mutants of *spo12* or *spo13* with *rad50*, *rad52*, or *spo11* produce two diploid spores but show no recombination. *rad52 spo13* strains produce two inviable spores (78). It has been possible to order the action of recombination functions in meiosis by using *spo13*. Since the double mutant *rad52 spo13* produces no viable spores, but the triple mutant *rad50 rad52 spo13* produces viable spores, it can be concluded *rad50* acts prior to *rad52* in the recombination pathway (78). Similarly, *spo11* has been shown to act before *rad52*, and *rad50* also acts before *rad57* (Esposito, personal communication). The double mutant *rad6 spo13* cannot be rescued by any of the other *rad* mutants (Esposito, personal communication). *spo13* permits haploids disomic for chromosome III to form viable ascospores, providing what should prove to be a powerful approach to the isolation of both dominant and recessive meiotic recombination mutants.

MITOTIC RECOMBINATION

Mitotic recombination was initially described as resulting from crossing over (for review see reference 141). However, Roman (114) definitively demonstrated that heteroallelic mitotic recombination occurred predominantly by gene conversion. He selected prototrophs in diploids heteroallelic for *ade3* or *ade6* and then determined the genotype of the prototrophs by sporulating, dissecting, and backcrossing the spores to the haploid parents of the diploid (Fig. 9). Since the prototrophic diploids never retained both alleles (Fig. 9A), but did contain one or the other (Fig. 9B), the prototrophs arose from gene conversion and not from a reciprocal crossover. The frequency of prototroph appearance was too high to be due to reversion of the alleles. Although mitotic crossing over and conversion have been described in a number of fungi (for review see reference 141), the most extensive characterization of possible mechanisms has been done in *S. cerevisiae*. Our review therefore emphasizes work in this organism.

Induction of Mitotic Recombination

Mitotic gene conversion and crossing over occur at levels several orders of magnitude lower than meiotic levels for the same interval. Mitotic recombination can be induced by X rays or UV irradiation, treatment with chemical mutagens such as mitomycin C (51), or thymidylate starvation (for review see reference 69). Whereas these induction studies suggest that recombination is induced by physical lesions in the DNA, there is evidence that induction of recombination competence may occur. Fabre and Roman (26) mated an X-ray-irradiated α-haploid strain of *S. cerevisiae* containing two *ade6* mutations to an unirradiated **a/a** (*MAT* homozygous) *kar1* strain heteroallelic for *ade6*. Because the *kar1* mutation prevents nuclear fusion, the induction of gene conversion in the unirradiated diploid nucleus by mating to the irradiated haploid strongly argues for the induction of diffusible factors responsible for mitotic recombination. Analysis of UV-induced mitotic recombination for genes on different chromosomes showed joint conversions of genes at frequencies too high to result from independent events, also suggesting an induction of recombination occurs (29).

Recombination at the Two-Strand Stage

Meiotic recombination occurs after DNA replication when four DNA duplexes are present, but most mitotic gene conversion seems to occur in the G_1 stage of the cell cycle, before DNA replication. Theoretically the recombination reaction could be resolved and completed in G_1 or after DNA replication in G_2. An elegant experiment by Fabre (25) demonstrated that gene conversion events could occur in G_1. He used a diploid strain heteroallelic for *cdc4*, a cell cycle mutation which blocks in G_1 at the nonpermissive temperature, and tested whether he could obtain *CDC4*$^+$ recombinants at the nonpermissive temperature. The *cdc4* diploid is arrested in G_1 and can only proceed past the block if the wild-type *CDC4* gene is generated in G_1. Fabre obtained *CDC4*$^+$ recombinants in cells at both the nonpermissive and the permissive temperature, thus establishing that gene conversion could occur in G_1.

Although mitotic recombination is more difficult to study than meiotic in that the genotype of a recombinant must be determined by sporulation, dissection, and backcrosses, analysis of the constitution of prototrophic and especially of colonies prototrophic for one marker and sectored for another has provided much information on the mechanism of mitotic recombination. Such an analysis of spontaneous sectored, prototrophic colonies provided further evidence that mitotic recombination could occur at the two strand stage (22, 40). Specific predictions about the genotypes of sectored, prototrophic colonies arise from a G_1 or G_2 event (Fig. 10 and 11). G_2 events must produce sectored colonies with the markers (m_1 +/ + +), (+ +/ + m_2). Although this class of colony also can arise by a G_1 event with symmetric heteroduplex DNA, G_1 events will result in eight other classes of marker segregations. For example, a genotype of (+ +/m_1 +), (+ +/ m_1 +) or (+ +/ + m_2), (+ +/ + m_2) is

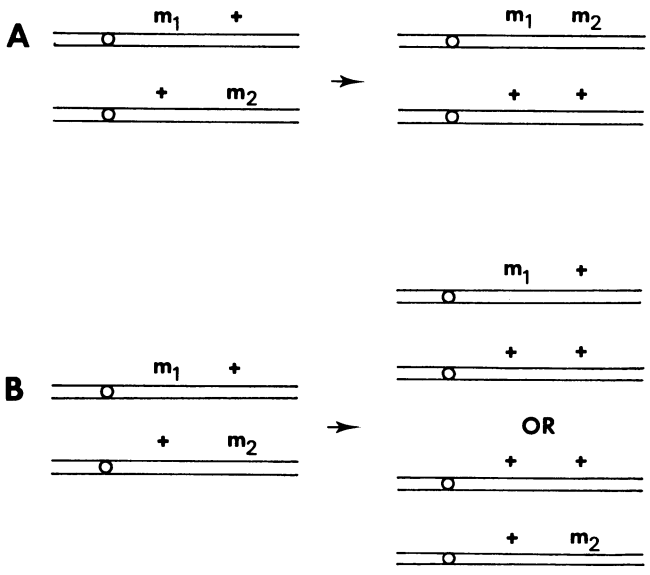
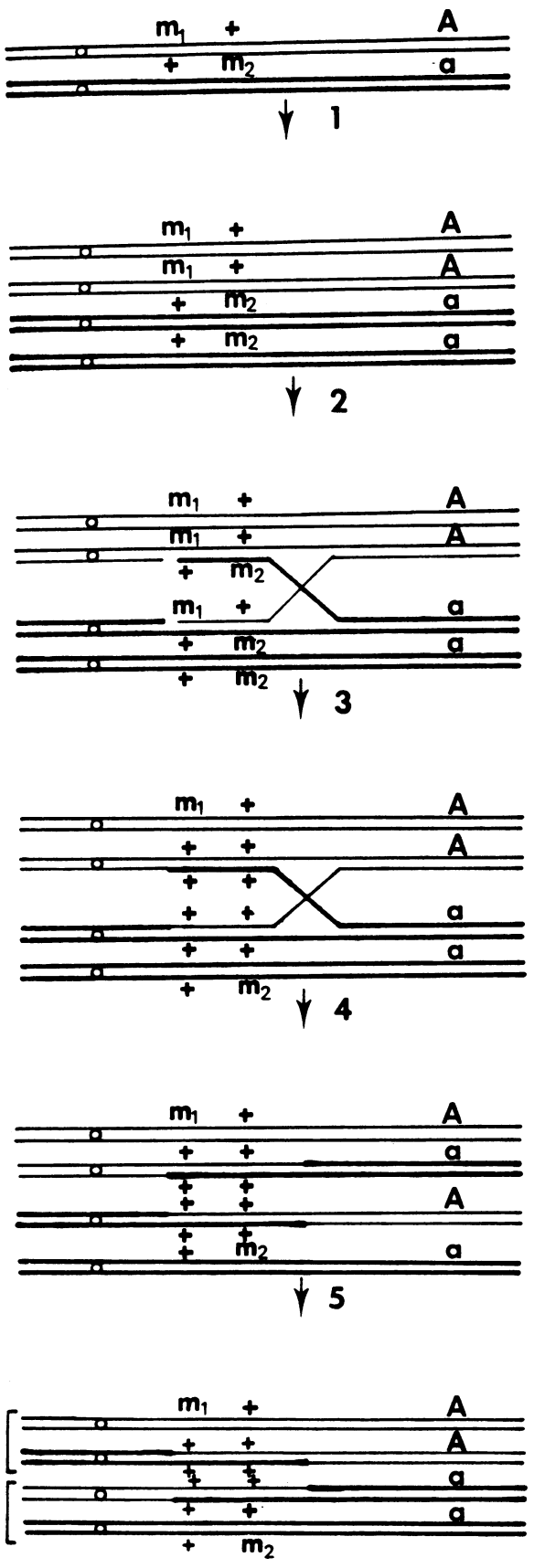


FIG. 9. Analysis of mitotic prototrophs. Mitotic prototrophs can be generated in a heteroallelic diploid by reciprocal recombination (A) or gene conversion (B). If a reciprocal crossover has occurred between the two alleles to produce the prototroph, then both alleles will be present in the prototrophic diploid (A). In contrast, only one of the alleles is retained after gene conversion (B).



diagnostic of a G_1 event. Analysis of heteroallelic gene conversion at *leu1* or *trp5* resulted in classes of genotypes that could arise only by G_1 events; 70 of 71 TRP^+ prototrophs and 20 of 20 LEU^+ prototrophs were produced by G_1 conversions.

In an analysis of sectored, prototrophic colonies similar to that performed by Esposito, Roman and Fabre (117) recently demonstrated that, although most X-ray-induced convertants occurred in G_1, it was possible for the events to occur in G_2. This study analyzed the same genotypic classes as in Esposito's experiments, but the study was done with either G_1 cells or cells arrested in G_2 with the drug methyl benzymidol-2yl-carbamate. The number of prototrophs belonging to the one genotypic class which arises from a G_2 or G_1 event rises from 2 of 32 from G_1 to 12 of 45 for methyl benzimidol-2yl-carbamate-blocked cells. Therefore, yeast cells are competent to undergo gene conversion in G_2.

Symmetric Heteroduplex DNA

Assuming that mitotic gene conversion occurs in G_1 via a heteroduplex DNA intermediate, analysis of the genotypes of sectored, prototrophic colonies can reveal the nature of the heteroduplex DNA at the convertant site. The genotypes (+ +/ m_1 +), (+ +/ + m_2) or (+ +/ + +), (+ +/ + +) are two genotypes indicative of symmetric heteroduplex DNA. However, there are no classes unique for asymmetric heteroduplex DNA; the two classes predicted by asymmetric heteroduplex (+ +/ m_1 +), (+ +/ m_1 +); (+ +/ m_2), (+ +/ + m_2) can also occur by correction of symmetric heteroduplex. Therefore, calculations of the extent of symmetric heteroduplex DNA by this analysis necessarily represent a minimum estimate. Golin and Esposito (40) found that at least 30% of spontaneous conversions at *leu1* or *trp5* arose from symmetric heteroduplex DNA. In similar experiments with *ade6*, Roman (116) found that at least 10% of X-ray-induced *ade6* convertants arose from symmetric heteroduplex DNA. The apparent frequent occurrence of symmetric heteroduplex DNA in mitotic gene conversion is in marked contrast to its rarity in meiosis.

Length of Heteroduplex DNA Tracts

Spontaneous *LEU1*$^+$ *TRP5*$^+$ co-conversion events were detected by Golin and Esposito (40, 41) at frequencies 1,200 times higher than predicted by multiplying independent rates of *LEU1* and *TRP5* conversion. This observation led them to postulate heteroduplex DNA formation over long regions in mitotic recombination. Enhanced co-conversion seems to be a distance-dependent phenomenon, in that the stimulation is 1,200-fold for *LEU1* and *TRP5* (18 cM apart), but only 200-fold for *LEU1* and *MET13* (94 cM apart). However, Roman and Fabre (117) were never able to observe co-con-

FIG. 10. Mitotic recombination at the four-strand G_2 stage. A sectored, prototrophic colony can be produced from a G_2 event only if symmetric heteroduplex DNA is formed and corrected to wild type. This type of an event results in a colony that retains both of the mutant alleles. (1) Replication of the diploid DNA, producing four chromatids. (2) Strand exchange to form heteroduplex DNA on each of the two recombining chromatids. (3) Mismatch repair of the heteroduplex DNA to wild type. (4) Resolution with a reciprocal exchange of the flanking marker A. (5) Segregation of chromatids 1 and 3 from 2 and 4 to produce a prototrophic colony, sectored for marker A. Where symbols appear both above and below a chromatid, they refer to the two strands of the chromatid.

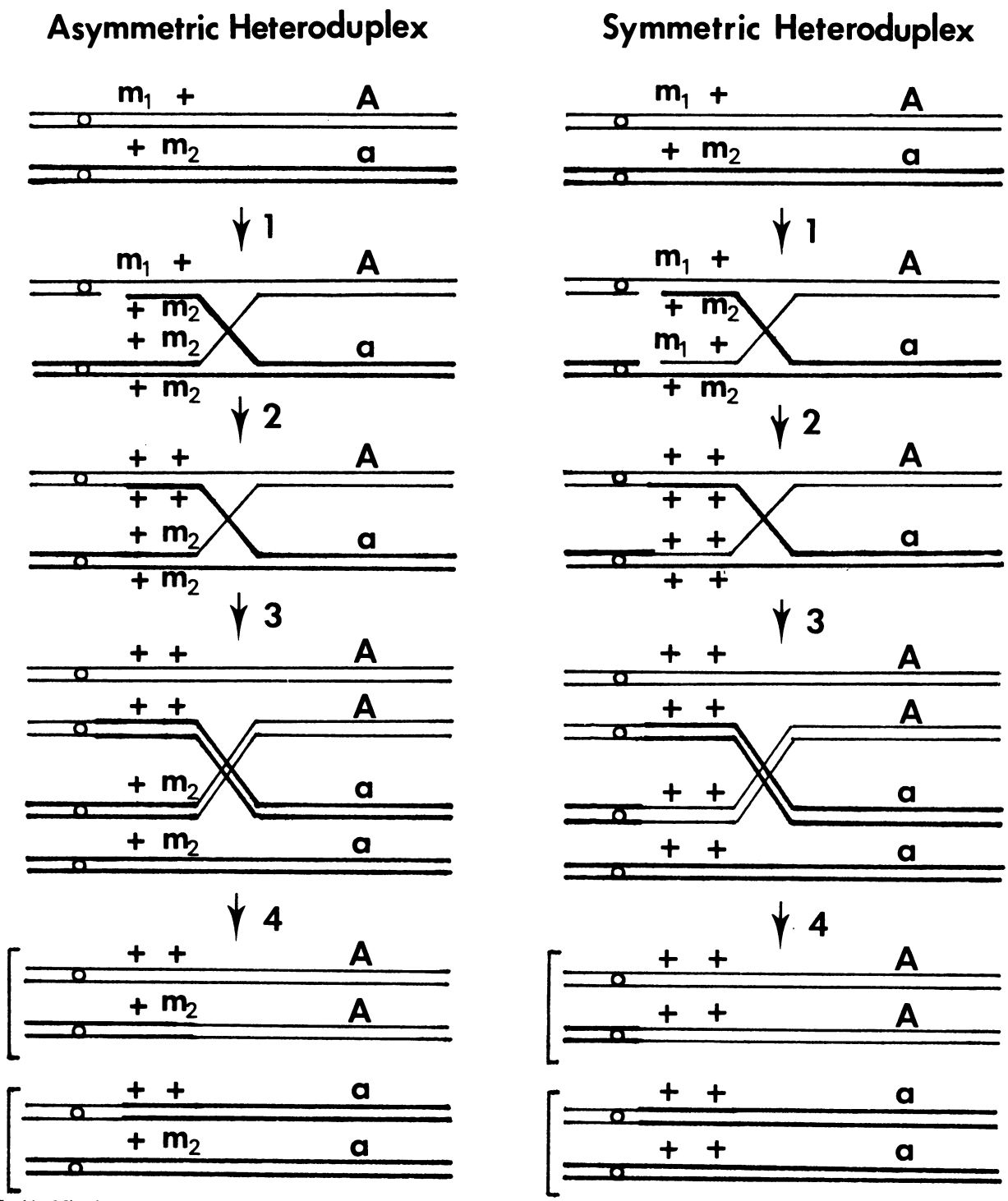


FIG. 11. Mitotic recombination at the two-strand G_1 stage. A sectored, prototrophic colony can be produced from a G_1 conversion event mediated by either asymmetric or symmetric heteroduplex DNA. Nine classes of marker segregations are possible, only two of which are shown here. A sectored, prototrophic colony containing only one of the two alleles is diagnostic of a G_1 event. (1) Heteroduplex DNA is formed by homologous strand exchange. (2) Mismatch repair of the heteroduplex DNA (in the case of symmetric heteroduplex, only one of the chromatids need be corrected to wild type to result in a sectored, prototrophic spore). (3) DNA replication through the crossover resolves the recombination event. (4) Chromatids 1 and 3 segregate from 2 and 4 to produce the sectored colony. Symbols above and below a chromatid refer to the two strands of the chromatid.

version between the *ade6* and *cly8* genes also on chromosome VII in their X-ray-induced studies. It is possible that X-ray-induced events involve shorter conversion tracts than spontaneous events.

In addition, the presence of a subpopulation of cells which are capable of high levels of recombination probably accounts for part of the enhanced co-conversion observed by Golin and Esposito. The existence of a recombination-com-

petent subpopulation has been invoked to explain the 10- to 100-fold enhancement of co-conversion observed for markers on different chromosomes (29, 86, 87).

Association of Crossing Over

Association of crossing over with mitotic gene conversion has been observed for a number of genes in *S. cerevisiae*, with frequencies of crossing over ranging from 10 to 55% (for review see reference 21), approximately the range observed for meiotic recombination. A rigorous comparison between frequencies of meiotic and mitotic associated crossing over awaits characterization of the same alleles in meiotic and mitotic cells. The degree of crossing over associated with mitotic gene conversion varies with the type of heteroduplex DNA formed. A total of 69% of gene conversions at *ade6* resulting from symmetric heteroduplex DNA are associated with crossing over, whereas only 15% of conversions likely to arise from asymmetric heteroduplex DNA are associated with crossing over (116). This observation is analogous to that obtained for meiotic events at the *b8* locus of *A. immersus* (95).

An association between mitotic gene conversion and crossing over is also supported by the observation that inducing agents stimulate both recombination events (27, 29). However, inducing agents can alter the frequencies of conversion and crossing over to differing extents, suggesting that the two types of events can be uncoupled (16, 115).

Two other types of experiments suggest that in mitotic recombination in *S. cerevisiae* conversion and crossing over are associated but separable. A total of 30% of the sectored prototrophs obtained by Roman and Fabre (117) had genotypes that only can be explained by a G_1 conversion event associated with a G_2 crossover (unless these events are actually independent events occurring in a subpopulation of cells with high recombination levels). Thus, although the two events are physically associated, they appear to be able to be temporarily dissociated with respect to the cell cycle. Since only 6 of the 92 prototrophs characterized by Esposito required a G_2 crossover explanation, X-ray induction may increase the occurrence of such events. Some recombination mutants also separate the two reactions. All types of gene conversions require the *rad52* gene product, but crossovers between homologs are decreased to a smaller extent by the *rad52* mutation (77, 104). Other reciprocal crossovers are *rad52* independent; for example, reciprocal, intrachromosomal exchange between two duplicated *his4* genes (56), sister chromatid exchange (103, 143), and circular plasmid integration (97). The *rec1* and *rec3* mutations (111) are defective in X-ray-induced intragenic gene conversion but not in X-ray-induced intergenic crossing over. The ability of mutants to separate some types of crossovers implies that in mitosis some exchanges are independent of the gene conversion mechanism. However, this independence is not complete in that other mutants depress both types of recombination (see below).

Recombination Mutants

Some mutants isolated as radiation sensitive or sporulation or cell cycle defective (or both) are mitotic hypo- or hyperrecombination mutations. Examination of the effects of recombination mutants on intragenic and intergenic recombination reveals that certain types of reciprocal crossovers can occur when gene conversion is blocked. As previously mentioned, *rad52* has been shown to be deficient in mitotic gene conversion, but the mutation appears to depress crossing over between homologs to a lesser extent (77, 104). *rec1* and *rec3* also block mitotic gene conversion (111), but only *rec3* affects sporulation. *rad51* and *mms1* depress both mitotic gene conversion and crossing over and meiotic recombination (88). *rad50* depresses meiotic recombination but elevates both mitotic gene conversion and crossing over (78). *rec4*, an *arg4*-specific recombination mutation, reduces both mitotic and meiotic heteroallelic recombination (111). *rad3*, *-6*, and *-18* are mitotic hyper-rec mutants (5, 58). *cdc9* and *-21* are also hyper-rec (37); the phenotype of these latter, hyper-rec mutations presumably results from an increase in recombinogenic lesions in the DNA. Finally, *spo11* gives higher rates of intergenic crossing over at specific intervals (8, 9).

Mitotic recombination mutants have been isolated by the use of disomic strains. A strain containing a disome of chromosome VIII heteroallelic for *arg4* was used to isolate dominant *MIC* mutants hyper-rec for gene conversion (81). Esposito and Bruschi (19) constructed a disome of chromosome VIII with markers enabling detection and identification of gene conversion, crossovers, nondisjunction, and chromosome loss. UV-induced mutants fell into five phenotypic groups: (i) depressed for both intra- and intergenic recombination, (ii) depressed for intergenic recombination only, (iii) hyper-rec for intergenic recombination, (iv) decreased for conversion only, and (v) hyper-rec for both. Again, these classes of mutants demonstrate that conversion and crossing over require some of the same functions but also are separable.

The semidominant *rem1* mutation, originally isolated as a mutator, has been shown to be hyper-rec for mitotic intra- and intergenic recombination, but to have no effect on meiotic recombination (39a). In the sectored, prototroph selection described above, $LEU1^+$ or $TRP5^+$ prototrophs occurred at frequencies 20- to 100-fold higher in a *rem1/rem1* diploid than in wild type. In addition, the minimum percentage of symmetric heteroduplex rose to 60 to 70% in the *rem1* mutant (40). Interestingly, the double mutant *rem1 rad50* or *rem1 rad52* is inviable, implying that *rem1* may generate lesions in DNA requiring the recombination repair system of *rad50 rad52* (80). *rem1 spo11* or *rem1 rad6* is viable and shows *rem1* levels of recombination (80). The double mutant *rad52 cdc9* is also inviable (87).

The yeast-repeated sequence element delta flanks the transposable element Ty, and delta-delta recombination can lead to Ty excision (112). Solo delta elements (not associated with Ty elements) also exist in many places in the yeast genome. Recombination between these delta elements can lead to a variety of genomic rearrangements including deletions and inversions (122). Rothstein (Cold Spring Harbor Symp. Quant. Biol., in press) has selected for mutants with altered delta-delta recombination. One mutation, *edr* (enhanced delta recombination), appears to be specifically hyper-rec for recombination events involving delta elements, in that it does not affect recombination between other short repeats. This mutation also stimulates meiotic gene conversion in genes known to lie close to delta elements.

Comparison of Mitotic and Meiotic Recombination

Mitotic recombination may differ mechanistically from meiotic recombination in several ways. Perhaps the strongest evidence for differences is provided by mutations showing differential effects on mitotic and meiotic recombination. *spo11* abolishes meiotic recombination, but has only small effects on mitotic recombination in specific intervals; in

contrast, *rad50* is meiotically hypo-rec and mitotically hyper-rec. The *con1*, *-2*, and *-3* mutants are meiotic specific (36), whereas the *MIC* and *rem1* genes are mitotic specific. However, the two types of recombination clearly use some of the same functions since some mutations block both.

Several specific differences between meiotic and mitotic recombination have been characterized. Spontaneous recombination levels are as much as 1,000-fold lower during mitotic growth than in meiosis; the elevated meiotic levels may be due to the generation of recombinogenic lesions in meiosis. As discussed above, some mitotic crossovers are dissociated from gene conversion genetically and temporally in the cell cycle. Meiotic and mitotic recombination occur at different times in the cell cycle: meiotic recombination occurs after DNA replication, whereas mitotic recombination can occur before DNA replication. Mitotic recombination appears to frequently have symmetric heteroduplex DNA. Polarity at the *his1* gene is abolished in mitosis (54). Similarly, the two alleles of *leu1* and *trp5* used in the sectored prototroph experiments show different gene conversion frequencies in meiosis, but equal conversion in mitosis (40). The absence of polarity in mitotic recombination could be the result of the formation of long heteroduplex regions that include both alleles. Alternatively, initiation sites for recombination used in meiosis may not be recognized in mitosis. The latter explanation is favored by the observation that the distribution of exchange events differs in mitosis and meiosis. A higher fraction of recombination events occurs in centromere-proximal intervals in mitosis than in meiosis (the distribution in mitotic *rem1* cells resembles that of meiosis) (79). The existence of a centromere effect on meiotic recombination is indicated by an experiment in which the centromere of chromosome III was moved to a different position on the chromosome. Recombination in an interval near the original position of the centromere increase by four- to fivefold, whereas recombination in an interval near the new position of the centromere decreased by four- to fivefold (B. Lamb and G. S. Roeder, personal communication).

Recombination is fundamental to the meiotic process; an attempted reductional division in the absence of recombination leads to lethality. In contrast, mitotic recombination may simply be a secondary result of DNA repair or of DNA replication past single-strand nicks. It also appears that there may be two pathways of mitotic recombination: a *RAD52*-dependent pathway responsible for gene conversion and some reciprocal crossovers (presumably those associated with gene conversion), and a *RAD52*-independent pathway for reciprocal crossing over.

SPECIFIC RECOMBINATION EVENTS

Plasmid-Chromosome Recombination

Yeast cells can be transformed with exogenous plasmid DNA (49). This allows the introduction of recombination substrates of defined structure into the cell. High-frequency transformation occurs if the plasmids contain chromosomal fragments conferring on the plasmid the ability to be maintained extrachromosomally (3, 132). Such *ARS* (autonomously replicating sequence)-containing plasmids presumably transform at high frequencies because integration of the plasmid into a homologous site in the genome, a low-frequency event (49), is not required. Plasmid integration results from a reciprocal recombination event between the homologous regions on the plasmid and chromosome. In approximately 20% of the low-frequency transformants the chromosomal information is simply replaced by homologous plasmid information without plasmid integration. These substitution transformants could arise either by gene conversion or a double-crossover event.

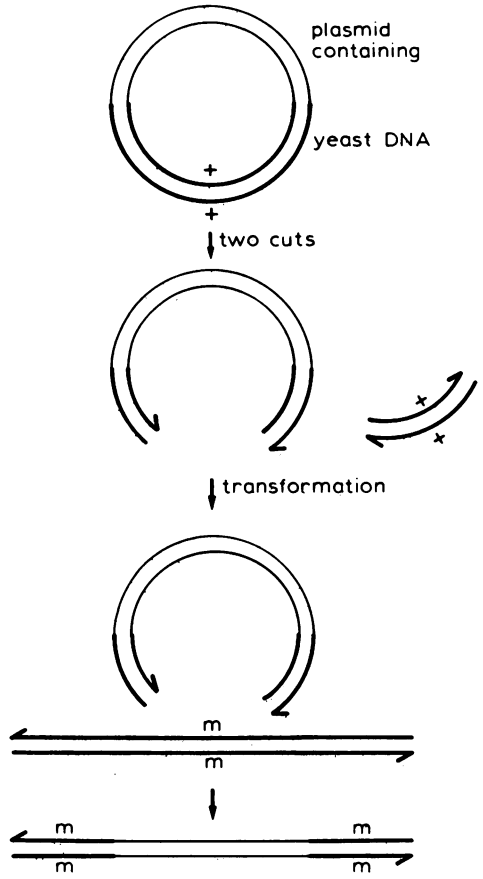


FIG. 12. Double-strand gap repair in yeasts. An *E. coli* plasmid containing a segment of yeast DNA is cut with restriction enzymes to produce a double-strand gap within the yeast DNA. The gapped plasmid is efficiently integrated into the homologous chromosomal locus, and the gap is repaired from chromosomal information during integration.

The introduction of a double-strand break into yeast sequences on plasmid DNA by restriction enzyme digestion before transformation stimulates transformation frequencies as much as 3,000-fold (97). Since the structures of the integrated plasmids, as determined by Southern blot restriction mapping, are identical when cut and circular plasmids are used, the double-strand break is repaired precisely. The observed stimulation in transformation frequency was shown to be a result of the direct interaction of the double-strand ends with homologous chromosomal sequences by a targeting experiment (97). Intact circular DNA of a plasmid containing two regions of homology with the yeast genome integrated at either of the sites of homology. When the plasmid was cut with a restriction enzyme, integration occurred only at the locus homologous to the region of the plasmid containing the double-strand break.

Surprisingly, if a double-strand gap is made by digestion of the plasmid DNA at two sites within one region of yeast DNA, the gapped molecule not only transforms at high frequency but also is repaired during integration (97). Transformation with gapped plasmids never results in the recovery of an integrated deletion corresponding to the original

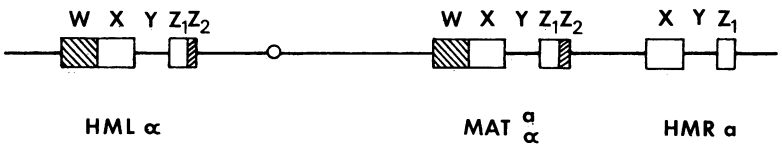


FIG. 13. Yeast mating-type loci. The expressed mating-type locus, *MAT*, comes in two forms, **a** and α, which differ in the DNA segment labeled Y. The silent mating-type loci, *HMR*, and *HML*, are homologous with *MAT* to the extents indicated.

gap. However, a deletion on circular plasmid DNA is readily integrated. The DNA information used by gap repair is from the homologous region on the chromosome, since a chromosomal mutation in the region homologous to the plasmid gap is transferred to the plasmid by gap repair (Fig. 12) (98). The fidelity of the double-strand gap repair process is demonstrated by the efficient regeneration of a functional gene from a gapped gene during plasmid integration. The repair of a double-strand gap involves the transfer of genetic information of two strands of DNA information from one DNA duplex to another and is therefore a gene conversion event.

Several aspects of gene conversion by double-strand gap repair are similar to chromosomal gene conversion. It is associated with crossing over in approximately 50% of the events. When a gap is made on a plasmid that contains an *ARS* element and transformants resulting from correct gap repair are selected, similar numbers of integrated and nonintegrated plasmids are recovered (96). Integrated plasmids arise by gap repair with crossing over, whereas nonintegrated plasmids result from gap repair without crossing over. Gap repair is associated with the formation of heteroduplex DNA adjacent to the DNA ends (Orr-Weaver and Szostak, submitted for publication). Restriction site mutations were made in vitro in the *his3* gene, and the chromosomal *HIS3* gene was replaced with one of these mutations. The *his*$^-$ strain was transformed with a linear plasmid containing another *his3* restriction site mutation; both mutations were on the same side of the double-strand break. Occasional *HIS*$^+$ transformants were obtained. Analysis of these demonstrated that they resulted either from transfer of plasmid information to the chromosome or from transfer of chromosomal information to the plasmid (Orr-Weaver and Szostak, manuscript in preparation). These conversion events occurring adjacent to the region of double-strand break repair appear to result from the formation and repair of heteroduplex DNA flanking the break. Finally, the processes of double-strand break and gap repair require the product of the *rad52* gene (97). The integration of linear or gapped, but not circular, plasmids is blocked by the *rad52* mutation.

The demonstration that gene conversion in mitotic cells can occur by double-strand gap repair, by the coordinated transfer of two strands of information from one DNA helix to another, suggests that chromosomal gene conversion need not always occur through a heteroduplex DNA intermediate. This point is discussed at greater length in the section on models.

Mating-Type Switching

Haploid strains of *S. cerevisiae* exist in one of two mating types, **a** or α. Homothallic strains (*HO*) are capable of switching mating type, whereas heterothallic (*ho*) strains are incapable of such switching (for reviews see references 48, 92). The expressed mating-type information is present at the *MAT* locus on chromosome III, and two "silent" loci on chromosome III, *HML* and *HMR*, contain unexpressed information. During switching, the information from one of these silent cassettes is transferred to the *MAT* locus in what can be viewed as a gene conversion event. This gene conversion event differs from other conversion events in several aspects: (i) it occurs at high frequency (as often as once per cell cycle); (ii) it is a unidirectional event in that information is transferred from the silent cassettes to *MAT* and only rarely in the opposite direction; and (iii) normal switching is not associated with reciprocal crossovers which would generate either a large deletion or a circular chromosome. Mating-type switching requires the *RAD52* gene product; switching in *rad52* cells is lethal (77, 139).

The physical structures of *MAT*, *HML*, and *HMR* have been determined. *HMR* and *MAT* share two regions of homology (X and Z) that flank a central region (Y) containing the mating-type-specific information (Fig. 13). *HML* has these two regions but contains on each side an additional region homologous to *MAT*. *MAT***a** cells have a Y-**a** fragment which is replaced by a larger Y-α fragment in *MAT*α cells. The Y–Z boundary has been demonstrated to be important in the switching process by the observations that mutations blocking switching (*mat-inc*) map to this region (140), deletions of this region on plasmids block switching of *MAT* on the plasmid (131), and populations of actively switching cells contain a double-strand break in this region (131).

The double-strand break appears to initiate the switching event in that plasmids cut near the Y–Z border with a restriction enzyme are able to be recipients of mating-type information even in *ho* strains (J. Strathern, personal communication). Thus mating-type switching appears to be similar to the gapped plasmid repair described above, except that it occurs without associated crossing over. Recently an endonuclease has been isolated from *HO* cells that makes a specific double-strand cleavage near the Y–Z border of *MAT* (68). The efficiency and directionality of switching are therefore readily explained by *HO* control; the switch frequency reflects the efficiency of cutting at *MAT*, and the break forces *MAT* to be the recipient of information from the silent loci. Both *HO* mRNA and the endonuclease activity appear transiently in the G_1 phase of the cell cycle of mother cells, so that the timing of switching is most likely regulated by the synthesis of the endonuclease (93). The switch event could arise by formation of a double-strand gap at *MAT* and gap repair from silent cassette information. Alternatively, heteroduplex DNA formed in the Y region could be repaired in a directional manner, such that the information at *MAT* is always lost.

Mating-type switching in *S. pombe* occurs by the transfer of information from one of the two silent cassettes into the expressed *mat1* locus (2). A double-strand break at *mat1* at the site of a previously defined switch mutation, *smt*, initiates the recombination event. In contrast to *S. cerevisiae*, this break is stable throughout the cell cycle and may even be inherited (2).

2μm Recombination

The 2μm circle is an endogenous plasmid present in most strains of *S. cerevisiae* at 60 to 100 copies per cell (for review

see reference 6). The plasmid contains two repeats of 599 base pairs (bp) in inverted orientation, separating two unique regions. Recombination between the inverted repeats occurs at high frequency, such that the unique regions become inverted with respect to each other. Thus the 2μm circle exists in two forms. This site-specific interconversion is catalyzed by a 2μm-encoded protein, the *FLP* gene product. The region within the inverted repeats necessary for the interconversion reaction has been determined by analyzing the effect of Tn*5* insertions at various sites in the inverted repeat. These studies demonstrated that the crossovers occurred within a 120-bp sequence of the inverted repeat. Deletion studies further delimited the necessary sequence to a 65-bp region; moreover, a 4-bp deletion in the middle of this region abolishes *FLP*-mediated interconversion (7). The 65-bp recombination site is a region of dyad symmetry composed of four repeats of 16 bp in alternating orientation. The 16-bp sequence is implicated in interconversion by its presence in the inverted repeats of Tn*5*, also a substrate for *FLP* recombination (7).

FLP catalyzes recombination both inter- and intramolecularly and also on a substrate in which the recombination sites are in direct orientation (15). When expressed in *Escherichia coli*, *FLP* has been shown to catalyze recombination between the 2μm inverted repeats present on plasmids inside the bacterial cell (15). Thus *FLP* is the only yeast protein required for this site-specific recombination event, it is able to act in *trans*, and it does not require native chromatin configuration.

Further analysis of the mechanism of *FLP*-mediated recombination will be facilitated by the recent development of an *in vitro FLP* recombination system. Extracts of cells overexpressing *FLP* catalyze recombination between both direct and inverted copies of the inverted repeat sequence. The enzyme is active on circular substrates relaxed by topoisomerase I and on linear plasmids; supercoiling or circular DNA constraints are therefore unimportant (138).

Recombination Between Repeated Genes or Elements

The genes encoding rRNA in *S. cerevisiae* are present as 140 tandem repeats of a 9-kilobase unit. Meiotic crossing over within the *rDNA* repeat cluster is lower by a factor of 100 to 300 than in normal chromosomal DNA. The genetic length of the entire cluster (about 1,300 kilobases) is only 1 to 2 cM. However, by integrating a plasmid containing a selectable marker into the repeat, it has been shown that unequal crossing over occurs in the *rDNA* locus in both mitotic (136) and meiotic (101) cells. Recently it has been demonstrated that a DNA fragment containing half of the *rDNA* repeat is able to stimulate mitotic recombination between *his4* heteroalleles 10-fold when inserted at the chromosomal *his4* locus. This stimulation is observed only if the *rDNA* insert is homozygous and only when it is in one orientation. The *rDNA* fragment increases mitotic but not meiotic recombination (57a). These results present the intriguing possibility that the *rDNA* repeat contains a recombination hotspot evolved to maintain homogeneity of the gene family.

Sequences located on different chromosomes are able to recombine in *S. cerevisiae*. The Ty elements are repeated transposable elements. Homologous recombination occurs between elements in different genomic locations. Gene conversion results in the transfer of information between elements, and crossing over results in translocations, inversions, deletions, and the excision and reintegration of one Ty element into another (for review, see reference 113). Several investigators have inserted a second copy of a gene on another chromosome. Recombination between the two copies occurs, and gene conversions, translocations, and complex recombination events are observed (85, 102, 133).

ENZYMOLOGY OF RECOMBINATION

Little is known of the enzymology of recombination in the lower fungi. The best-characterized enzyme known to be involved in recombination is the *rec1* gene product of *Ustilago maydis*. Its activities are similar to those of the *recA* protein of *E. coli* (65). The *rec1* protein is an ATPase that catalyzes homologous strand transfer. Although the presence of ATP is required for synapsis, its hydrolysis is needed only for strand transfer (66). The *rec1* protein, like the *recA* protein, shows a polarity in the direction of strand transfer. The *rec1* protein catalyzes a reaction in which a single-strand circle pairs with the 5′ end of a linear duplex, and strand transfer displaces the 3′ end of the duplex. Thus *rec1*-driven strand transfer has a directionality opposite to that catalyzed by the *recA* protein (66). In a strand transfer reaction between a circular single-stranded molecule and a linear duplex whose ends are not homologous to the circle, the molecules pair but the strands cannot become interwound. The formation of the resulting paranemic joints appears to be coupled to the formation of left-handed Z DNA (67). The *rec1* activity is absent in *rec1 U. maydis* strains; *rec1* is a mutation that blocks both induced DNA repair and recombination (53).

rad52 strains of *S. cerevisiae* are deficient in an endo-exonuclease, present in wild-type cells, that shows cross-reactivity with the *N. crassa* single-strand DNA endo-exonuclease (14). The *S. cerevisiae* nuclease is induced during meiosis, with maximum activity appearing at the time of commitment to recombination. The protein has been purified, and based on the size of the polypeptide chain it appears to be too large to be encoded by the *RAD52* gene itself (Resnick et al., in press). Thus this activity may be encoded by a gene under the regulatory control of *RAD52*.

An in vitro recombination system from mitotic *S. cerevisiae* cells has been recently developed (134). These cell-free extracts catalyze both gene conversion and reciprocal recombination between circular or circular and linear substrates. The latter reaction is depressed fivefold in extracts prepared from *rad52* cells. This in vitro system should permit the purification of recombination proteins by complementation assays, using mutant strains.

RECOMBINATION MODELS

In this section we discuss the Holliday and Meselson-Radding models for recombination. We also describe the double-strand break repair model, a new model that was formulated from the demonstration of gene conversion by double-strand gap repair of plasmids in mitotic yeast cells. We compare the ability of the models to account for the properties of mitotic and meiotic recombination.

The Holliday Model

The Holliday model represents a crucial step in our understanding of recombination because it introduced the idea that heteroduplex DNA might be generated during the breakage and rejoining of chromatids (50). Holliday proposed that two homologous chromatids are nicked at the same site on strands with the same polarity. The strands of each chromatid are separated over some variable distance

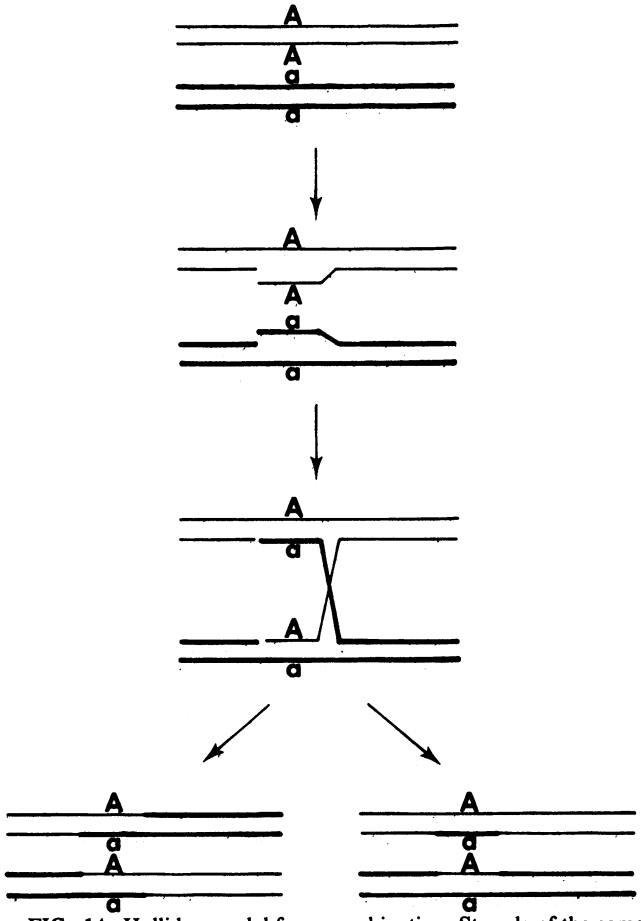


FIG. 14. Holliday model for recombination. Strands of the same polarity are nicked at homologous sites and are then exchanged to produce symmetric heteroduplex DNA. The crossed strand, or Holliday junction, can be resolved either with or without exchange of flanking markers. If the symmetric heteroduplex produced by strand exchange is not repaired, an aberrant 4A;4a segregation results.

and then pair with strands from the other chromatid, forming heteroduplex DNA (Fig. 14). If the symmetric heteroduplex DNA formed by the strand exchange is unrepaired, aberrant 4:4 segregation results. Repair of the mismatch on one chromatid produces 5:3 segregation, whereas repair of the heteroduplex on both chromatids in the same direction (e.g., mutant to wild type) gives 6:2 segregation. Repair of both chromatids in the opposite direction results in a normal 4:4 event. Gene conversion in the Holliday model arises by repair of heteroduplex DNA.

The second conceptual advance of the Holliday model was that it defined a mechanism for the association of crossovers with aberrant segregation events. After the pairing of strands of the two chromatids, a cross-strand, or Holliday junction, is formed. This junction can be resolved by cutting and resealing the crossed strands to give aberrant segregation without an associated crossover. Cleavage and religation of the outer strands cause a reciprocal exchange of flanking markers.

Analysis of Holliday junctions, using space-filling models, showed that such junctions can be formed with all bases of both duplexes remaining paired (126). The crossed and uncrossed strands of the junction are equivalent, being interconvertable by isomerization without bond breakage (Fig. 15). Therefore, cleavage of the Holliday junctions would be predicted to yield crossover and non-crossover products at equal frequencies. It was recognized that the cross connection could diffuse along the helices, with bases from one chromatid being exchanged to the other (126). Rotary diffusion could drive the branch migration process at rates sufficient to generate long tracts of heteroduplex DNA (83).

The genetic demonstration of asymmetric heteroduplex DNA demanded an extension of the Holliday model. The Meselson-Radding, or Aviemore, model provided such a mechanism.

The Meselson-Radding Model

The Meselson-Radding model (84) differs from the earlier Holliday model in that strand exchange is initiated on only one chromatid, and strand transfer is driven by DNA synthesis. In this model asymmetric heteroduplex DNA, symmetrical heteroduplex, and crossing over all follow from one initiation event. Recombination is initiated by a nick on one chromatid (Fig. 16). The 3′ end of the nick serves as a primer for DNA synthesis, displacing a single strand which then invades the other chromatid. The resulting D-loop is degraded and the invading strand is ligated in place. Asymmetric heteroduplex DNA can then be expanded by concerted DNA synthesis on the donor duplex and the exonucleolytic degradation of the recipient duplex. After the enzymatically driven production of asymmetric heteroduplex DNA stops, either branch migration or isomerization can bring the 5′ and 3′ single-stranded ends into apposition so they can be

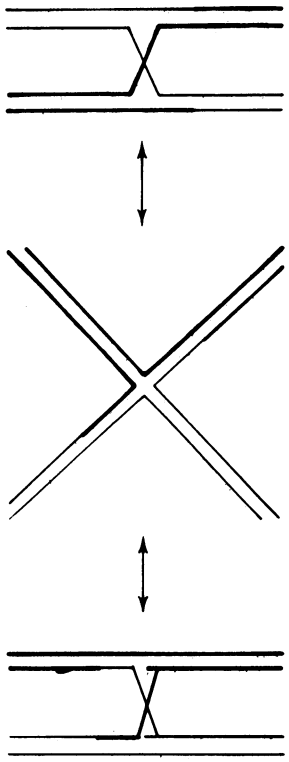


FIG. 15. Isomerization of a Holliday junction. The Holliday junction can isomerize through a symmetrical intermediate without bond breakage. Therefore, resolution can occur by cutting either the originally crossed strands or the noncrossed strands.

ligated. The resulting Holliday junction can migrate by branch migration to form symmetrical heteroduplex. Resolution can yield either the crossover or the non-crossover configuration. The Meselson-Radding model has two phases, an enzymatically driven asymmetric phase and a symmetric phase generated by branch migration. As in the Holliday model, gene conversion results from mismatch repair.

The Double-Strand Break Repair Model

The double-strand break repair model differs from the Meselson-Radding model in four ways: (i) the recombination event is initiated by a double-strand break; (ii) the initiation chromatid is the recipient rather than the donor of information; (iii) gene conversion can result from transfer of two strands of information to a gapped chromatid without a heteroduplex intermediate or from mismatch repair in heteroduplex DNA adjacent to the region of gap repair; and (iv) the recombination mechanism produces a region of gene conversion flanked by two Holliday junctions (135).

The simplest version of the model is shown in Fig. 17. Recombination is initiated by a double-strand break. This is enlarged by exonucleases to a gap with 3′ single-stranded ends. One of the free 3′ ends then invades a homologous region on the intact chromatid, displacing a small D-loop. This D-loop is enlarged by repair synthesis primed by the invading 3′ end. After continued repair synthesis the DNA homologous to the other 3′ end will become single-stranded. This 3′ end then pairs and serves as a primer for repair synthesis on the other strand. Thus the gap is repaired by two rounds of repair synthesis. There are two regions of asymmetric heteroduplex DNA formed by the pairing of the 3′ ends of the gap, and two cross strand junctions flank the gap repair region. The left-hand junction of Fig. 17 is identical to the junction in the Meselson-Radding model, so asymmetric heteroduplex on this side may be extended by concerted repair synthesis and exonucleolytic degradation. Branch migration of the two Holliday junctions will form symmetric heteroduplex.

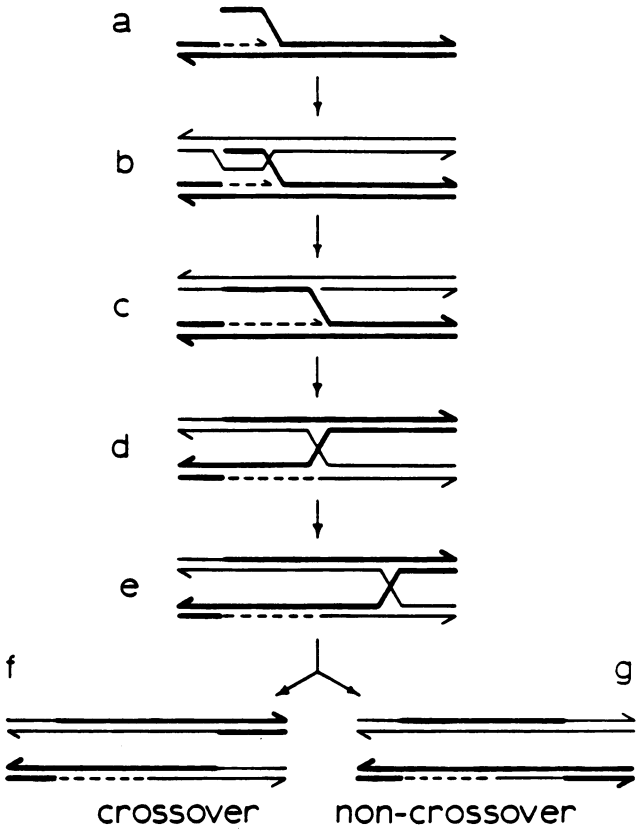


FIG. 16. Meselson-Radding model. (a) Recombination is initiated by a single-strand nick which serves as a primer for DNA repair synthesis. This displaces a single strand that can then pair with a homologous region on the other chromatid (b). The resulting D-loop is degraded, and the asymmetric heteroduplex DNA is enlarged by DNA synthesis on the donor chromatid coupled with degradation on the recipient duplex (c). Branch migration and ligation of the nicks produces a Holliday junction which can be isomerized (d). Symmetric heteroduplex DNA can be formed by branch migration of the Holliday junction (e). Resolution can yield either the crossover (f) or the non-crossover (g) configuration.

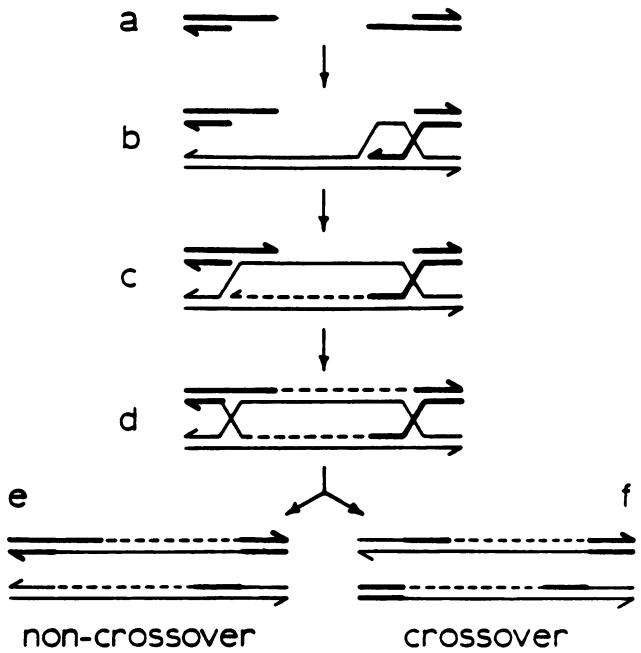


FIG. 17. Double-strand break repair model. (a) A double-strand cut is made in one duplex, and a gap flanked by 3′ single strands is formed by the action of exonucleases. (b) One 3′ end invades a homologous duplex, displacing a D-loop. (c) The D-loop is enlarged by repair synthesis until the other 3′ end can anneal to complementary single-stranded sequences. (d) Repair synthesis from the second 3′ end completes the process of gap repair, and branch migration results in the formation of two Holliday junctions. Resolution of the two junctions by cutting either inner or outer strands leads to two possible non-crossover (e) and two possible crossover (f) configurations. In the illustrated resolutions, the right-hand junction was resolved by cutting the inner, crossed strands.

Gene conversion can arise if a marker falls within a double-strand gap; in this case the marker is converted by a double-strand transfer of information. Alternatively, if the marked site falls within either of the flanking regions of asymmetric heteroduplex, mismatch correction will produce a 6:2 segregation. Heteroduplex DNA in which a mismatch is not repaired yields a postmeiotic segregation.

Gene conversion by double-strand gap repair results in an associated crossover when the two Holliday junctions are resolved in the opposite sense (i.e., cutting the inner strands of one junction and the outer strands of the other). Resolution in the same sense gives the non-crossover configuration of flanking markers.

Comparison of Meselson-Radding and Double-Strand Break Repair Models

Both models account for the aberrant segregation patterns observed in the fungi. The absence of symmetric heteroduplex in *S. cerevisiae* can be explained in both models by assuming that branch migration of the Holliday junction(s) does not occur in yeast. The *b2* locus of *A. immersus* clearly follows all of the predictions of the Meselson-Radding model. However, if in *A. immersus* the double-strand gap is always small (for example, if the activities of exonucleases are low or if strand invasion and annealing occur rapidly), an event initiated by a double-strand break would be genetically indistinguishable from a Meselson-Radding type of event.

Co-conversion events result either from both alleles being included in a double-strand gap and thus corepaired or from a mismatch repair event in which the excision tract covers both mutations. The latter mechanism offers a simpler explanation for the effect of one mutation on the segregation pattern of another. An allele showing 6:2 segregation is recognized efficiently by the mismatch repair machinery and can therefore trigger an excision event extending across an adjacent allele that normally shows 5:3 segregation. The result would be an increase in 6:2 segregations for the second allele. If the mutations are in heteroduplex DNA adjacent to a region of gap repair, then an allele giving 6:2 segregation could also affect the segregation of an allele normally showing 5:3 segregation by altering the probability of mismatch repair. However, it has been observed that alleles showing 6:2 segregation and located on either the high or the low conversion side of the normally 5:3 *arg4-16* allele reduce the level of postmeiotic segregation for *arg4-16* (35). If recombination is initiated at *arg4* by an enzyme that recognizes a site and then moves and initiates recombination at various distances from that site (see below), the gap could be on either the high or the low conversion side of *arg4-16*. Thus an allele on either side could be included in heteroduplex DNA along with *arg4-16*.

If conversion events in both *A. immersus* and *S. cerevisiae* occur as in the Meselson-Radding model, then the mismatch repair systems must be quite different. Base substitution mutations show mainly postmeiotic segregation in *A. immersus*, but such segregation is rare in yeasts. Also, all types of mutations show approximate parity in yeasts, in contrast to the extreme disparity observed for small insertions and deletions in *Ascobolus* sp. If most gene conversion in *S. cerevisiae* occurs by double-strand gap repair, then postmeiotic segregation would be rare and parity in conversion would simply reflect an equal probability of initiation on either chromatid. The restoration-conversion experiment of Hastings (46, 125) presents the problematic result that if conversion in yeasts occurs via heteroduplex DNA then the correction system has to recognize and repair in favor of the invading strand. The problem is avoided if gene conversion occurs by gap repair, and the problem does not arise in *A. immersus*, which shows no bias in restoration versus conversion. Hastings (Cold Spring Harbor Symp. Quant. Biol., in press) has recently proposed that mismatch repair in yeasts occurs via double-strand gap repair. In this model, mismatches are recognized by an enzyme that cleaves the DNA; the resulting break is enlarged to a gap, which is then repaired from the intact chromatid. The net result is that the genotype of the invading strand is the template for repair.

In both *A. immersus*, and *S. cerevisiae* large deletions show parity of conversion. Radding (105) has proposed a model for the conversion of deletions and insertions by a mechanism that involves the formation of heteroduplex DNA that includes a large heterology. If repair synthesis of the single-stranded loop always occurs, resolution of the resulting Holliday junction would lead, with equal probability, to conversion to wild type or mutant. Parity of deletion conversion can also be explained by the double-strand break repair model. The generation on the deletion chromatid of a gap that covers the position of the deletion will lead to conversion to the wild type by repair, since the wild type is the only template available. Formation of a gap on the wild-type chromatid that overlaps the site of the deletion must lead to conversion to the deletion. This is because the wild-type gapped chromosome cannot interact with the other chromatid until the wild-type sequences have been removed by continued exonucleolytic enlargement of the gap, so that sequences with homology to the other chromatid are exposed.

Although the frequency of associated crossovers can be explained by either model, the position of associated crossovers is more simply explained by the double-strand break repair model. Crossovers in polar genes are observed to occur at either the high or the low conversion side of an allele showing postmeiotic segregation. Since the two Holliday junctions formed by gap repair flank the site of segregation, the crossover may appear genetically to lie on either side of the site showing aberrant segregation. In contrast, the Meselson-Radding model predicts that crossovers will occur on the low conversion side of the gene. The results can be explained within the context of this model by assuming that the Holliday junction branch migrates back towards the initiation site. However, in yeasts this explanation demands the constraint that Holliday junctions can branch migrate in only one direction. Branch migration away from the initiation site cannot occur since this would generate symmetric heteroduplex DNA. Alternatively, initiation of recombination could occur by an enzyme which binds a specific site and then moves along the DNA for a variable distance before nicking and initiating strand transfer (13). If asymmetric heteroduplex DNA could form to either side of the nick, the crossover could appear to lie on either side of a particular stretch of heteroduplex DNA.

In as many as 30% of the associated crossover events, the crossover is separated from a converted allele by an allele that shows normal 4:4 segregation. Holliday (52) proposed that such events could be accounted for by the presence of two Holliday junctions. It is assumed that the converted allele lies in between the two junctions (as in the double-strand break repair model) and the unconverted allele is outside of the junctions. Branch migration of the two junctions in the same direction until both have crossed over the second allele (the allele giving normal 4:4 segregation), followed by resolution of the junctions, will produce the observed genotype. To explain this class of crossovers in terms of the Meselson-Radding model, an independent, although associated, second strand transfer must be initiated on the other side of the second allele and be resolved with a crossover (141). It is not clear how such multiple, independent events could occur.

Both models explain chiasma interference as a result of steric hindrance against the isomerization of two closely spaced Holliday junctions.

The version of the double-strand break repair model presented in Fig. 16 does not explain the low frequency of aberrant 5:3 segregation observed in some of the lower fungi. If asymmetric heteroduplex DNA is present on both chromatids, in the absence of mismatch repair an aberrant

5:3 segregation may occur. To exclude this possibility two constraints must be imposed on the model: (i) the asymmetric heteroduplex must be present on only one chromatid; and (ii) the resolution of the Holliday junctions must be restricted. The asymmetric heteroduplex can be limited to one chromatid if, after formation of the initial heteroduplex DNA and the initiation of repair synthesis, branch migration occurs (Fig. 18). Alternatively, if the heteroduplex formed by the initial strand invasion is quite small, while the heteroduplex at the other side of the gap is extended enzymatically, essentially all observed postmeiotic segregations would be derived from this longer region of heteroduplex DNA (Fig. 18). Another possible explanation is that the initiation of recombination could occur by the formation of a single-strand gap which is then processed into a double-strand gap with overhanging 5′ and 3′ ends. Both 5′ and 3′ ends would invade the homologous duplex, forming two regions of heteroduplex on the same chromatid (Fig. 19).

In the models presented in Figure 18 all heteroduplex DNA is present on the recipient chromatid. At least one Holliday junction must be resolved by cutting the crossed strands to avoid producing an aberrant 5:3 segregation event. If two closely spaced Holliday junctions constrain each other and prevent isomerization, the first junction may be resolved by an enzyme that cleaves only crossed strands. The second junction would then be free to isomerize and could be resolved in either plane. In the alternative model shown in Fig. 19 one of the junctions would have to be resolved by cleavage of the outer strands.

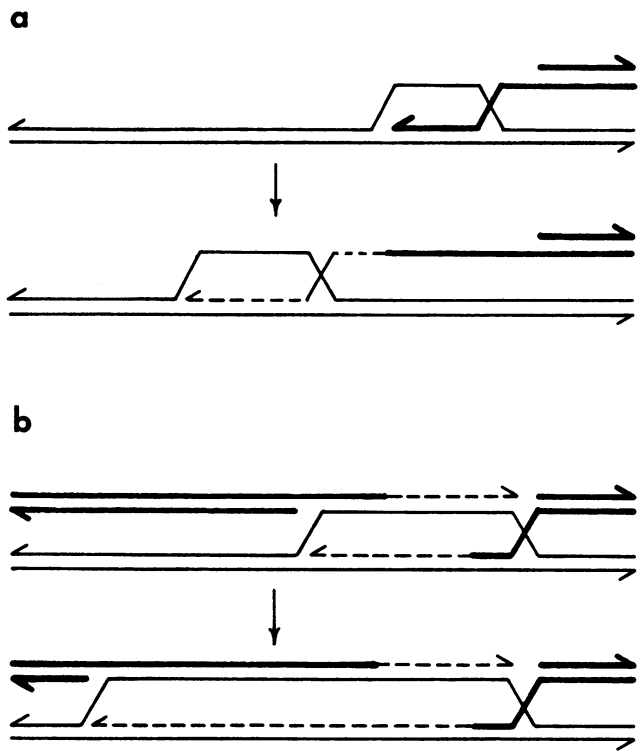


FIG. 18. Formation of asymmetric heteroduplex DNA on only one chromatid. Initiation as shown in Fig. 17 creates heteroduplex DNA on the right-hand side of the gap by strand invasion and on the left-hand side of the gap by strand annealing. To avoid the formation of aberrant 5:3 asci, all heteroduplex DNA must be confined to one chromatid, and one Holliday junction must be resolved in a defined way. The right-hand heteroduplex DNA may be removed by single-stranded branch migration as the first round of repair synthesis is in progress (a). Alternatively, the right-hand region of heteroduplex may be quite short, and the left-hand region may be extended enzymatically, so that essentially all observed postmeiotic segregation derives from the left-hand heteroduplex DNA (b).

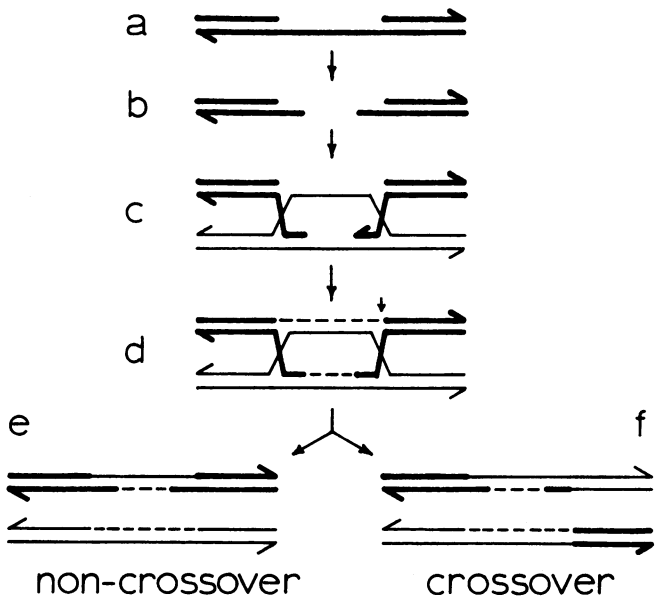


FIG. 19. Initiation from overhanging 5′ and 3′ ends. Initiation occurs by the formation of a single-strand gap (a), which is then cleaved by nucleases to produce overhanging 5′ and 3′ ends (b). These invade the homologous duplex (c), and the double-strand gap is repaired by two rounds of single-stranded repair synthesis (d). This initiation model confines all heteroduplex DNA to the donor chromatid; to avoid the generation of aberrant 5:3 asci, one Holliday junction must be resolved by cutting the outer strands. After the completion of the second round of repair synthesis, one of the outer strands of the right-hand Holliday junction is already nicked. This nick could bias the resolution of the Holliday junction towards cutting of the outer strands. Resolution of the left-hand junction can then result in a non-crossover (e) or crossover (f) configuration.

In the three characterized recombination initiation sites, *M26*, *YS17*, and *cog*$^+$, the initiating chromatid is the recipient of genetic information. Both *M26* and *YS17* show strong disparity in conversion to wild type, and the *cog*$^+$ chromosome is usually the recipient. This behavior is predicted by the double-strand break repair model but contradicts the Meselson-Radding model. Radding (106) has proposed a modification of the model in which nicking is followed by degradation to form a single-strand gap. Strand invasion then leads to the formation of asymmetric heteroduplex DNA on the initiating chromatid. Another proposal is that an initiation enzyme recognizes the initiation site and introduces a nick not at that site, but at the homologous site on the other chromatid (82). Both of these explanations postulate the formation of heteroduplex DNA at the initiation site. The observation that *YS17* shows postmeiotic segregation in *rec1*$^-$ strains, but only rarely gives such segregation when acting as an initiation site, strongly suggests the absence of heteroduplex DNA at the *YS17* initiation site. This result is more consistent with the generation of a double-strand gap at *YS17*.

Polarity can be explained in two ways by both the double-strand break repair model and the Meselson-Radding model. If initiation occurs at fixed sites, then polarity reflects the variable size of the regions of gap repair and het-

eroduplex DNA. Thus a site at an increasing distance from the initiation site has a decreasing probability of falling within a gap or within heteroduplex DNA. If initiation is a two-step process, in which an enzyme binds at a specific site, but moves along the DNA before cutting and initiating recombination, then polarity reflects a decreasing probability of initiation with increasing distance from the central binding site. The *M26* and *YS17* initiators appear to act as fixed initiation sites since they are converted at high frequencies and because adjacent mutations on either side of *M26* are co-converted with *M26*. In contrast, cog^+ acts as a recognition site, and actual information transfer occurs at a distance from cog^+. cog^+ is able to stimulate information transfer across a translocation break point. Therefore, it seems likely that at least in different fungi both types of recombination initiation mechanisms are used.

Recent electron microscopic observations on DNA from meiotic yeast cells are consistent with the appearance of Holliday junctions in pairs. Bell and Byers (4) isolated DNA from meiotic cells at the time of recombination, cross-linked the strands to prevent branch migration, cleaved the DNA with a restriction enzyme, and examined it in the electron microscope. Most chi forms (molecules joined at homologous sites) were joined not by a single Holliday junction, but by a fused region or by two junctions separated by 100 to 1,000 bp. These molecules, if indeed they represent true recombination intermediates, are clearly unexpected by the Meselson-Radding model, but are explained simply by the double-strand break repair model.

Three mutations that cause an elevation in postmeiotic segregations have been isolated in *S. cerevisiae* (33; M. Williamson and S. Fogel, personal communication). These *cor* mutants are postulated to be defective in mismatch repair. Their phenotype of increasing 5:3, while decreasing 6:2 segregation for a given allele, can be explained within the context of the double-strand break repair model by assuming that they affect the activity of the exonucleases that create the double-strand gaps. For example, a defective single-strand-specific exonuclease could increase the size of the single-stranded tails at the expense of the double-strand gap region. This explanation, however, does not readily account for the mitotic mutator phenotype of the *cor* mutants, unless the same exonucleases are involved in mitotic mismatch repair.

Resnick has described the accumulation of single-strand interruptions in *rad52* cells of *S. cerevisiae* in meiosis (108). The significance of these breaks to recombination is not yet clear. A search for the appearance of site-specific single-strand or double-strand breaks during the period of commitment to meiotic recombination may provide direct information about the mechanism of initiation.

Recent evidence indicates that gene conversion by double-strand break repair can occur on mitotic chromosomes. Resnick and Haber (43; M. Resnick and J. Haber, personal communication) X-irradiated a haploid *lys2* strain of *S. cerevisiae*, mated it to a haploid strain containing a different *lys2* allele, and selected LYS^+ diploids. They found that it was almost always the irradiated *lys2* allele that was lost in the diploid; i.e., the chromatid on which initiation took place was the recipient of genetic information. Furthermore, at least 15% of the conversion events were associated with reciprocal crossovers. Mitotic recombination appears to occur by two pathways, a *RAD52*-dependent pathway for gene conversion and a *RAD52*-independent pathway for reciprocal crossing over. The *RAD52*-dependent pathway may involve the repair of double-strand breaks. *RAD52*-independent reciprocal crossovers could be initiated by single-strand nicks or, alternatively, might be the result of topoisomerase action.

Mating-type switching is initiated by a double-strand break (131). A simple double-strand break repair model for mating-type switching is that the initial double-strand break is enlarged to a gap by degradation of the old *MAT* information and that the gap is subsequently repaired, using one of the silent cassettes as a template. The low frequency of crossing over associated with mating-type switching must mean that switching is resolved differently than other double-strand break repair events. If two Holliday junctions are formed, as in our model, crossing over would be suppressed if both junctions were always resolved in the same plane. This could be achieved if the resolution enzyme cut, for example, only the crossed strands and if isomerization were suppressed. Alternatively, the double Holliday structure could be resolved by replication without crossing over.

CONCLUDING REMARKS

Despite the extensive genetic characterization of many kinds of recombination events, and the even more extensive speculation on potential mechanisms of recombination, few specific functions have been ascribed to recombination. The most likely explanation for the existence of mitotic recombination is that it is simply a consequence of DNA repair activities. Mismatches introduced by DNA replication errors, double-strand breaks, pyrimidine dimers, and other lesions in DNA are all removed or repaired, and many of the repair pathways are known to result in recombination. It appears that the double-strand break pathway has been evolutionarily adapted and modified to mediate mating-type switching in yeasts. It is interesting to note that this high-frequency mitotic recombination event has in common with meiotic recombination a role in the sexual cycle: it is essential for the efficient generation of diploids in the homothallic yeasts.

What, however, is the function of meiotic recombination? The question of the role of recombination in increasing genetic diversity and bringing together new combinations of alleles has bedeviled generations of population geneticists and evolutionary thinkers, and we will not add to the confusion here. A second, unrelated role for recombination in meiosis is that it is necessary for proper chromosome disjunction in the reductional division. This conclusion is strongly supported by the genetic evidence in yeast cells and *Drosophila melanogaster* and by cytological evidence in many organisms. Chiasmata, the result of crossing over, hold homologs together while they become oriented on the meiotic spindle. What are chiasmata, and how are they generated? Murray and Szostak (unpublished data) have speculated that one consequence of recombination mediated by the double-strand break repair pathway (but not by the Meselson-Radding pathway) is topological linkage of the recombining molecules. This linkage results from DNA repair synthesis occurring over the same region on the two recombining chromatids. Chiasmata, in this model, are localized regions of catenation between homologous chromosomes. Chiasmata could be released either by topoisomerase activity or by diffusion of the catenation off the ends of the chromosomes (terminalization). Whatever the actual mechanism by which recombination generates chiasmata, and thereby facilitates correct chromosome disjunction, the possibility exists that recombination plays what is essentially a structural role in meiosis and that its genetic consequences are in fact secondary to this role.

The mechanism of the initiation of meiotic recombination is certainly one of the most interesting unanswered questions in the field. Unfortunately, no simple genetic test has yet been devised that is capable of distinguishing between initiation by single versus double-strand breaks. A closely related, and perhaps more tractable, question is whether initiation involves the introduction of lesions into DNA at specific sites, or is a two-step process involving the recognition of a site and the subsequent introduction of lesions at some distance from the site. Several laboratories are now in the process of using recombinant DNA technology in an attempt to identify and characterize recombination initiation sites from *S. cerevisiae*. The identification of such sites will facilitate the analysis of the initiation process by allowing the genetic identification of proteins that act at the initiation site and by serving as substrates for the biochemical analysis of initiation.

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