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Review

Sex and the single cell: Meiosis in yeast

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ABSTRACT Recent studies of *Saccharomyces cerevisiae* have significantly advanced our understanding of the molecular mechanisms of meiotic chromosome behavior. Structural components of the synaptonemal complex have been identified and studies of mutants defective in synapsis have provided insight into the role of the synaptonemal complex in homolog pairing, genetic recombination, crossover interference, and meiotic chromosome segregation. There is compelling evidence that most or all meiotic recombination events initiate with double-strand breaks. Several intermediates in the double-strand break repair pathway have been characterized and mutants blocked at different steps in the pathway have been identified. With the application of genetic, molecular, cytological, and biochemical methods in a single organism, we can expect an increasingly comprehensive and unified view of the meiotic process.

The budding yeast *Saccharomyces cerevisiae* is an ideal organism for studies of meiosis for a multiplicity of reasons. When diploid yeast cells are starved for carbon and nitrogen, almost all cells in the population enter meiosis, and they proceed through the meiotic divisions in a fairly synchronous manner. This efficiency and synchrony permits temporal analyses of the meiotic process using biochemical, molecular, and cytological assays. Thanks to sophisticated genetics and a powerful transformation system, yeast meiotic mutants are easy to isolate, and the corresponding genes can be rapidly cloned and disrupted. Artificial chromosomes and synthetic constructs integrated into authentic chromosomes provide invaluable tools in studies of recombination, homolog pairing, and chromosome segregation. Last, but not least, the ability to recover and analyze all four products of individual meioses allows detailed investigation of the mechanisms of meiotic recombination.

Studies of yeast meiosis have benefited from recent improvements in cytology. Despite the small size of yeast chromosomes, it is now possible to visualize meiotic chromosomes in nuclear spreads (Fig. 1), to localize proteins to chromosomes (Fig. 2), and to assess chromosome pairing by fluorescent *in situ* hybridization (FISH) (Fig. 3). Concurrent with these improvements in cytology has been the development of numerous physical assays that detect recombination intermediates and products. By combining these cytological and physical techniques with classical genetic methods, it has been possible to test canonical theories about the role of the synaptonemal complex (SC) and the relationship of recombination to homolog pairing and chromosome segregation. The often surprising results have necessitated abandonment of long-standing theories and inspired new ones.

SC Structure

The pairing of homologous chromosomes during meiotic prophase culminates in the formation of the SC, which is a ribbon-like, proteinaceous structure that holds homologous chromosomes in close apposition along their entire lengths (6). Yeast SCs can be visualized clearly when meiotic nuclei are surface spread, stained with silver nitrate, and viewed in the electron microscope (Fig. 1*A*). Early in the pathway of SC assembly, each pair of sister chromatids develops a common proteinaceous core called an axial element. Concurrent with the development of axial elements, the formation of mature SC initiates at a few sites along each chromosome pair (7). At these positions, the protein components of the central region of the SC assemble between the axial elements, which then become the lateral elements of the SC. Bidirectional SC extension results in complete synapsis. Most DNA is located outside the SC and is organized into chromatin loops that emanate from the lateral elements. The distance between lateral elements is ≈100 nm. In yeast, the average chromatin loop is 500 nm in length and contains 20 kbp of DNA (8).

The best-characterized structural component of the yeast SC is the Zip1 protein. Zip1 is localized continuously along the lengths of mature SCs (Fig. 2*B*) but is not present in unsynapsed axial elements, indicating that Zip1 is a component of the central region (3). In the *zip1* null mutant, axial elements are full-length and homologously paired, but the distance between them is greater and more variable than the distance between lateral elements in mature SC (Fig. 1*B*). Each pair of axial elements is closely associated at a few positions (Fig. 1*B*), postulated to be sites at which Zip1 and other components of the central region begin to polymerize (3). In some organisms, subunits called transverse filaments have been observed to lie perpendicular to the long axis of the SC and to bridge the space between lateral elements (6). The Zip1 protein is predicted to form a rod-shaped dimeric molecule and the predicted length of the Zip1 dimer corresponds approximately to the distance between lateral elements in the SC (3). Mutations that increase the length of the Zip1 dimer lead to corresponding increases in the distance between lateral elements, suggesting that Zip1 is a component of transverse filaments (9).

The *RED1* and *HOP1* genes encode proteins associated with the lateral elements of the SC and with unsynapsed axial elements. The Red1 and Hop1 proteins are not always localized continuously along the length of the SC; thus, there is not a perfect correspondence between the appearance of lateral elements as defined by silver staining and the pattern of Red1/Hop1 deposition (Hop1: ref. 10, F. Klein and B. Byers, personal communication; Red1: A. Smith and G.S.R., unpublished data). The Red1 protein is required for the formation of axial elements (11) and may be involved in nucleating the formation of these protein cores. Unsynapsed axial elements assemble in the absence of the Hop1 protein (F. Klein and B. Byers, personal communication) and genetic (12) and physical (13) analyses indicate that Hop1 plays an important role in promoting interhomolog interactions. Perhaps Hop1 localizes to preassembled axial elements and subsequently promotes chromosome synapsis. Overexpression of the *RED1* gene suppresses a subset of mutant alleles of the *HOP1* gene, suggesting that the Red1 and Hop1 proteins physically interact with each other (14, 15).

Homology Searching and Presynaptic Alignment

Chromosome synapsis is preceded by a homology search that results in the side-by-side alignment of homologs at a distance that exceeds the width of the SC (6). In yeast, this presynaptic alignment has been visualized by FISH (Fig. 3) using

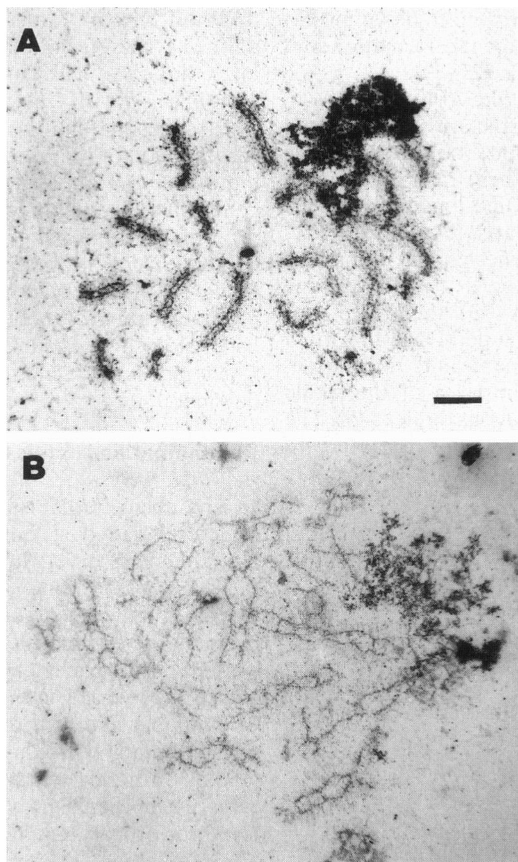

FIG. 1. Electron micrographs of yeast meiotic chromosomes. Meiotic nuclei from wild type (*A*) and the *zip1* mutant (*B*) were surface spread and stained with silver nitrate (1). (Bar = 1 μm; micrographs provided by Mary Sym.)

chromosome-specific DNA probes (5, 16). A significant, albeit reduced, level of meiotic homolog pairing is observed in mutants defective in SC formation (4, 16–18).

The problem of homolog alignment is generally assumed to be specific to meiotic cells; however, recent FISH studies indicate that homologs are paired in a substantial fraction of yeast cells prior to the induction of meiosis (4, 16, 19). Changes in mitotic chromatin structure that depend on homozygosity of the affected sequences provide additional evidence for premeiotic interactions between homologs (S. Keeney and N. Kleckner, personal communication). The vegetative pairing detected by FISH is temporarily disrupted during premeiotic DNA replication and then reestablished (16). It has been proposed that premeiotic and meiotic pairing involve the formation of unstable side-by-side joints between intact DNA duplexes (16, 19). Such reversible associations would provide a mechanism to deal with the interchromosomal tangles that result when uncondensed chromosomes initiate pairing at multiple sites. As meiosis progresses, SC formation establishes stable interhomolog connections that can withstand the forces of meiotic chromatin condensation.

FISH using short DNA probes has provided evidence that premeiotic pairing involves interactions at multiple sites along each chromosome pair (16). The total number of interactions per nucleus (≈190) is similar to the number of meiotic recombination events, leading to the hypothesis that meiotic recombination initiates at the sites of early pairing (16, 19). The notion that pairing connections precede (and therefore possibly promote) recombination, and not vice versa, is supported by the following observations. (*i*) Pairing is observed in vegetative cells in which the DNA presumably contains no strand interruptions (16). (*ii*) Some meiotically induced pairing is observed in mutants that fail to initiate recombination (4, 16–18). (*iii*) In a particular mutant, the number of pairing connections exceeds the number of exchanges initiated (16).

Surprisingly, meiotic recombination is not confined to sequences at the same position on homologous chromosomes. Two copies of a gene positioned on nonhomologous chromosomes recombine almost as frequently as allelic genes (20, 21). These ectopic recombination events imply the operation of a genome-wide homology search that allows even short stretches of DNA (≈2 kbp) to find any and all homologous counterparts. Interactions between sequences on nonhomologous chromosomes are evidently not excluded either by premeiotic pairing or by SC formation. The timing of these ectopic recombination events (22) suggests that dispersed recombining sequences are physically associated throughout the period when homologous chromosomes are fully synapsed. Such ectopic interactions may take place in chromatin loops that are distant from the protein cores engaged in SC formation.

Double-Strand Cleavage Initiates Meiotic Recombination

There is compelling evidence that double-strand breaks (DSBs) initiate meiotic recombination in yeast. Meiotically induced DSBs have been observed at a number of recombination hot spots that display elevated levels of genetic exchange and cis-acting mutations that reduce DSBs at these sites decrease the frequency of recombination (23–26). Analysis of whole meiotic chromosomes by pulsed-field gel electrophoresis has demonstrated that DSBs are widespread, but there are a number of preferred sites of cleavage on each chromosome (27, 28). DSBs appear and disappear with the kinetics expected for an early intermediate in the exchange process (7). Meiotic DSBs do not occur at a specific DNA sequence but rather are dispersed throughout a region of ≈150–180 bp at each locus (89–91). Comparison of the yeast genetic map with the distribution and frequency of double-strand cuts suggests that DSBs initiate most, if not all, meiotic recombination events (29).

Features of chromatin structure established during mitotic growth play an important role in determining the sites of meiotic DSBs (29). Sites with a high probability of breakage during meiosis correspond to nucleosome-free regions (as defined by nuclease hypersensitivity) present in chromatin isolated from vegetative cells (29). In addition, cis- and trans-acting mutations that lead to chromatin remodeling at a specific locus alter the frequency and distribution of DSBs (29). Almost all breaks occur in intergenic regions that contain transcription promoters (29). However, transcription *per se* is not required for DSB formation (30) and transcription through the promoter can interfere with recombination hot spot activity (31). Two observations suggest that an open chromatin configuration is not the only determinant of DSB formation (32). (*i*) There is not a strong correlation between the level of nuclease hypersensitivity and the probability of cleavage. (*ii*) DSB formation can be affected by sequences located several kilobase pairs away.

Despite extensive studies of DSBs, the endonuclease(s) responsible for cleavage remains elusive. Genetic analysis indicates that several yeast mutants are defective in the initiation of meiotic recombination (33); at least five of these (*rad50, spo11, xrs2, mre11*, and *mer2*) have been shown to be defective in DSB formation by physical

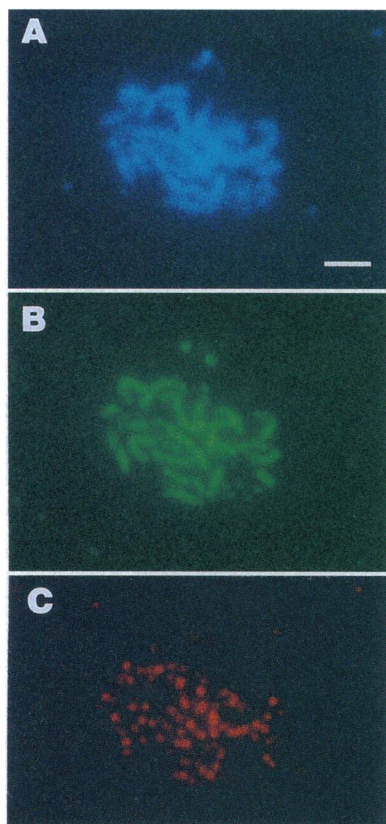

FIG. 2. Immunolocalization of the Zip1 and Msh4 proteins. Shown is a spread meiotic nucleus from a strain producing an epitope-tagged version of the Msh4 protein. Chromosomes were stained with a DNA-binding dye (4′,6-diamidino-2-phenylindole) (*A*), anti-Zip1 antibodies (*B*), and antibodies against an epitope-tagged Msh4 protein (*C*) (2, 3). Anti-Zip1 and anti-epitope antibodies were detected with appropriate secondary antibodies tagged with fluorescein and Cy3, respectively. (Bar = 2 μm; photographs provided by Petra Ross-Macdonald.)

assays (18, 23, 34–36). However, it remains to be determined whether the implicated gene products participate directly in DSB formation or whether they establish preconditions necessary for DSB induction. The Rad50 protein has DNA-binding activity (37) and acts in conjunction with Mre11 in a complex that also includes Xrs2 (36).

DSB Processing and Recombination Intermediates

The occurrence of meiosis-specific DSBs provides strong support for the DSB repair model of recombination (38, 39), as diagramed in Fig. 4. Many intermediates postulated by the DSB repair model have been demonstrated physically and mutants blocked at different steps in the repair process have been identified (Fig. 4). A non-null allele of the *RAD50* gene allows the formation of DSBs, but the broken molecules are not processed and therefore accumulate (34). This allele, called *rad50S*, has proved to be an invaluable tool in studies of the formation and distribution of DSBs (27–29).

In three mutants, *rad51* (40), *dmc1* (41), and *sep1* (42), DSBs are processed to expose single-stranded tails with 3′ termini (Fig. 4), but these tails accumulate and eventually become longer than their wild-type counterparts. The *DMC1* (41) and *RAD51* (40) genes encode homologs of the bacterial RecA strand exchange enzyme. Like RecA (43), the Rad51 protein coats single-stranded DNA to form a nucleoprotein filament (44) and subsequently promotes invasion of the single strand into a homologous duplex (45). The Sep1 protein, although unrelated to RecA, has also been shown to promote strand transfer *in vitro* (46). Thus, it is reasonable to suppose that the accumulation of DSBs in some or all of these mutants results from a defect in the first step in the repair of DSBs (i.e., strand invasion).

Soon after DSBs disappear, branched molecules that contain information from both recombining chromosomes can be detected by two-dimensional gel electrophoresis (13, 47). These joint molecules contain two full-length nonrecombinant strands from each parental duplex (13), but digestion with a Holliday junction-cleaving enzyme generates crossover products (A. Schwacha and N. Kleckner, personal communication). Thus, these molecules appear to contain two Holliday junctions, as predicted by the DSB repair model (Fig. 4 *f* and *g*).

If strand exchange occurs in a region where the parental chromosomes are genetically nonidentical, heteroduplex DNA (hDNA) containing one or more mismatched base pairs results. Unexpectedly, physical assays fail to detect hDNA until relatively late in meiotic prophase, around the same time as mature crossover products (17, 48). According to the DSB repair model (Fig. 4), however, hDNA should be produced early in the recombination process and certainly should be present in joint molecules. Thus, it is necessary either to abandon the model or to postulate that hDNA is present at earlier times but not detected in current assays. hDNA present in recombination intermediates may be lost due to branch migration during DNA extraction or it might be contained in branched molecules that fail to migrate as a single species during gel electrophoresis (48).

According to the DSB repair model, resolution is a stochastic process and each recombination intermediate has an equal probability of being resolved to generate either the crossover or the noncrossover configuration of flanking markers (Fig. 4 *h* and *i*). However, the behavior of certain meiotic mutants suggests that resolution in favor of crossing-over specifically requires additional proteins. There are four different yeast mutants in which recombination events initiate at the wild-type level, but crossing-over is reduced 2- to 10-fold (2, 49, 50). One of these mutants is *zip1*, suggesting that the SC can influence the direction of resolution (50). Two other mutants, *msh4* (2) and *msh5* (92), define genes that encode homologs of the bacterial MutS protein, which is involved in the correction of mismatched base pairs. Since Msh4 and Msh5 play no role in mismatch repair, these homologs must have evolved to acquire a new function, which may include interacting with Holliday junctions.

Recombination Nodules

In a number of organisms, electron-dense structures called recombination nodules have been observed in association with meiotic chromosomes (51). Nodules are classified as early if they are present in zygotene, when synapsis initiates. Nodules present during pachytene, when homologs are fully synapsed, are classified as late. The number and distribution of late nodules correlate with those of crossovers, leading to the hypothesis that these structures are multienzyme complexes that catalyze reciprocal exchange. Early nodules are more abundant and have been postulated to mark the sites of all strand exchange reactions. The Dmc1 and Rad51 proteins colocalize to discrete spots on chromosomes prior to synapsis and may therefore be components of early nodules (52). Rad51 has been shown to associate physically with Rad52 (40), suggesting that the latter protein is also a component of early nodules. Msh4 localizes to discrete

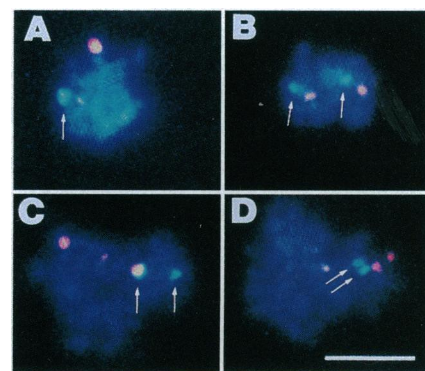

FIG. 3. FISH analysis of chromosome pairing. Spread meiotic nuclei were painted (4, 5) with composite probes specific for chromosome I (red) and chromosome III (green). DNA was stained blue to reveal spread chromatin. (*A*) Nucleus in which chromosomes I and III are homologously paired. (*B*) Nucleus in which both sets of homologs are unpaired. (*C*) Nucleus in which two nonhomologous chromosomes are accidentally associated. (*D*) Nucleus in which chromosomes III are tightly aligned, while chromosomes I are unpaired. (Bar = 5 μm; photographs provided by Harry Scherthan.)

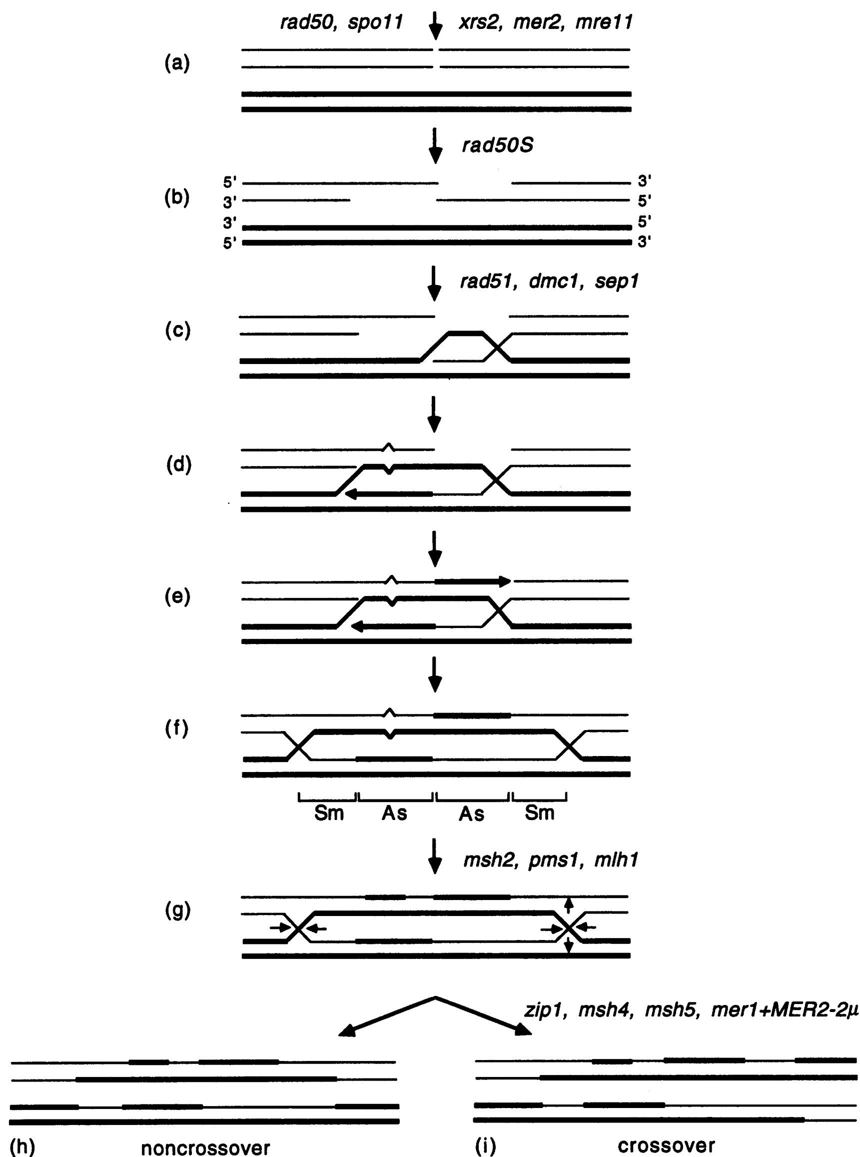

FIG. 4. DSB repair model of recombination (38, 39). (*a*) One of the two recombining DNA duplexes sustains a DSB. (*b*) The 5′ ends of the broken duplex are exonucleolytically degraded to expose single-stranded tails with 3′ termini. (*c*) One of the tails invades an uncut homologous duplex, resulting in the displacement of a D loop. (*d*) The D loop is enlarged by repair synthesis primed by the invading 3′ end until the other single-stranded tail can anneal with complementary sequences in the D loop. (*e*) DNA synthesis primed by the second 3′ end completes repair. (*f*) Branch migration results in the formation of two Holliday junctions; regions of asymmetric (As) and symmetric (Sm) strand exchange are indicated. (*g*) A mismatched base pair, diagramed in *d–f*, is repaired by excision and resynthesis. Mismatch repair might occur earlier than diagramed (i.e., immediately upon formation of hDNA). (*h*) If both Holliday junctions are resolved in the same direction (i.e., by cleavage of inner or outer strands in both cases), then the parental configuration of flanking markers is preserved. (*i*) Resolution of the two Holliday junctions in opposite directions results in a reciprocal crossover between markers that flank the region of strand exchange. For the Holliday junction shown on the right in *g*, the cuts indicated by the horizontal or vertical arrows generate the products shown in *h* or *i*, respectively. Mutants blocked at particular steps are indicated.

foci on synapsed chromosomes (2) and is thus a candidate for a late nodule component (Fig. 2*C*).

Relationship Between Recombination and Chromosome Synapsis

Temporal studies have demonstrated that meiotic recombination and SC assembly proceed concurrently. DSBs with single-stranded tails appear early in prophase, prior to the formation of tripartite SC, and they disappear in zygotene (7). Joint molecules are evident during pachytene (13). Crossover products and hDNA are detected at the end of pachytene or soon thereafter, as the SC disassembles (7, 17, 48).

Studies of yeast have challenged the traditional view that chromosome synapsis is required for meiotic recombination. Gene conversion occurs at the wild-type level in *zip1* strains (3, 50), suggesting that the initiation of recombination is unaffected, despite the absence of mature SC. In the *red1* mutant, in which there is no SC or any obvious SC precursors, recombination occurs at ≈25% of the wild-type rate (11), which is orders of magnitude above the mitotic background level. The observed high frequencies of ectopic recombination also argue that fully synapsed chromosomes are not an exclusive venue for recombination (20, 21). Finally, DSBs are observed during meiosis in haploid yeast, demonstrating that the initiation of recombination does not depend on prior interactions between homologs (53, 54).

A number of observations suggest that synapsis may depend on DSB processing. DSBs with single-stranded tails appear prior to the initiation of synapsis and their processing to a later intermediate appears to be coincident with SC initiation (7). The *rad50S* mutation prevents the processing of DSBs to expose single-stranded tails (34) and also abolishes (34) or substantially reduces (4) SC formation. Of the many yeast mutants characterized, none has been found to make SC in the absence of DSBs. Finally, a recent study suggests that synapsis initiates at the sites of DSB processing defined by Dmc1- and Rad51-staining foci (93). The Dmc1 and Rad51 proteins are required to establish the interhomolog connections observed in *zip1* strains (Fig. 1*B*) and the number of these connections (3) corresponds to the number of Dmc1- and Rad51-staining foci (52). SC formation in *dmc1* and *rad51* mutants is delayed and incomplete, as expected if the observed interhomolog connections normally serve as sites of synaptic initiation (41, 93).

Mismatch Correction

The machinery responsible for correction of mismatches during meiotic recombination in yeast is closely related to the *Escherichia coli* machinery involved in the repair of replication errors. Components of the bacterial mismatch repair system include the MutS protein, which binds to mismatched base pairs, and MutL, which interacts with MutS to expand the DNA footprint (55). In yeast, genes encoding six MutS (refs. 2, 56, 57, and 92; GenBank data base) and three MutL (58, 59) homologs have been identified. Msh2 is the functional counterpart of bacterial MutS (60). In contrast to *E. coli*, two different MutL proteins, Pms1 and Mlh1, are required for mismatch repair in yeast (58, 59). The Pms1 and Mlh1 proteins physically associate with each other and the resulting Pms1/Mlh1 complex then interacts with Msh2 protein that is bound to hDNA (61).

According to the DSB repair model (Fig. 4), the invasion of single-stranded tails results in hybrid DNA on one of the two recombining DNA duplexes (i.e., asymmetric strand exchange), while the branch migration of Holliday junctions results in hybrid DNA on both duplexes (i.e., symmetric strand transfer). In yeast, most non-Mendelian segregations are gene conversions (i.e., 6:2 segregations) (33), and cis- and trans-acting mutations that inhibit mismatch repair result in a predominance of 5:3 segregations, indicative of a single heteroduplex (60, 62, 63). Thus, most hDNA appears to be confined to the single-stranded tails that flank DSBs, and mismatch repair is extremely efficient.

A number of yeast genes display a polarity gradient in which the frequency of gene conversion is high at one end and low at the other (25, 64, 65). The polarity gradient generally is assumed to reflect the probability of heteroduplex formation; the closer an allele is to the DSB, the higher the probability it will be included in heteroduplex. However, recent studies indicate that inhibition of mismatch correction (e.g., by a *msh2* mutation) results in a uniformly high frequency of conversion throughout the gene (62, 64). One model that accounts for these unexpected findings suggests that a polarity gradient reflects the direction of mismatch repair rather than the probability of heteroduplex formation (64). According to this view, mismatches near the DSB are usually repaired in favor of the invading strand (leading to gene conversion), whereas mismatches far from the DSB are generally repaired in favor of the invaded duplex (and therefore undetected). An alternative model suggests that polarity gradients reflect the transition from asymmetric to symmetric heteroduplex (Fig. 4 *e* and *f*) (62). Mismatch repair enzymes are postulated to be coupled to the machinery that promotes symmetric strand exchange, such that exchange is terminated (and perhaps reversed) whenever a mismatch is encountered. The mismatch repair machinery is known to act as a barrier to recombination between divergent DNA sequences in yeast (66) and bacteria (67), supporting the hypothesis that mismatch repair enzymes can prevent or reverse strand transfer.

Chromosome Segregation

The meiosis I division is distinct from mitosis and meiosis II in that sister chromatids remain associated, while homologous chromosomes segregate from each other. Proper reductional segregation depends on crossing-over to establish chiasmata, which are chromatin bridges between nonsister chromatids that persist after the SC has disassembled. During prometaphase, homologous centromeres can become attached to spindle fibers from opposite spindle poles or (at least transiently) to fibers from the same pole. Spindle fiber attachment is stabilized only when homologs become attached to opposite spindle poles and the opposing poleward forces exerted on the chromosomes are resisted by chiasmata. Chiasma function is thought to depend on sister-chromatid cohesion distal to crossovers, on chiasma-binding proteins positioned at crossovers, or both.

The SC has been postulated to play a role in meiosis I chromosome segregation. Remnants of SC have been observed at chiasmata, suggesting that SC proteins may bind chiasmata (6). In addition, studies of maize have suggested that synapsis is necessary to establish meiotic sister-chromatid cohesion (68). In the *zip1* mutant, meiosis I nondisjunction events are confined to nonrecombinant chromosomes, indicating that chiasmata are functional in the absence of tripartite SC (50). In addition, *zip1* strains display only a modest defect in sister-chromatid cohesion (50). Although these results demonstrate that tripartite SC is not essential for meiosis I chromosome segregation, they do not preclude a role for SC components in segregation. In the *red1* mutant (which fails to make axial elements), even recombinant chromosomes missegregate (11).

Studies of yeast artificial chromosomes provide additional evidence that crossing-over is not sufficient to ensure correct disjunction. Crossovers that occur in yeast or human sequences promote proper segregation of artificial chromosomes, whereas crossovers in bacteriophage λ DNA do not (69, 70). Thus, apparently only those crossovers that occur in the context of a particular sequence or structure lead to functional chiasmata.

An alternative pathway of meiosis I chromosome segregation, called distributive disjunction, promotes the segregation of chromosomes that cannot (or did not) recombine. The distributive system requires neither crossing-over nor sequence homology. Two nonhomologous artificial chromosomes (71), or two genuine yeast chromosomes that lack homologs (72), segregate to opposite poles in ≈90% of meioses. Disjunction is preceded by physical pairing between the chromosomes during meiotic prophase (73). The distributive system probably ensures proper disjunction of those rare chromosomes that fail to recombine in wild-type diploids but is apparently inadequate to handle multiple pairs of nonrecombinant chromosomes.

Regulation of Crossover Distribution

The distribution of meiotic crossovers is nonrandom in two respects (51). (*i*) Two crossovers on the same chromosome arm rarely occur close together. (*ii*) Every pair of chromosomes (no matter how small) undergoes at least one reciprocal exchange. These two phenomena, referred to as crossover interference and obligate chiasma, may be mechanistically related. By preventing excess crossovers on large chromosomes, interference might ensure that small chromosomes cross over (74, 75).

Crossover interference has been postulated to involve the transmission of an inhibitory signal from one crossover site to nearby potential sites of crossing-over (75–77). The SC is an obvious conduit along which such a signal might travel. In support of this hypothesis, the *zip1* mutation abolishes crossover interference, resulting in a random distribution of crossovers along chromosomes (50). Small chromosomes in the *zip1* mutant frequently fail to recombine and therefore missegregate (50), indicating an absence of the obligate chiasma. A role for the SC in crossover interference is further supported by two exceptional organisms, *Schizosaccharomyces pombe* and *Aspergillus nidulans*, that fail to make SC and do not exhibit interference (78, 79).

In yeast, small chromosomes sustain more crossovers per kilobase pair than large chromosomes (74, 80). Chromosome I is a small chromosome whose density of meiotic crossovers is more than twice that of the genomic average. When chromosome I is bisected to produce two smaller chromosomes, recombination in chromosome I sequences increases even further. When chromosome I is fused to a larger chromosome, recombination decreases. These results demonstrate the existence of a control mechanism that responds directly to chromosome size (74).

Meiotic Cell Cycle Checkpoints

The events of the mitotic cell cycle are ordered into pathways in which the initiation of late events depends on the successful completion of earlier events (81). Control mechanisms that enforce dependency are called checkpoints. Studies of mutants indicate that checkpoints also operate during the meiotic cell cycle and identify the transition from pachytene as a critical control point. Several mutants that arrest at the start point of the mitotic cell cycle arrest in the pachytene stage of meiosis (82). Cells in pachytene still have the option of reverting to mitotic cell division, whereas cells that have progressed beyond this point are irreversibly committed to reductional chromosome segregation.

Several mutants with specific defects in meiotic chromosome metabolism—*zip1* (83), *dmc1* (41), *top2* (84), and *sep1* (42)—arrest in the pachytene stage of meiosis. All of these mutants retain viability at the arrest point, as expected if a checkpoint is operating. Some or all of these mutants probably arrest in response to the accu-

mulation of intermediates in the recombination and/or synapsis pathway. In support of this hypothesis, the prophase arrest of the *dmc1* (41), *top2* (85), and *zip1* (3) mutants is alleviated by mutations that block the initiation of recombination and synapsis. The complete absence of recombination does not trigger a checkpoint as evidenced by the fact that mutants defective in DSB formation sporulate efficiently.

The Rad9 and Rad24 proteins of yeast are responsible for arrest in the G_1 (86) and G_2 (87, 88) stages of the mitotic cell cycle in response to DNA damage, such as DSBs. A *rad9* mutation does not bypass the meiotic arrest of the *dmc1* (41), *sep1* (42), and *zip1* (M. Sym and G.S.R., unpublished data) mutants; however, a *rad24* mutation does restore sporulation at least to *dmc1* (D. Lydall, Y. Nikolsky, D. Bishop, and T. Weinert, personal communication). Thus, mitotic cell cycle checkpoints do operate during meiosis, but meiosis-specific factors may modify the signals generated and the response machinery.

Studies of meiotic mutants suggest differences between laboratory strains of yeast in the operation of cell cycle checkpoints. For example, the *zip1* mutant sporulates in a strain background in which the *dmc1* mutant displays prophase arrest and vice versa (3, 41, 50, 93). The strain-specific behavior of *zip1* and *dmc1* suggests that meiotic arrest in these mutants is triggered by different signals or effected by different mechanisms.

Overview

Over the past several decades, the meiotic process has been studied extensively at the cytological level in a variety of organisms. Due in large part to recent studies of meiosis in yeast, we now have molecular handles on the structures and processes observed microscopically. Structural components of the SC have been identified and enzymatic components of the recombination machinery are in hand. Cytological entities that have been the topics of speculation and theory building for many years are now the subjects of rigorous experimental tests.

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