

Does Crossover Interference Count in *Saccharomyces cerevisiae*?

Franklin W. Stahl,^{*,1} Henriette M. Foss,^{*} Lisa S. Young,^{*} Rhona H. Borts,[†]
M. F. F. Abdullah[‡] and Gregory P. Copenhaver[§]

^{*}Institute of Molecular Biology, University of Oregon, Eugene, Oregon 97403-1229, [†]Department of Genetics, University of Leicester, Leicester LE1 7RH, United Kingdom, [‡]Department of Microbiology, Mara University of Technology, 40450 Shah Alam, Malaysia and [§]Department of Biology and The Carolina Center for Genome Sciences, University of North Carolina, Chapel Hill, North Carolina 27599

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ABSTRACT

We previously proposed a “counting model” for meiotic crossover interference, in which double-strand breaks occur independently and a fixed number of noncrossovers occur between neighboring crossovers. Whereas in some organisms (group I) this simple model alone describes the crossover distribution, in other organisms (group II) an additional assumption—that some crossovers lack interference—improves the fit. Other differences exist between the groups: Group II needs double-strand breaks and some repair functions to achieve synapsis, while repair in group I generally occurs after synapsis is achieved; group II, but not group I, has recombination proteins Dmc1, Mnd1, and Hop2. Here we report experiments in *msh4* mutants that are designed to test predictions of the revised model in a group II organism. Further, we interpret these experiments, the above-mentioned differences between group I and II meiosis, and other data to yield the following proposal: Group II organisms use the repair of leptotene breaks to promote synapsis by generating double-Holliday-junction intermediates that lock homologs together (pairing pathway). The possible crossover or noncrossover resolution products of these structures lack interference. In contrast, for both group I and group II, repair during pachytene (disjunction pathway) is associated with interference and generates only two resolution types, whose structures suggest that the Holliday junctions of the repair intermediates are unligated. A crossover arises when such an intermediate is stabilized by a protein that prevents its default resolution to a noncrossover. The protein-binding pattern required for interference depends on clustering of sites that have received, or are normally about to receive, meiotic double-strand breaks.

A key feature of meiosis in most organisms is crossing over, the process in which homologous chromosomes exchange segments during the repair of programmed double-strand breaks (DSBs) in their DNA. The frequencies of crossing over provide the basis for genetic linkage mapping (STURTEVANT 1913), in which the distance between genes (in morgans) is defined as the average number of points of crossing over in the interval that separates the genes (HALDANE 1919). STURTEVANT (1915) and MULLER (1916) noted that crossovers occurring during *Drosophila melanogaster* oogenesis show a kind of territoriality—a relatively equitable, nonrandom distribution—among and within chromosomes. This property, which they called “interference,” is a widespread phenomenon, which may have been selected for its ability to encourage at least one crossover on each chromosome, without undue increase in the mean number of crossovers. Interference can act over great distances (*e.g.*, about half the length of the *Drosophila* X linkage

map) and still beguiles geneticists, microscopists, and mathematicians alike.

A mathematical model that effectively describes linkage data from the X chromosome of *Drosophila* (MCPECK and SPEED 1995; ZHAO *et al.* 1995) was put forth by COBBS (1978) and STAM (1979). Their model, notable for its simplicity and mathematical tractability, was foreshadowed by several others (reviewed in BAILEY 1961). It describes the probability distribution for the linkage distances (in morgans) between adjacent crossovers as a scaled chi-square probability distribution with an even number of degrees of freedom. Such a distribution gained biological appeal from the suggestion that crossovers may be portrayed as successful outcomes of independently distributed *attempts* to cross over, and that adjacent crossovers are separated by a fixed number of failed outcomes. However, in the absence of an expressed view of what the “attempts” or “failures” might be, the model languished until FOSS *et al.* (1993), elaborating a suggestion by MORTIMER and FOGEL (1974), proposed that the products of all programmed meiotic DSB repair (DSBR)—observable as gene conversions (see MALKOVA *et al.* 2004, accompanying article, this issue)—represent crossover *attempts* and that the gene

¹Corresponding author: Institute of Molecular Biology, 1370 Franklin Blvd., 1229 University of Oregon, Eugene, OR 97403-1229.
E-mail: fstahl@mollbio.uoregon.edu

conversions unaccompanied by crossing over represent the failures.

Evidence that attempts are, indeed, distributed independently (*i.e.*, at random with respect to each other) is provided for *Neurospora crassa* by STADLER (1959) and for (budding) yeast by MORTIMER and FOGEL (1974) and MALKOVA *et al.* (2004). The most direct test of independence would be a demonstration that conversions (crossovers plus noncrossovers) manifest no interference with each other, either positive or negative. The practical difficulty of obtaining adequate data for such a test restricted those authors to asking whether conversions unaccompanied by crossing over (failed attempts) repress nearby crossovers, as do conversions that are accompanied by crossing over. They did not.

A major implication of this “counting model” is that the number of noncrossover gene conversions postulated to lie between adjacent crossovers may be determined experimentally as well as theoretically, and that the two measurements should yield comparable values (Foss *et al.* 1993). To test this prediction, published interference data collected from *Drosophila* and *Neurospora* were subjected to a best-fit analysis of the counting model (MCPEEK and SPEED 1995) to determine the number of failures between adjacent “successes.” Foss *et al.* (1993) compared these numbers with the experimentally observed fraction of noncrossovers among gene conversions. For *Drosophila*, the observed fraction of noncrossovers is close to 0.80, as determined in a Herculean analysis at one locus (HILLIKER and CHOVIK 1981; HILLIKER *et al.* 1991). For *Neurospora*, the observed fraction of noncrossovers is close to 0.67, based on the average of numerous observations (PERKINS *et al.* 1993). As predicted, both values are in agreement with the optimal fits to the interference data determined by MCPEEK and SPEED (1995), who calculate the number of obligate failures between adjacent crossovers to be four for *Drosophila* and two for *Neurospora*.

The success with which the counting model describes interference in *Drosophila* and *Neurospora* inspired FOSS and STAHL (1995) to test another powerful prediction of the model—that, relative to the general population, progeny with two close crossovers should show an enhanced frequency of gene conversion unaccompanied by crossing over in the interval between the two crossovers. Testing the prediction in the only organism in which such an experiment was feasible, *Saccharomyces cerevisiae*, they obtained an unambiguously negative result and concluded that the counting model was either wrong or not applicable to yeast.

The companion article to this work (MALKOVA *et al.* 2004), however, supports a third possibility—that interference in wild-type yeast, while approximating the rules described by MCPEEK and SPEED (1995), is better described if that model includes a set of additional crossovers that are not subject to interference (and see DE LOS SANTOS *et al.* 2003). Moreover, for linkage data from

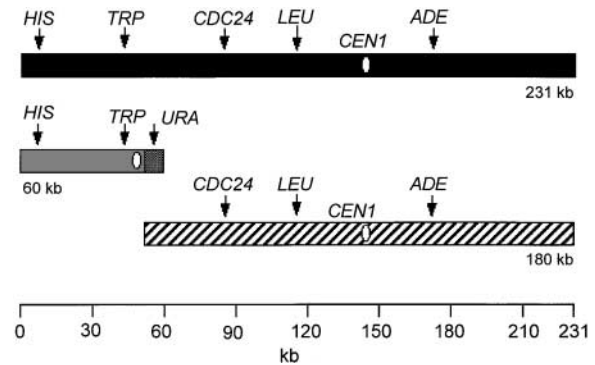


FIGURE 1.—Bisected chromosome I (after KABACK *et al.* 1999). Chromosome I (black) was bisected by KABACK *et al.* (1992). The *URA* marker on the shorter derivative (gray), cloned on a plasmid with two telomeres, a centromere, and a segment of chromosome I located to the right of *TRP*, facilitated selection of the bisection strain.

humans (HOUSWORTH and STAHL 2003) and *Arabidopsis thaliana* (COPENHAVER *et al.* 2002) too, the good fit of the counting model is improved if a fraction of meiotic crossovers is assumed to lack interference. In contrast, the same analysis applied to data from *Drosophila* and *Neurospora* suggests that all crossovers in these organisms show interference (COPENHAVER *et al.* 2002). Below, we offer a context for these observations.

MATERIALS AND METHODS

Strains: Haploid *S. cerevisiae* strains carrying genetically marked, bisected (JL51) and full-length (JL52) versions of chromosome I, diagrammed in Figure 1, are described by KABACK *et al.* (1999). JL51 strains are *MAT α* [*FUN43-CEN1-URA3*] *fun43::TRP1 his3 leu2 ura3 trp1 arg4*, *MATa* [*FUN43-CEN1-URA3*] *YAL067 HIS3 cdc24 fun30::LEU2 ade1 trp1 ura3 his3 arg4 leu2*; JL52 strains are *MAT α* *fun43::TRP1 his3 leu2 ura3 trp1 arg4*, *MATa* *YAL067::HIS3 cdc24 fun30::LEU2 ade1 trp1 ura3 his3 arg4 leu2* (mating-type designations were reversed in the original article; D. KABACK, personal communication). In both JL51 haploids the 60-kb (light gray) derivative of chromosome I carries a *URA3* gene that is not present in the 231-kb full-length chromosome I-containing JL52 haploids. To avoid possible complications resulting from differences in uracil auxotrophy, we transferred both JL52 strains from *ura3* to *URA3* with a 1.1-kb *Sma*I fragment from pJJ242 (JONES and PRAKASH 1990) to yield JL53 haploids. Results obtained with JL52 and JL53 diploids were statistically indistinguishable and were pooled.

Strains precisely deleted for the *MSH4* ORF were made with the *loxP-kanMX-loxP* disruption cassette followed by excision of the *kanMX* module by induction of the Cre recombinase from plasmid pSH47 (GÜLDENER *et al.* 1996). Oligonucleotides used to effect *MSH4* deletion were 5'-AGTTATAGCATTGAAA TCTGTAGCTGATCAACGCAAACCTATATGCATCGACAACC CTTAATATAACTTCG-3' and 5'-CAGAAATAATGGATTATATGTTTAAGCTAAGCGGAAAAGCCAAAATCACCTAATAAC TTCGTATAGC-3'.

The *MSH4*-targeted *loxP-kanMX-loxP* disruption cassette was generated by polymerase chain reaction (PCR) using the EXPAND high-fidelity kit (Roche Diagnostics, Indianapolis) following the manufacturer's directions. The following oligonucleotides were used to verify *MSH4* replacement with *loxP*-

TABLE 1
Two groups of eukaryotes

Group	Candidates	Noninterfering crossovers	Evidence for two periods of DSBR	DMC1 ^{a,m} (<i>HOP2</i> , <i>MND1</i>)	Resolution types
I: DSBR not required for <i>synapsis</i> ^a (NA for <i>N. crassa</i>)	<i>Drosophila</i>	No ^{h,c}	No ^h	No	NA
	<i>C. elegans</i>	No ^d	No ⁱ	No	NA
	<i>N. crassa</i>	No ^b	NA	No	Two types ⁿ
II: DSBR required for <i>synapsis</i> ^a	<i>S. cerevisiae</i>	Yes ^e	Yes ^j	Yes	Five types ^o
	<i>Homo/Mus</i>	Yes ^f	Yes ^k	Yes	NA
	Green plants	Yes ^g	Yes ^l	Yes	NA

NA, data not available.

^a Reviewed in COPENHAVER *et al.* (2002).

^b FOSS *et al.* (1993); COPENHAVER *et al.* (2002).

^c ZHAO *et al.* (1995).

^d MENEELY *et al.* (2002).

^e MALKOVA *et al.* (2004).

^f HOUSWORTH and STAHL (2003).

^g For *Arabidopsis*, COPENHAVER *et al.* (2002).

^h JANG *et al.* (2003); PAGE and HAWLEY (2001); LIU *et al.* (2002).

ⁱ A. VILLENEUVE (personal communication).

^j XU *et al.* (1997) and see text.

^k MOENS *et al.* (2002).

^l For *Lilium*, TERASAWA *et al.* (1995).

^m TAKANAMI *et al.* (2000); GERTON and DERISI (2002); BORKOVICH *et al.* (2004); [http://www.informatics.jax.org/mgihome/](http://www.informatics.jax.org/mgihome/MGD/aboutMGD.shtml)MGD/aboutMGD.shtml; <http://www.yeastgenome.org/>; <http://fly.ebi.ac.uk:7081/>; <http://www.arabidopsis.org/home.html>; <http://www.broad.mit.edu/annotation/fungi/neurospora/>; http://www.sanger.ac.uk/Projects/C_elegans/.

ⁿ STADLER and TOWE (1963).

^o e.g., FOSS *et al.* (1999).

kanMX-loxP using standard PCR: 5'-GTTTTGGTATGGGATGA CATTGTTTTACGTAG-3' (472 bp upstream of the *MSH4* ORF) and 5'-TCTCAAGGTGATTTGGAGGCAGACG-3' (896 bp downstream of the *MSH4* ORF).

Media, diploid construction, sporulation, and tetrad analysis: Media used were as described in MCCUSKER and HABER (1988). To reduce the tendency of the 60-kb portion of bi-sected chromosome I to undergo duplication, it proved necessary to avoid applying selection for *TRP1* or *HIS3* when constructing the diploids. Thus, diploids were generated by micromanipulating appropriate haploids together, allowing colonies to grow up, and screening for nonmaters. All subsequent manipulations were at 25°, except for detection of the *CDC24* allele, which was at 37°. Sporulation was induced on plates at 25° (MALKOVA *et al.* 2004). Analysis of tetrad data was facilitated by MacTetrad 6.9.1, available by Gopher from merlot.welcj.jhu.edu. Statistical analyses were carried out with the advice of Russell Lande. Online calculators are available at the Stahl Lab web site: <http://groik.com/stahl/>.

Estimation of interference: The chi-square probability distribution, or its gamma variation, has been shown to provide a good description of interference in a variety of organisms (e.g., FOSS *et al.* 1993; LANDE and STAHL 1993; MCPEEK and SPEED 1995; BROMAN and WEBER 2000). The chi-square distribution is fully determined by the value of a single parameter, which FOSS *et al.* (1993) called *m*. When *m* = 0, the chi-square distribution is exponential (i.e., no interference). *m*-values were determined from tetrad data as described in STAHL and LANDE (1995) with the aid of the online calculator at <http://www.molbio.uoregon.edu/%7Egraham/tetrad.html>.

A widely used measure of interference in two-factor tetrad data, the “NPD ratio” (PAPAZIAN 1952), measures a *consequence* of interference that necessarily changes value when the map

distance changes. For data that approximate the chi-square distribution, the *m*-value, calculated from the frequencies of tetrad types, increases with increasing interference and is independent of map distance.

RESULTS AND DISCUSSION

Two groups of eukaryotes: Some eukaryotes are known to depend on some meiotic DSBR functions for intimate pairing and synapsis, as well as for proper disjunction of homologous chromosomes (for reviews see KLECKNER 1996; ROEDER 1997; ZALEVSKY *et al.* 1999; WALKER and HAWLEY 2000; COPENHAVER *et al.* 2002; VIERA *et al.* 2004). Other eukaryotes, including *Drosophila* and *Caenorhabditis elegans*, achieve synapsis via *cis*-acting “homolog recognition centers” or “pairing centers” (VILLENEUVE 1994; DERNBURG *et al.* 1998; MCKIM *et al.* 1998; PAGE and HAWLEY 2001; LIU *et al.* 2002). Eukaryotes requiring DSBR functions for synapsis share a set of additional meiotic features, including the prediction of noninterfering crossovers (Table 1). Thus, it is economical to hypothesize (1) that, together, these features characterize a process that potentiates synapsis and (2) that noninterfering crossovers are a (by)product of this process.

Additional evidence for the existence of noninterfering crossovers in wild-type *S. cerevisiae*: ZALEVSKY *et al.* (1999) raised the possibility that, in wild-type yeast but

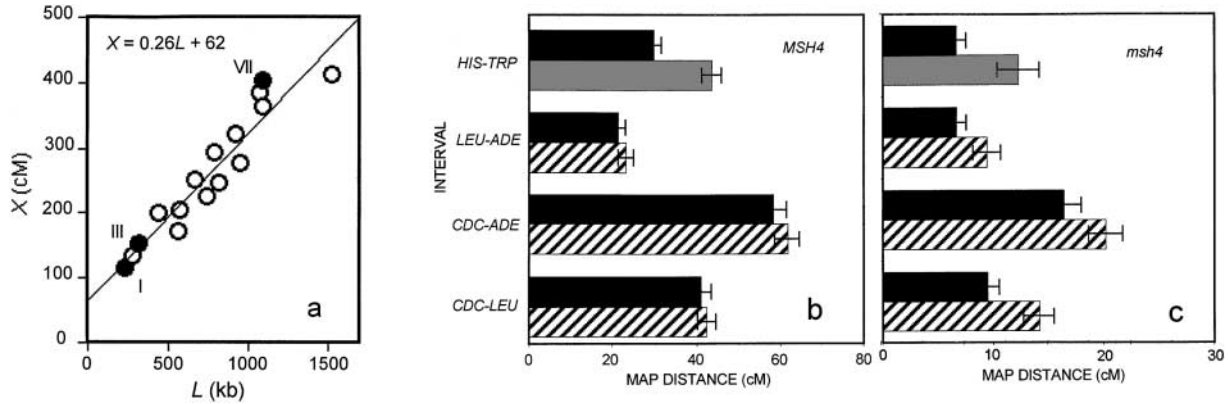


FIGURE 2.—Relationships among chromosome length, crossover density, and *msh4* phenotype. (a) Chromosome length in centimorgans as a function of length in kilobases with the least-squares regression of the data for *S. cerevisiae*. The y -axis intercept is above zero, indicating that crossover density is higher on short than on long chromosomes. Data are from Saccharomyces Genome Database: <http://db.yeastgenome.org/cgi-bin/SGD/PGMAP/pgMap>. Data for chromosomes of special interest in this article are indicated by solid points. (b) Bisection of chromosome I into a longer and a shorter derivative (see Figure 1) confirms the observation of KABACK *et al.* (1992) that, in *MSH4* (wild-type) cells, a defined interval is genetically longer when it resides on a shorter chromosome. For the *HIS3-TRP1* interval on the shorter derivative (60 kb), the increase in map length resulting from the bisection is significant. (c) Deletion of *MSH4* (which abolishes interference and reduces the map length of all intervals) enhances, rather than eliminates, the influence of chromosome length on crossover density. The relatively greater influence of chromosome length on the density of crossovers in the *msh4* mutant (significant for all intervals tested) suggests that chromosome-length dependence is a feature of the Msh4-independent crossovers. Solid bar, full-length chromosome (231 kb); shaded bar, shorter derivative (60 kb); hatched bar, longer derivative (180 kb). Figure 2, b and c, is based on the tetrad data tabulated in Table 2.

not in *C. elegans*, DSBR functions aiding in the establishment of synapsis yield noninterfering crossovers. These workers called attention to mutant phenotypes of the meiosis-specific *HIM-14* gene in *C. elegans* and those of its *S. cerevisiae* homolog, *MSH4*. In both yeast and *C. elegans*, *msh4/him-14* mutations reduce crossing over, apparently without affecting the formation or final level of repair of DSBs (ROSS-MACDONALD and ROEDER 1994; NOVAK *et al.* 2001; COLAIACOVO *et al.* 2003). In *C. elegans*, however, *him-14* mutations completely eliminate crossing over, while *msh4* mutations in yeast allow a conspicuous residuum of crossovers, and these crossovers lack interference. One interpretation of these data is that yeast, but not *C. elegans*, has a pathway of DSBR that promotes homolog pairing and produces noninterfering crossovers (and noncrossovers). Intermediates in this pathway do not depend on Msh4 for crossover resolution, although Msh4 may aid in stabilizing the intermediate during its formation as suggested (to us) by the delayed synapsis observed in *msh4* mutants (NOVAK *et al.* 2001; see also MOENS *et al.* 2002; SANTUCCI-DARMANIN *et al.* 2000; reviewed in HOFFMAN and BORTS 2004).

An alternative hypothesis, proposed by STORLAZZI *et al.* (1996; and see SYM and ROEDER 1994), was based on mutant phenotypes of *ZIP1*, a member of the yeast epistasis group that includes *MSH4* and, presumably, its partner *MSH5* (NOVAK *et al.* 2001). *ZIP1* is responsible for making the transverse elements of the synaptonemal complex, and *zip1* mutations, like *msh4* mutations, reduce crossing over and eliminate interference without

affecting the frequency of gene conversion. STORLAZZI *et al.* (1996, p. 9047) proposed that all crossovers in wild-type yeast are subject to interference and that in a *zip1* mutant “precrossover intermediates lacking their special promoting factors mature aberrantly and also, via randomization at the resolution stage, into both crossovers and noncrossovers.” Although the model of Storlazzi *et al.* accounts for the observed residual crossovers and their lack of interference, it does not predict the difference between the mutant phenotypes of *msh4* in yeast and those of *him-14* in *C. elegans*.

The presence of noninterfering crossovers could account for a phenomenon described by ZHAO *et al.* (1995). These workers demonstrated that the strength of interference in wild-type yeast varies significantly among different parts of the genome. We suggest that the weaker interference in some genomic regions simply reflects a relatively higher density of noninterfering crossovers in these regions. Conversely, regions of stronger interference would signal a relatively higher concentration of interfering crossovers. Since crossover interference is strictly dependent on genes in the *MSH4-MSH5-ZIP1* epistasis group, this hypothesis predicts that mutations in these genes should cause a greater reduction in crossing over in genomic segments with normally strong interference than in segments with weak interference in wild-type yeast. Several sets of data indicate that this is, indeed, the case (SYM and ROEDER 1994; NOVAK *et al.* 2001; Figure 3). The hypothesis that the lack of interference in *msh4* or *zip1* mutants was created by the relevant mutations (STORLAZZI *et al.* 1996) makes no predictions

TABLE 2
Tetrad data for Figure 2, b and c

Genetic interval	Chromosome size (kb)	Genotype	Tetrad type			Map distance (cM)
			PD	NPD	TT	
<i>CDC24-LEU2</i> (44 kb)	180	<i>MSH4</i>	201	21	415	42.5
		<i>msh4</i>	255	1	93	14.2
	231	<i>MSH4</i>	202	21	330	41.2
		<i>msh4</i>	243	0	57	9.5
<i>CDC24-ADE1</i> (97 kb)	180	<i>MSH4</i>	83	46	488	61.9
		<i>msh4</i>	207	1	131	20.2
	231	<i>MSH4</i>	114	41	389	58.4
		<i>msh4</i>	204	1	91	16.4
<i>LEU2-ADE1</i> (53 kb)	180	<i>MSH4</i>	375	9	232	23.2
		<i>msh4</i>	280	1	58	9.4
	231	<i>MSH4</i>	339	6	195	21.4
		<i>msh4</i>	259	0	40	6.7
<i>HIS3-TRP1</i> (43 kb)	60	<i>MSH4</i>	163	19	348	43.6
		<i>msh4</i>	222	2	57	12.3
	231	<i>MSH4</i>	276	10	270	29.7
		<i>msh4</i>	264	0	41	6.7

regarding the relationship between the density of Msh4-dependent crossovers and strength of interference.

Another phenomenon, illuminated by KABACK *et al.* (1999), may also be explained by the presence of noninterfering crossovers. These workers started with the observation that, in yeast, the shorter chromosomes have a higher crossover density than the longer ones (Figure 2a) and demonstrated that chromosome length *per se* affects both crossover density and interference. Specifically, they showed that, within a defined chromosome segment, crossover density is higher, and interference weaker, when that segment is embedded in a short chromosome than when it resides in a long chromosome.

KABACK *et al.* (1999) hypothesized that longer chromosomes are more susceptible to interference, and that interference suppresses crossover density. This interpretation predicts that the elimination of interference, *e.g.*, via deletion of *MSH4*, should remove the differential crossover suppression and, hence, abolish the chromosome-length dependence of crossover density. We tested this prediction. Using strains generously provided by D. Kaback, we examined four intervals (one of which is the sum of two smaller, adjacent intervals) embedded in full-length chromosome I (~231 kb) and in shorter derivatives of chromosome I (60 and 180 kb), created by bisection (Figure 1; MATERIALS AND METHODS). Crossover densities for each interval were measured in *MSH4* and *msh4* backgrounds. In wild-type cells (*MSH4*), all four

intervals examined showed a greater crossover density on the shorter chromosomes. The increase was significant for the *HIS3-TRP1* interval on the shorter derivative of chromosome I (Figure 2b). However, instead of abolishing the chromosome-length dependence of crossover density observed in wild-type strains, the *msh4* defect appeared to significantly *enhance* the length dependence in each case (Figure 2, b and c, and Table 2).

Only tetrads with four viable spores were used for the data presented in Figure 2. Such tetrads constituted 72% of the total in the *MSH4* diploid, but only 11–13% in the *msh4* diploid. To guard against the possibility that this small subclass misrepresented the total *msh4* tetrad population, we measured the effect of chromosome length on crossover density in *MSH4* and *msh4* tetrads with fewer than four viable spores and in *MSH4* and *msh4* tetrads with four viable spores. The fraction of tetrads with at least one spore recombinant for *HIS3* and *TRP1* was used as a relative measure of recombination activity. In each case, deletion of *MSH4* resulted in a relative increase in the observed chromosome length dependence of such activity (data not shown), arguing that the poor spore viability of the *msh4* diploid did not affect our results. Together, our results argue against the notion that suppression of crossing over by interference accounts for the lower density of crossing over on longer chromosomes.

On the other hand, the notion that yeast has two kinds

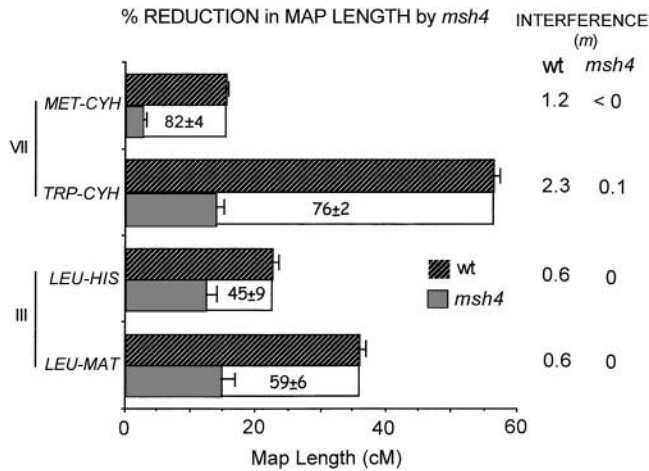


FIGURE 3.—Relationships among interference, chromosome length, and *msh4* phenotype. Two intervals in the relatively short (320 kb) chromosome III and two in the longer (1100 kb) chromosome VII (Figure 2a) were characterized with respect to map length and interference in *MSH4* and *msh4* meioses. The fraction of crossovers that were Msh4 dependent was greater for the intervals in the longer chromosome, in which interference, measured as m , was stronger. In the absence of interference, $m = 0$ (MATERIALS AND METHODS). Data are from ABDULLAH *et al.* (2004) with additional unpublished data from E. PHILPOTT, M. F. F. ABDULLAH and R. H. BORTS.

of crossovers can readily account for the results presented above. The data suggest that one kind of crossover occurs at a roughly fixed number per kilobase, whereas the other occurs at a roughly fixed number per chromosome. The latter kind would cause the shorter chromosomes to have a relatively higher density of crossovers. The enhanced chromosome-length dependence of crossover density apparent in the *msh4* mutants also argues that this length dependence is a feature primarily of the Msh4-independent crossovers, *i.e.*, the noninterfering crossovers. Thus, the longer chromosomes, with their relatively higher proportion of interfering (Msh4-dependent) crossovers, would suffer a relatively greater reduction in crossing over from loss of Msh4 than would shorter chromosomes. We tested this prediction by analyzing linkage data from two intervals on a long chromosome (VII) and two intervals on a short one (III) in both *MSH4* and *msh4* backgrounds. The intervals on the long chromosome proved to have stronger interference than those on the short one, and, as predicted, the *msh4* mutations eliminated interference in each case and removed relatively more crossovers from the longer than from the shorter chromosome (Figure 3).

These results are supported by studies implicating the Mus81-Mms4 endonuclease in the regulation of the Msh4-Msh5-independent crossovers. DE LOS SANTOS *et al.* (2003) report that *mms4* mutations reduce or eliminate a subset of crossovers, especially those on the shorter chromosomes. Unlike the crossovers remaining in *msh4-msh5* mutants, those in *mms4* mutants show interference.

As proposed by the authors, these results suggest that there are crossovers of (at least) two types: those that depend on Msh4-Msh5 and exhibit interference and those that depend on Mms4-Mus81, lack interference, and occur at a higher density on shorter chromosomes.

The data described above argue that the overall distribution of crossovers may be expressed as $X = aL + b$, where X equals map length (in morgans), L equals chromosome length (in base pairs), aL represents the component of crossovers (hypothesized to be interfering) whose number increases with chromosome length, and b represents the crossovers (hypothesized to be noninterfering) whose number per chromosome is insensitive to chromosome length. Figure 2a estimates b at 62 cM and implies that the fractions of length-insensitive crossovers on chromosomes VII and III are 0.16 and 0.43, respectively. These values are compatible with the independently derived averages of 0.21 and 0.48 (Figure 3) for the fractions of Msh4-independent (hence, noninterfering) crossovers on chromosomes VII and III, respectively. Presumably, the frequencies of Mms4-dependent crossovers reported by DE LOS SANTOS *et al.* (2003) also represent the frequencies of Msh4-independent crossovers. Their values were 0.11 and 0.34 for VII and III, respectively. Moreover, a fourth independent method of analysis, described in the companion article (MALKOVA *et al.* 2004), yields values of 0.08–0.12 as the fraction of noninterfering crossovers on chromosome VII. These numbers, arrived at by very different routes, are compatible with the hypothesis that wild-type *S. cerevisiae* meioses generate two populations of crossovers, one of which occurs independently of Msh4, lacks interference, and is distributed at a roughly constant number per chromosome.

For humans, as for Arabidopsis (COPENHAVER *et al.* 2002) and for the yeast chromosome analyzed by MALKOVA *et al.* (2004), the frequency distributions of inter-crossover distances were significantly better described when crossovers assumed to be free of the interference were added to the chi-square distribution (HOUSWORTH and STAHL 2003). Most of the exceptional chromosomes (*i.e.*, those for which the fit of the data was not improved by assuming a fraction of noninterfering crossovers) in the human and Arabidopsis data sets share an architectural feature: the presence of large rDNA arrays, which are thought to aid pairing and synapsis of homologous chromosomes (COPENHAVER *et al.* 2002). This feature may allow these exceptional chromosomes to achieve synapsis with a minimum of DSBR. It is not obvious that chromosome I has a synapsis-promoting feature that could account for our observation that the implied value of ~ 0.30 Msh4-independent crossovers falls short of the ~ 0.56 predicted by the data shown in Figure 2a (but see *Clustered intermediates*, below).

As with yeast, the best estimates for the frequencies of the interference-free crossovers in humans, which differed for the two sexes, were compatible with the values

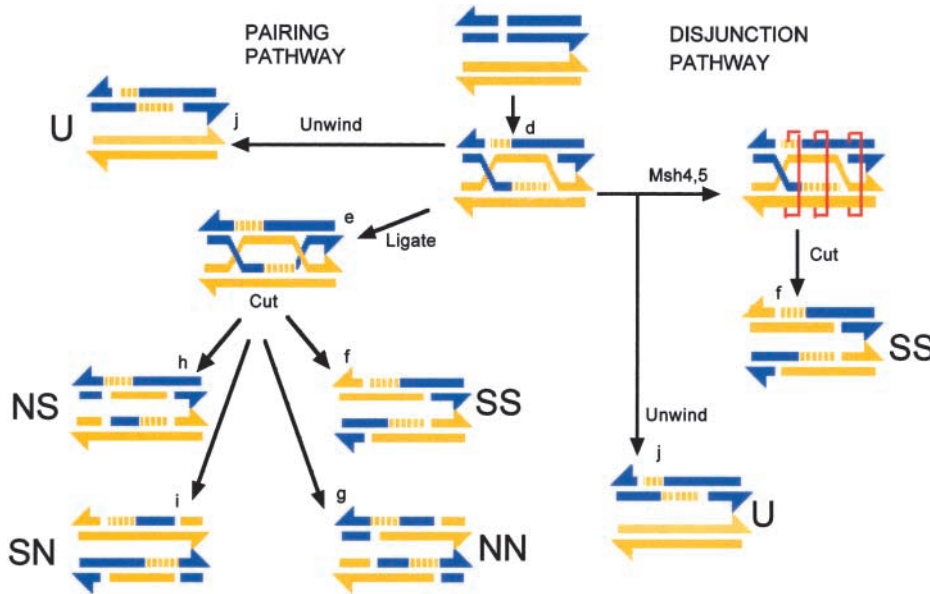


FIGURE 4.—Double-strand-break repair pathways. We propose that DSBs occurring in the pairing pathway give rise to noncrossovers (h and i) and noninterfering crossovers (f and g) via the cutting of fully ligated double Holliday junctions of joint-molecule intermediates (e). These breaks could also give rise to noncrossovers of type j via helicase-dependent unwinding of either ligated (e) or as yet unligated (d) intermediates. DSBR in the disjunction pathway involves only joint-molecule intermediates with unligated Holliday junctions that are either unwound, to form noncrossovers (j), or cut, to form interfering crossovers (f). Lowercase alphabetic designations of resolution products are after HILLERS and STAHL (1999). Uppercase designations indicate the implied mode of resolution of the intermediates. U indicates unwinding

(with or without the aid of a topoisomerase), as deduced from the presence of a heteroduplex on only one of the participating duplexes. The letters S or N (ordered from left to right) refer to the mode of resolvase cutting at the left and right junctions, respectively. S, cutting of the strand that has newly synthesized DNA at the junction and/or the co-polar strand; N, cutting of the pair of co-polar strands that includes *no* newly synthesized DNA at the junction. Additional repair products (not shown) that could have resulted from “synthesis-dependent strand annealing” or “single-end invasion” are discussed in PAQUES and HABER (1999) and HUNTER and KLECKNER (2001), respectively.

for b obtained by fitting the data for each sex to the relationship $X = aL + b$. Such agreement supports the view that interference-free crossovers occur at an average number per chromosome that is independent of chromosome length (L). As mentioned above, similar analyses applied to *Drosophila* and *Neurospora* do not indicate a class of noninterfering crossovers for these organisms. Together, the data support the idea that noninterfering crossovers are unique to creatures that need DSBR functions to achieve synapsis, *i.e.*, group II organisms.

Two periods of DSBR: In group I organisms such as *Drosophila* and *C. elegans*, evidence of repair of meiotic DSBs is seen primarily in pachytene cells, *i.e.*, those in which synapsis of homologous chromosomes is complete (PAGE and HAWLEY 2001; LIU *et al.* 2002; JANG *et al.* 2003; COLAIÁCOVO *et al.* 2003). In contrast, group II organisms depend on DSBR functions to establish synapsis. During leptotene these organisms enjoy programmed DSBs, the repair of which may have reached the stage of “double Holliday junctions” (Figure 4e) before pachytene (reviewed in ZICKLER and KLECKNER 1998) or not (HUNTER and KLECKNER 2001).

Reports (ATCHESON *et al.* 1987; CHU *et al.* 1998) that the yeast transcript of *SPO11*, a gene conserved among eukaryotes and required for DSB formation, reaches its highest level in pachytene lends credibility to the possibility of a round of DSBs at that stage. As in yeast, *SPO11* transcription in mouse occurs from leptotene through pachytene with its maximum level in pachytene (SHANNON *et al.* 1999). This led SHANNON *et al.* (1999, p. 334) to write, “One possibility is that *SPO11* acts in

both the early and middle stages of meiotic prophase,” with the implication that its role at both stages was to make DSBs. The observation by ROMANIENKO and CAMERINI-OTERO (2000) that Spo11 is localized on pachytene chromosomes supports the view that it has a role, although the authors, in the absence of evidence, eschewed the possibility that the pachytene role of Spo11 is to make DSBs. In a different interpretation of a similar experiment, LIU *et al.* (2002), working with the *Drosophila* protein MEI-P22 (which they demonstrated to be required for meiotic DSB formation), took the appearance of MEI-P22 foci on synapsed chromosomes as evidence that, in flies, DSBs are formed after synapsis. The appearance of γ -H2AX, indicative of DSBs, only in the pachytene stage of *Drosophila* supports that conclusion (JANG *et al.* 2003; and see VIERA *et al.* 2004).

Whether or not group II organisms produce DSBs at pachytene, observations made on non-yeast group II organisms suggest that they, like those of group I, engage in a round of DSBR during pachytene. HOTTA and STERN (1971) used DNA-DNA hybridization, density label substitutions, and sensitivity of DNA synthesis to hydroxyurea to demonstrate that “repair synthesis” occurs during pachytene in meioses of lily. Other workers used microscopy to detect the presence of labeled proteins involved in early stages of meiotic DSBR (*e.g.*, the strand-exchange proteins Rad51 and/or Dmc1). These proteins may appear as “foci” or “painted regions” on independently labeled chromosomes, indicating the occurrence of DSBR (see ROEDER 1997 for review). Such methods applied to lily (TERASAWA *et al.* 1995) and

mouse (IKEYA *et al.* 1996) meiosis indicate that foci of labeled Rad51 and Dmc1 localize to the chromatin loops during leptotene/zygotene, while Rad51, but not Dmc1, appears to paint the chromosome cores during pachytene.

Using similar methods, MOENS *et al.* (2002) provided additional evidence for two periods of DSBR in mouse. These authors showed that the sites of DSBR initiated in leptotene did not coincide, in time or space, with a second set of DSBR sites that acquired (during pachytene) foci of Mlh1, a protein required for crossing over in mouse.

Working with yeast, BYERS and GOETSCH (1982) and DAVIDOW and BYERS (1984) used temperature elevation to prolong pachytene and then returned the cells to permissive temperatures to allow sporulation and genetic analysis. The authors reported that longer times spent in temperature-induced pachytene arrest resulted in higher levels of recombination and suggested that this phenomenon represents an extension of normal events, rather than a temperature-induced novel process. Together, the data suggest that group II organisms resemble *Drosophila* and *C. elegans* in undergoing a round of DSBR (and DSBs?) during pachytene (disjunction pathway), but that group II organisms are unique in also undergoing a round of presynaptic DSBR (pairing pathway). We predict that only those crossovers that are derived from the disjunction pathway exhibit interference.

Dmc1p appears to be a group II-specific protein: The hypothesis summarized in Table 1 correlates presynaptic DSBR with a special set of proteins that include Dmc1 and Mnd1. These meiosis-specific proteins have been identified in protists, several yeasts, *Arabidopsis*, mice, and humans; in *Drosophila* and *C. elegans* (TAKANAMI *et al.* 2000; GERTON and DERISI 2002; RINALDO *et al.* 2002) and in *Neurospora* (BORKOVICH *et al.* 2004) they were looked for but not found. Mnd1's meiosis-specific partner, Hop2, which appears to form a complex with Mnd1 (TSUBOUCHI and ROEDER 2002), is another candidate for a protein that occurs uniquely in group II organisms. Only the strand-exchange protein Dmc1, however, has been studied widely enough to inspire a hypothesis as to its function in promoting synapsis. Dmc1 (Lim15 in lily) and its relative Rad51 are eukaryotic homologs of the bacterial protein RecA, which catalyzes homology-dependent exchange between DNA segments (WEST 1992). While Rad51 functions in both vegetative and meiotic cells of all eukaryotes examined, Dmc1 is meiosis specific and appears to be limited to group II organisms (see COPENHAVER *et al.* 2002 for review).

The phenotypes of *dmc1* mutants in *S. cerevisiae* and other group II organisms suggest that Dmc1 promotes synapsis by allowing early DSBs to be processed into intermediates that topologically bind homologous chromatids together. Such intermediates were isolated by SCHWACHA and KLECKNER (1995) and were shown to

have the joint-molecule structure diagrammed in Figure 4e. In meiosis of "SKI" strains of *S. cerevisiae*, *dmc1* mutants fail to form these double-Holliday-junction intermediates, except under special circumstances discussed below (SCHWACHA and KLECKNER 1997; HUNTER and KLECKNER 2001). Instead, these *dmc1* mutants accumulate unrepaired DSBs, and their progress through meiosis is arrested.

"BR" strains of yeast have also been used to examine *dmc1* phenotypes. In these strains the *dmc1* mutations allow significant DSBR and recombination. ROCKMILL *et al.* (1995) demonstrated that BR *dmc1* (and *rad51*) mutants are deficient in chromosome pairing as measured by fluorescence *in situ* hybridization (FISH). Moreover, electron micrographs of silver-stained *dmc1 zip1* chromosomes failed to show "axial associations," the intimate connections between homologous chromosomes visible in *zip1* single mutants. Thus, in group II, presynaptic pairing and subsequent progress through meiosis are normally dependent on Dmc1-mediated DSBR.

Two conditions, identified by SCHWACHA and KLECKNER (1997), allow *dmc1* yeast mutants to undergo DSBR and form joint-molecule intermediates: (1) the absence of the meiosis-specific protein Red1 (and, perhaps, other members of the RED1 epistasis group) and (2)—possibly a special case of (1)—the return of meiotic cells to growth in rich medium. The following section includes a proposal for the significance of these observations.

Issues of special concern to group II: The transition from mitosis to meiosis includes a change in repair templates used for homology-based DSBR—sister chromatids are the preferred templates in mitosis, whereas homologs serve predominantly as templates in meiosis. In yeast, and possibly all group II organisms, DSBs follow promptly upon chromosome replication, at which time homologs are unpaired. In due course, homologous chromosomes realign themselves in a process that is independent of DSB formation or repair (reviewed in BURGESS *et al.* 1999). Until then, however, homologous sequences on sister chromatids (acting as in mitosis) or in nonallelic (ectopic) positions could be serious competitors to allelic homologies as templates for DSBR (GOLDMAN and LICHTEN 2000; WALKER and HAWLEY 2000). Thus, since synapsis depends on allelic inter-homolog interactions, it would appear to be important that group II organisms, especially, have a mechanism for avoiding DSBR until the homologs are the primary candidates for repair template. In pursuit of that possibility, we ask whether extant data in yeast are subject to an interpretation that might solve that problem. In doing so, we do not claim that our interpretation is driven by the data, and we recognize that others will interpret these data differently.

Some phenotypes of mutants in the yeast *RED1-MEK1-HOP1* epistasis group suggest that these genes play a role in preventing premature DSBR. That Red1 prevents

DSBR is readily discernible in *dmc1* mutants, which normally arrest in meiotic prophase with an accumulation of unrepaired DSBs (SCHWACHA and KLECKNER 1997). The absence of Red1 allows *dmc1* mutants to repair their DSBs and progress through meiosis (SCHWACHA and KLECKNER 1997; XU *et al.* 1997). In a *DMC1* background, too, Red1 may delay DSBR as suggested by the RED1-dependent preference for DSBR involving the homolog as opposed to the sister chromatid (SCHWACHA and KLECKNER 1997).

Of course, even in *RED1* cells, the early DSBs need to be repaired in due time. The mutant *red1* and *dmc1* phenotypes suggest that the accumulation of the meiosis-specific protein Dmc1 is required for alleviating the postulated Red1-induced block to DSBR. Presumably, by the time Dmc1 has accumulated sufficiently to overcome the block to repair, the homologs will be closely aligned so that interhomolog, rather than intersister or ectopic, interactions are predominant. In the context of Table 1, the dependence on Dmc1 of timely DSBR implies that the Dmc1-facilitated joint-molecule intermediates (Figure 4e) yield noninterfering crossovers. Suppression of the recombination phenotype in *dmc1* mutants by overexpression of Rad51 (Tsubouchi and Roeder 2003) or Rad54 (Shinozaki *et al.* 2003) suggests that the mechanism by which Dmc1 overcomes the Red1-imposed barrier is to empower strand invasion by Rad51 with the help of Rad54.

Ligated and unligated DSBR intermediates? The “canonical” DSBR model (Szostak *et al.* 1983, as modified by Sun *et al.* 1991) has served as a useful basis for studies of meiotic recombination. Molecular and genetic studies of yeast meiosis have provided evidence in support of the major features of the model: (1) DSBs, (2) 5′-3′ resection of the broken DNA ends, (3) the bimolecular, ligated intermediate (Figure 4e), and (4) the predicted resolution products (Figure 4, f, g, h, and i) (Sun *et al.* 1989, 1991; Schwacha and Kleckner 1995; Gilbertson and Stahl 1996; Hillers and Stahl 1999; for reviews see Roeder 1997; Zickler and Kleckner 1998).

Several observations, however, suggest that the canonical DSBR model describes the DSBR required for synapsis in group II organisms, rather than the type of DSBR that results in crossover interference. For example, the observed ligated bimolecular intermediate is normally dependent on Dmc1, a protein apparently lacking in group I organisms. Moreover, three of the four canonical resolution products are underrepresented in yeast and may be lacking altogether in *Neurospora*, a group I candidate (Table 1, and see below). Conversely, at least two features documented for DSBR in yeast as well as in *Neurospora* were not predicted by the model: (1) the predominance among noncrossovers of resolutions of the type labeled j in Figure 4 and (2) the predominance among crossovers of type f resolutions (Figures 4 and 5; Stadler and Towe 1963; Gilbertson and Stahl 1996; Foss *et al.* 1999; also see data, if not text, from

Merkler *et al.* 2003). Below, we discuss these “noncanonical” features and the mechanism for crossover interference suggested by their presence.

An early hint of noncanonical DSBR comes from an analysis by Stadler and Towe (1963) of the four haploid products from individual acts of meiosis in *Neurospora*. If we grant the order of markers proposed by the authors, their data are simply interpreted as indicative of only two resolution types—essentially all of the crossover resolutions that could be typed were of type f, and none of the noncrossovers showed any sign of reciprocal transfer of markers between the participating chromosomes (as in h’s or i). Instead, all appeared to be of type j, as if the two participants in the intermediate had simply slid apart from each other (Figure 5; h’s would not have been detected in this study because the markers monitoring the conversion were only on the right side of the DSB; their absence is inferred from the absence of i’s with which they share molecular symmetry).

The notion that participants can slide apart suggests that the Holliday junctions, instead of being fully formed as in the canonical model (Figure 4e), have failed to execute the final, ligation, step. Such a failure would mean that the participants are not topologically locked together (Figure 4d) and so could be separated without the involvement of either a topoisomerase or a junction-cutting “resolvase.” If, however, the bimolecular intermediate were stabilized so as to prevent its members from sliding apart, it would become a substrate for a junction-cutting resolvase. As elaborated below, a feature that appears typical of resolvases clarifies how the concept of nonligation of the bimolecular intermediate can account for the possible absence in *Neurospora* (and paucity in yeast) of three out of the four canonical resolution types and suggests a mechanism for interference.

When presented, *in vitro*, with a fully ligated Holliday junction, the most thoroughly characterized resolvases, RuvC from *Escherichia coli* and endonuclease VII from phage T4, are equally likely to cut the two “Watson” or the two “Crick” strands (see, for example, Schwacha and Kleckner 1995). However, when presented with a junction that is nicked (unligated or precut) on one strand, the resolvases always cut the intact strand of corresponding polarity (Fogg and Lilley 2000; Birkenbihl and Kemper 2002). The same principle appears to govern the action of the Mus81-Eme1 complex in fission yeast (Gaillard *et al.* 2003). In a stabilized, unligated intermediate (Figure 4d), the “nick” to be recognized by the resolvase is necessarily adjacent to the 3′ end of the newly synthesized DNA at each junction. This limits, at each junction, the resolvase’s substrate to the strand of the same polarity as that carrying the newly synthesized DNA (S in Figure 4) and dictates that the outcome is, inevitably, a crossover of type f (SS). Candidates for proteins to stabilize bimolecular recombination intermediates include Msh4 and Msh5 (Zallev-

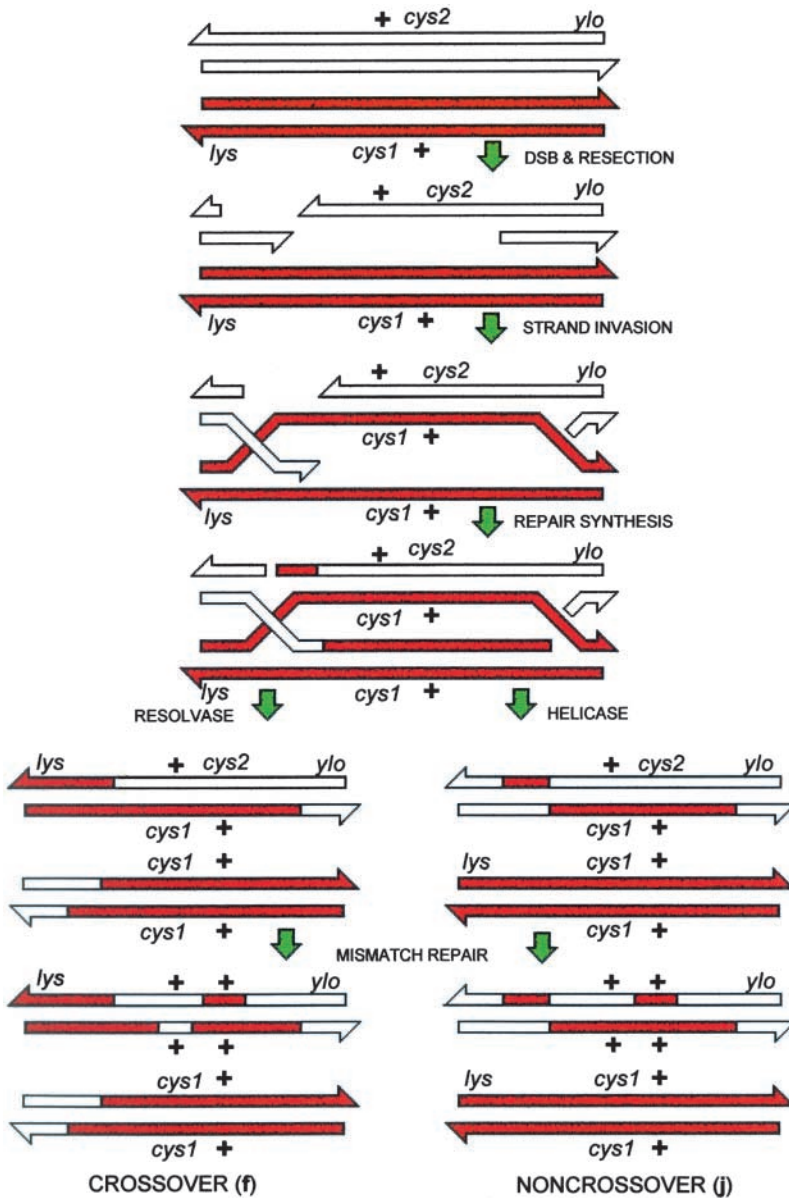


FIGURE 5.—Conversion and crossing over in the *cys* gene of *Neurospora*. The example, which shows only the two interacting chromatids, is for *cys*⁺ arising by conversion at a right-hand site in *cys*. That results when the white (*cys2*) parent is cut, at the hotspot left of *cys*, and resection extends rightward beyond *cys2*. Junction cutting is directed by the strand discontinuities, so that in common parlance, “the crossover is always to the left of the gene” (f, Figure 4). In noncrossovers, the participants slide apart (j, Figure 4). In these studies, postmeiotic segregation was rare, so *cys*⁺ is generally the result of mismatch repair to + at both sites. For both crossovers and noncrossovers, these rules account for “Reciprocal crossing over accompanying cysteine recombination nearly always results from an exchange at the left of the cysteine locus” (p. 1323), and “The striking result that [in the ++ spore pair] the right-hand marker (*ylo* locus) almost always identifies the *cys* mutant which has segregated 3:1” (STADLER and TOWE 1963, p. 1332). (These rules apply as well when the red parent is cut and conversion to + occurs at the *cys1* site.) [Note that in the filamentous fungi examined, unlike in *S. cerevisiae*, meiotic mismatch repair is not directed by strand discontinuities, as revealed by the strong conversion disparities demonstrated for frameshift markers by ROSSIGNOL and PAQUETTE (1979).]

SKY *et al.* 1999; KELLY *et al.* 2000). This speculation is supported by evidence that the Msh4-Msh5 heterodimer binds specifically to Holliday junctions and is then activated by ATP to slide as a clamp, stabilizing the junction (SNOWDEN *et al.* 2004).

Our hypothesis, that unligated bimolecular intermediates characterize DSB in the disjunction pathway (Table 1), gains support from a study of chromosomal DNA isolated from pachytene yeast (BELL and BYERS 1983). These authors used electron microscopy to characterize branched chromosomal DNA structures, which they expected to possess Holliday junctions. As controls they used structures known to have Holliday junctions. They observed, however, that the branch points of the chromosomal pachytene structures failed to reveal the open centers typical of these junctions. Accordingly, they stated that the four-way junctions “. . . could, for example, be nicked Holliday junctions. . .” (p. 838).

Resolution types: If the *in vivo* behavior of resolvases mimics their *in vitro* behavior, the resolution of an unligated intermediate must yield a crossover of type f (SS) if the intermediate is stabilized or a noncrossover of type j (U) if it is not (Figure 4). In contrast, the resolution of a ligated intermediate has no such limitations. The ligation serves both to stabilize the intermediate and to abolish the nick that would have directed the resolvase to one substrate only. Thus, the newly synthesized DNA in ligated intermediates does not signal a substrate for resolvase, allowing indiscriminate (though not necessarily equal) resolution into types SS, SN, NS, or NN, as expected of the canonical DSB model (Figure 4).

If resolution products g (NN), h (NS), and i (SN) are unique to the postulated canonical, leptotene/zygotene DSB, genomic regions or loci that normally show relatively weaker interference should coincide with a rela-

tively higher incidence of such products. Support for this prediction comes from studies in yeast of the *ARG4* locus on chromosome VIII, where interference is strong, and the *HIS4* locus or neighborhood on chromosome III, where interference is weak (KING and MORTIMER 1991; HOFFMANN 2002). At *ARG4*, GILBERTSON and STAHL (1996) found a strong predominance of resolution types f and j, reminiscent of *Neurospora*. At *HIS4*, in contrast, HILLERS and STAHL (1999) and HOFFMANN (2002) found a relatively greater fraction of the products predicted by the canonical DSB model.

Clustered intermediates: While the presence or absence of Msh4-Msh5 binding may guide the resolution fate of a DSB intermediate (ZALEVSKY *et al.* 1999), crossover interference according to the counting model will result only if the bound intermediates routinely flank a quasi-constant number of unstabilized intermediates. How might such a pattern be created? Perhaps (as part of the process of chromosome condensation?), neighboring intermediates (-to-be) are gathered into clusters of more or less fixed size (STAHL 1993) in which one member, in a favored position, competitively commandeers the multiple Msh4-Msh5 heterodimers needed for stabilization. Then, only that member, for instance the middle one, becomes a crossover.

Direct evidence for clustering of sites of DSB in yeast comes from experiments that address the relationship between meiotic DSB and “synapsis initiation complexes” (SICs—colocalizations of Zip3 and Zip2 and other proteins required for normal frequencies of crossing over; FUNG *et al.* 2004). HENDERSON and KEENEY (2004) measured the fate of SICs, presumed sites of crossing over, as a function of decreasing frequencies of DSBs achieved through the use of *SPO11* hypomorphs. They found that loss of DSBs down to ~40–20% of wild type had little or no effect on the survival of Zip3 foci. DSB frequencies of <~20%, however, caused a steep decline in Zip3 foci. They report that crossovers were lost with similar kinetics. Such kinetics imply that each wild-type SIC has multiple DSB sites and that a mutant SIC, and its associated crossover, will be lost only if every site in that SIC fails to receive a DSB. The equation for this model fits the data of HENDERSON and KEENEY (2004) if its single adjustable parameter—cluster size of one crossover plus m noncrossovers—is set at five (Figure 6). Those authors suggest (K. A. HENDERSON and S. KEENEY, personal communication) that their data are better described as compatible with a range of N -values from 4 to 9. Using the two-pathway model to analyze crossover interference in tetrad data from yeast chromosome VII, MALKOVA *et al.* (2004) estimated $m + 1 = 4$. Uncertainties in the two methods of calculating m are such that the estimates appear compatible with each other.

This view of SICs, provoked by the data of HENDERSON and KEENEY (2004), demands that, in wild-type strains, the items clustered are guaranteed to receive a DSB without regard to whether the DSBs occur prior to or

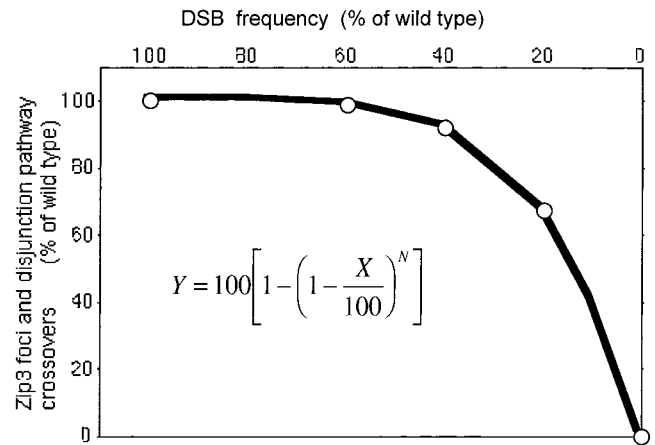


FIGURE 6.—Theoretical dependence of SIC formation (and crossing over in the disjunction pathway) on DSB rate for five-membered clusters (equivalent to $m = 4$ in the counting model). The model assumes that a synapsis initiation complex (SIC) will form and a crossover in the disjunction pathway will occur as long as one member of the cluster receives a DSB. Y and X refer to the axes. The points were hand calculated from the equation with N , the number of recombination intermediates in the cluster, $= 5$ and values for X chosen at convenient intervals. The curve was drawn by kaleidograph; it appears to be a good model for the experimentally based curve in Figure 5C of HENDERSON and KEENEY (2004).

following the clustering. The SICs, which we take to be clusters of “attempts”, manifest nonrandom spacing characteristic of chiasma interference and occur at a frequency of about two-thirds the overall frequency of crossing over (ROCKMILL *et al.* 2003; FUNG *et al.* 2004), making them eligible candidates for sites of crossing over in the disjunction pathway. FUNG *et al.* (2004) observed that the spacing of SICs is unaffected by *zip1* mutations, which eliminate crossover interference, suggesting that the absence of Zip1, and hence of Msh4-Msh5 proteins (NOVAK *et al.* 2001), causes all DSB intermediate in each SIC to be resolved, by default, as noncrossovers (j’s, Figure 4). The concept of clustering is further boosted by the demonstration that “late recombination nodules,” each of which, in group I organisms, demonstrably corresponds to a single crossover, can be seen by electron microscopy (in pachytene of pigeon meiosis) to be composed of four to five morphologically equivalent subunits (PIGOZZI and SOLARI 1998). Each such subunit may represent a recombination intermediate with associated proteins.

A cluster version of the counting model raises concerns regarding “end effects.” Clusters at one end of the chromosome (or the other, or both) are apt to fall shy of the normal number of attempts. Crossovers in such shorted clusters would manifest reduced interference and would occur at increased density. This effect, which would have a greater impact on the analysis of interference in short chromosomes than in long ones, will be assessed in subsequent analyses. At this point we note that such an end effect could contribute to the

inverse relationship between crossover density and chromosome length (*e.g.*, Figure 2a) and might account for the quantitative discrepancy noted above between the observed and “expected” frequency of Msh4-independent crossovers on the very short chromosome I.

Implications and ramifications: An implication of the perspective presented here is that mitotic Rad51-dependent DSBR resembles the DSBR of the disjunction pathway, and that such repair occurs via an unligated joint-molecule intermediate. In the apparent absence of proteins to stabilize this intermediate, most mitotic products would be noncrossovers, as observed (for review, see IRA *et al.* 2003). Furthermore, crossovers should be resolution type f, as was observed by BAKER and BIRMINGHAM (2001) in their studies of *in vivo* homologous recombination between a linearized transfer vector and a mammalian chromosome. That no direct identification of joint-molecule intermediates has been reported for mitotic DSBR may reflect a short life span for such intermediates as compared with those detected in meiotic prophase.

The proposal summarized in Table 1 rests on the premise that the unique attributes of group II organisms all serve to promote synapsis between homologous chromosomes. It may be distilled into the following set of related hypotheses: (1) Interference, wherever it occurs, is governed by the rules of the counting model and is based on the patterning and resolution of repair intermediates; (2) the noncrossovers and the interfering crossovers arising from the disjunction pathway of DSBR represent the sole alternative resolution modes of intermediates with properties such as those of the unligated joint molecules pictured in Figure 4d (see also Figure 5); (3) crossover resolution of such intermediates requires proteins such as Msh4-Msh5 to stabilize the intermediate until its junctions are cleaved by a resolvase; (4) the pattern of Msh4-Msh5 binding required for interference is achieved through competition within clusters [the size of the clusters determines the intensity of interference (*m* in the counting model)]; (5) those creatures (group II) that use DSBR to promote synapsis are a subclass of organisms that have crossover interference; (6) synapsis-promoting DSBR generates canonical DSBR intermediates (which tie the homologs together), yielding noninterfering crossovers of types g and f and noncrossovers of types h, i, and, possibly, j (Figure 4); (7) the noninterfering crossovers found in mutants such as *msh4* in *S. cerevisiae* represent these noninterfering crossovers present in wild-type organisms; (8) chromosomes without pairing centers have a roughly fixed average number of Msh4-independent, noninterfering crossovers, regardless of chromosome length; and (9) genes in the *RED1* epistasis group prevent premature repair of DSBs.

The body of this article exposes the rationale leading to these interrelated postulates. We present our view

for its explanatory, predictive, and iconoclastic value and for its vulnerability to experimental test.

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