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Exchanges are not equally able to enhance meiotic chromosome segregation in yeast

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ABSTRACT **Homologous chromosomes pair, and then migrate to opposite poles of the spindle at meiosis I. In most eukaryotic organisms, reciprocal recombinations (crossovers) between the homologs are critical to the success of this process. Individuals with defects in meiotic recombination typically produce high levels of aneuploid gametes and exhibit low fertility or are sterile. The experiments described here were designed to test whether different crossovers are equally able to contribute to the fidelity of meiotic chromosome segregation in yeast. These experiments were performed with model chromosomes with which it was possible to control and measure the distributions of meiotic crossovers in wild-type cells. Physical and genetic approaches were used to map crossover positions on model chromosomes and to correlate crossover position with meiotic segregation behavior. The results show that crossovers at different chromosomal positions have different abilities to enhance the fidelity of meiotic segregation.**

Reciprocal recombination is critical for successful sexual reproduction in most eukaryotes. Haploid gametes are generated by meiosis in which a single round of DNA replication is followed by two successive rounds of chromosome segregation. In meiosis I, homologous chromosomes pair and recombine, and then segregate to opposite poles of the cell (disjoin) at anaphase (Fig. 1*a*) (for review see ref. 1). Crossovers between homologs are critical for the formation of chiasmata, the linkages between homologous chromosomes that can be observed in cytologically tractable organisms, which keep homologs joined until anaphase I. In the absence of chiasmata, homologous chromosomes have a greatly elevated chance of segregating independently of each other, often migrating to the same pole at anaphase I (nondisjunction) (Fig. 1*b*) (for a discussion of exceptions see ref. 1). Organisms with mutations that abolish meiotic recombination experience randomized partitioning of chromosomes at meiosis I, leading to aneuploid gametes and reduced fertility or sterility. In humans, the failure of chromosomes to experience exchange in meiosis is correlated with the generation of gametes aneuploid for certain chromosomes (2).

Exchange alone is not sufficient for securing paired homologous chromosomes until anaphase I. A second uncharacterized activity, termed chiasma binder, is needed to stabilize the linkage between homologs (3, 4). The requirement for chiasma binder is illustrated in Fig. 2*a*, which shows that a resolved crossover, by itself, would not be expected to hold a pair of homologous chromosomes together. There are two basic models for the way in which chiasma binder function is achieved (4). In the first model, chiasma binder maintains the association of sister chromatids distal to the chiasma (Fig. 2*b*). In the second model, chiasma binder acts at the chiasma (Fig. 2*c*). These models are not mutually exclusive, and evidence consistent with both of them has been described (for review see ref. 5).

Crossovers do not guarantee disjunction, rather they enhance the chances that it will occur. The observation of nondisjoined homologs that have experienced crossovers raises the possibility that some crossovers may have properties that cause them to form less effective chiasmata than other crossovers. The experiments described here were designed to test systematically whether different crossovers were equally able to enhance the fidelity of meiotic chromosome segregation. To test the ability of a chromosomal exchange to influence disjunction, it is necessary that the exchange be the only one between the homologs whose segregation is being monitored. This situation is rare in yeast, because even the smallest chromosomes typically experience two or more exchanges in most meioses. Yeast artificial chromosomes and derivatives of yeast natural chromosomes offer the advantage that they can be constructed such that they experience one (E_1) or zero (E_0) exchanges in most meioses (6–8). The experiments presented here show that exchanges in different genetic intervals of these model chromosomes have different abilities to enhance disjunction.

MATERIALS AND METHODS

Strains, Media, and Genetic Methods. Media was prepared as described (9). *Saccharomyces cerevisiae* strains are described in Table 1. Mini chromosomes and artificial chromosomes can vary in copy number. Sporulation and tetrad analysis was performed as described (8). Only four spore viable tetrads with two copies of each chromosome of interest were informative for these experiments. A tabulation of all of the classes of four spore viable tetrads observed in each experiment is found in the corresponding figure legend. Statistical analyses were performed using the G test (10) or comparisons of the 95% confidence limits of Poisson sampling variability (11).

Construction of Artificial Chromosomes and Mini III Chromosomes. The construction and features of the yeast linear plasmids (YLps) and the mini III chromosomes have been described (6, 8, 12). Chromosomal alterations were accomplished using one- and two-step gene replacement techniques (13, 14). The mini III chromosomes diagrammed in Fig. 4 are the same as those described in ref. 8. The artificial chromosomes diagrammed in Fig. 3 are derived from those described in ref. 8 with the following modifications. The left arm *Xba*I site on YLp201 has been removed so that crossover positions on the left arm can be determined with respect to the location of the *Xba*I site (Fig. 3). pA252p6 (6), which carries sequences corresponding to part of the left arm of the YLp including a telomere *LEU2* and a fragment of λ phage DNA with the *Xba*I site, was cut with *Xba*I, with flush ends created using T4 polymerase and recircularized to create pL29. A strain carrying a *leu2, trp1, URA3, ARG4, his3* YLp was transformed with a *Bam*HI fragment of pL29. This fragment includes the

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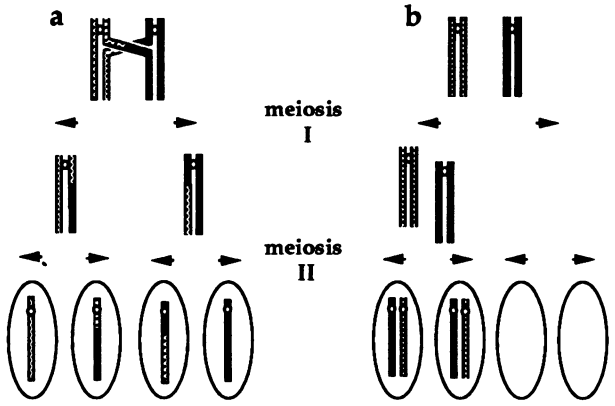

FIG. 1. Meiosis I disjunction (*a*). Meiosis I nondisjunction (*b*).

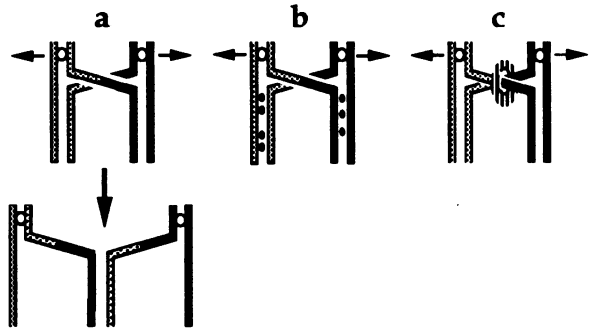

FIG. 2. Models for chiasma binder activity. (*a*) A pair of homologs joined by a chiasma. (*b*) Chiasma binder is represented as cohesion between sister chromatids. (*c*) Chiasma binder acting at the site of the exchange to fix the chiasma in place.

telomere, *LEU2*, and the λ DNA with the modified restriction site. After transformation, Leu$^+$ transformants were screened by Southern blot analysis for loss of the *Xba*I site. YLp200 is *leu2*, *TRP1*, *ura3*, *arg4*, *HIS3*, but has had the *Nhe*I site removed and replaced with three HO endonuclease recognition sites. The strains used in these experiments are *ho/ho,* and in this strain background the presence of the HO sites results in no detectable change in the levels of crossing-over in the left arm of the YLps (data not shown). The effect of double-strand breaks at these sites in meiosis will be described elsewhere. A 502-bp *Ssp*I to *Bam*HI fragment of λ bacteriophage DNA (λ map position 34,499 to 35,001) that carries the *Nhe*I site was cloned into *Ssp*I, *Bam*HI digested YIp5. The *Nhe*I site was cleaved and filled in with T4 polymerase. Three copies of a 24-bp oligomer with an HO endonuclease cut site and a *Kpn*I restriction site was ligated into the blunted site to create plasmid pD148 (a *URA3* vector). A strain carrying an *leu2*, *TRP1*, *ura3*, *arg4*, *HIS3* version of the artificial chromosome was transformed with pD148 digested with *Bst*XI, and a two-step selection was used to construct YLps that had been modified by the *Nhe*I/HO site sequences on pD148 (14). Candidates were then screened by Southern blot analysis for loss of the plasmid but maintenance of the *Kpn*I carrying inserts.

Extrapolation of Crossover Positions in the Left Arm. The positions of the 170 crossovers in the left arm relative to the dimorphic *Xba*I site adjacent to *LEU2* were predicted by determining the crossover positions in a subset of the 170 tetrads, using approaches described by others (15, 16) and extrapolating from these data. The only other *Xba*I site on the YLps is in the *TRP1* gene and the *trp1* allele on one of the YLps was created by removing this site. Therefore, by examining *Xba*I fragment sizes from YLps that had experienced an exchange in the left arm it was possible to determine whether it mapped to the left or right of the telomere proximal *Xba*I site. The positions were determined for crossovers on YLps from 28 randomly selected tetrads in which the YLps had experienced a left arm crossover and disjoined, and the four tetrads in which the left arm crossover YLps had nondisjoined. One of the 28 crossovers was associated with a conversion of the *Xba*I site making it impossible to determine on which side of the site it occurred.

RESULTS

Analysis of Exchange and Segregation of Homologous YLps. A pair of artificial homologs, or YLps, was used to determine whether a single exchange could enhance the fidelity of meiosis I segregation in yeast. The YLps are composed primarily of bacteriophage λ DNA, and carry yeast elements known to be necessary for chromosome replication and maintenance (Fig. 3*a* and refs. 6, 8, and 17). The YLps are differentially marked with yeast genes and an *Xba*I restriction site dimorphism making it possible to assign exchanges to one of five intervals (Fig. 3*a*). Genetic analysis of a strain containing these YLps (DL363) was used to determine the segregation fidelity of YLps that had experienced exchanges in each of the genetic intervals. The analysis was performed in three steps. (*i*) Tetrads were dissected on rich medium and spore colonies were replica-plated to media lacking leucine, tryptophan, uracil, arginine, histidine, or adenine. Artificial chromosomes that disjoined, either with or without an exchange, were identified by the growth of spores containing them on these plates. Additionally, this step identified tetrads in which the sister chromatids of one of the YLps had precociously segregated at meiosis I (PSSC), and tetrads in which one of the YLps had been lost from the cell or was carried in two copies prior to meiosis. (*ii*) We attempted to determine the genetic maps of chromosomes in spores containing more than one YLp as a result of nondisjunction or PSSC. Because of the mitotic instability of the artificial chromosomes, it was possible to isolate two derivatives of each spore carrying nondisjoined artificial chromosomes; cells that had lost one of the nondisjoined artificial chromosomes and cells that had lost the other. The growth characteristics of these two derivatives were tested, using the media described above, to determine whether the nondisjoined artificial chromosomes had parental or recombinant configurations of genes. (*iii*) We attempted to determine by Southern blot analysis the distribution of crossovers mapped to the left arm relative to the dimorphic *Xba*I site (15, 16). Genetic analysis revealed that there were 4 nondisjunction and 166 disjunction tetrads among the 170 tetrads with a single exchange between *TRP1* and *LEU2*. Southern blot analysis of DNA from the 4 nondisjunction tetrads and 28 randomly selected disjunction tetrads was performed to determine the distribution of exchanges on the left arm relative to the dimorphic *Xba*I site. These data were used to estimate the distribution of all 170 left arm exchanges.

Exchanges in Different Regions of the YLps Are Correlated with Different Levels of Nondisjunction. Among the 2516 tetrads with 4 viable spores and a single copy of each YLp upon entry into meiosis were 2 tetrad classes that were not informative with respect to the ability of individual exchanges to enhance segregation. The first included 156 tetrads in which 1 of the YLps had experienced PSSC. The second included 199 tetrads with multiple exchanges (the YLps disjoined in all of these tetrads). Among the 2334 informative tetrads were 1277 in which the YLps did not experience an exchange (E_0). The nondisjunction frequency of the YLps in these tetrads was 11.5% (Fig. 3 *b* and *c*). This nondisjunction frequency is typical of nonexchange chromosomes in yeast in which one pair of nonexchange or artificial chromosomes is typically partitioned at meiosis I with about 90% fidelity using unknown mechanisms (6, 18–21). In contrast, the YLps in the 1057 E_1 tetrads

Table 1. Strains used in this study

Strain	Genotype	Source
DD97	*MATa/MATα, trp1-1/trp1-1, ura3-52/ura3-52, his3-11, 15/his3-11, 15, ade1/ADE1, arg4-17/ARG4, leu2-3, 112/leu2-3, 112, lys2/lys2.* Mini III[*URA3, leu2-3, 112, trp1*(*Xba*I site fill-in)]. Mini III[*ura3* (*Nco*I site fill-in), *LEU2, TRP1*].	ref. 8
DL363	*MATa/MATα, trp1-1/trp1-1, ura3-52/ura3-52, his3-11, 15/his3-11, 15, ade1/ADE1, arg4-Hpa*I deletion/*arg4-Hpa*I deletion, *leu2-3, 112/leu2-3, 112.* YLp201[*leu2* (*Cla*I site fill-in), *TRP1, ura3* (*Nco*I site fill-in), *arg4* (AT removal at *Eco*RV site), *HIS3*]. YLp200[*LEU2, trp1* (*Xba*I site fill-in), *URA3, ARG4, his3* (*Hin*dIII site fill-in)].	This study
DL648	*MATa/MATα, trp1-1/trp1-1, ura3-52/ura3-52, his3-11, 15/his3-11, 15, ade1/ADE1, arg4-17/ARG4, leu2-3, 112/leu2-3, 112, lys2/lys2.* Mini III[*URA3, leu2-3, 112, trp1* (*Xba*I site fill-in)]. Mini III[*ura3* (*Nco*I site fill-in), *LEU2, TRP1*].	This study

showed significantly lower levels of nondisjunction (2.6%; see Fig. 3*c*). Therefore, exchange is correlated with enhanced segregation fidelity but does not guarantee disjunction.

To test whether all exchanges between YLps were equally able to enhance disjunction, the nondisjunction frequency of YLps that had experienced single exchanges in each of the five intervals was determined (Fig. 3*b*). If all exchanges are equally able to fail to ensure disjunction, then exchanges in each of the five intervals should be correlated with the same nondisjunction frequency (2.6%). This was not the case; nondisjunction frequencies of YLps with crossovers in the five intervals differed significantly from each other ($G = 36.1$, $df = 4$, $P < 0.005$). YLps with exchanges in their outermost intervals showed the highest nondisjunction frequencies (4.6% and 9.0%; see Fig. 3*b*). These frequencies were not significantly different than the nondisjunction frequency of 11.5% exhibited by YLps that had experienced no exchange (that is, the 95% Poisson confidence values are overlapping; see Fig. 3*b*). YLps with exchanges in the central three intervals showed significantly lower levels of nondisjunction than nonexchange YLps (1.6%, 0%, and 0.9% versus 11.5%; see Fig. 3*b*), or YLps with exchanges in interval 5 (Fig. 3*b*).

Not All Exchanges on Mini III Chromosomes Ensure Disjunction. The ability of exchanges between short derivatives of chromosome III (mini III chromosomes) to enhance segregation fidelity were evaluated using an approach similar to that described above. The mini III chromosomes used in these experiments are approximately 72 kb in length and have a central region of 55 kb of contiguous chromosome III sequences, which includes the centromere (Fig. 4*a*). The mini III chromosomes have genetic markers that make it possible to divide them into three intervals. Interval one includes 3 kb of pBR322 and 3 kb of *HMR* inserted between *URA3* and *LEU2*. Interval 2 is composed entirely of contiguous chromosome III

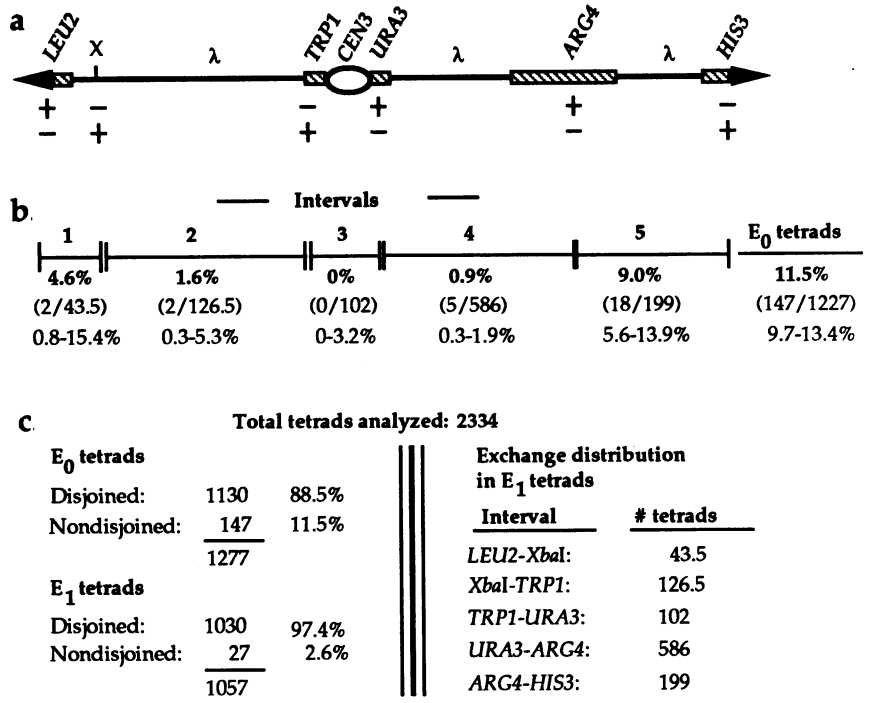

FIG. 3. Meiotic segregation of artificial chromosomes that have experienced a single exchange. (*a*) The artificial chromosomes used in this study; YLp200 (*LEU2, trp1, URA3, ARG4, his3*), and YLp201 (*leu2, TRP1, ura3, arg4, HIS3*). (*b*) Three values are shown for each interval. From top to bottom these values are: the nondisjunction frequency for YLps that experienced a single exchange in that interval (boldface type); the number of nondisjunction-E_1 tetrads over the total number of E_1 tetrads with exchanges in the indicated interval; the 95% confidence limits, expressed in terms of percent nondisjunction, based on the assumption that the number of nondisjunction tetrads exhibits Poisson sampling variability (11). (*c*) Summary of tetrads used to determine the ability of exchanges to enhance artificial chromosome segregation. Additional tetrad data: 3840 DL363 tetrads were dissected yielding 3037 with four viable spores. The segregation patterns in 59 of these tetrads suggested that one of the YLps had experienced a mitotic rearrangement or gene conversion. In 289 tetrads, 1 or both of the YLps had been lost or was carried in 2 copies per cell upon entry into meiosis. In 156 tetrads, 1 of the YLps experienced precocious separation of sister chromatids (PSSC), and in 199 tetrads there were multiple recombinations between the YLps. The remaining 2334 tetrads are described in the figure.

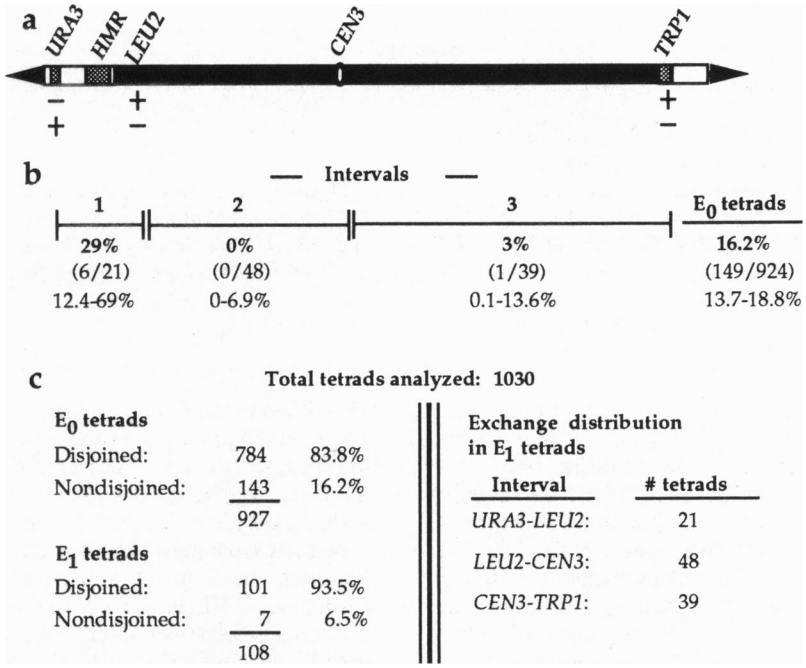

FIG. 4. Nondisjunction frequency of mini III chromosomes that have experienced a single exchange. (*a*) The mini III chromosomes used in this study. (*b*) Three values are shown for each interval as in Fig. 3. (*c*) Summary of tetrads used to determine the ability of exchange to enhance mini III chromosome segregation. To examine the correlation of exchanges and disjunction of E_1 and E_0 mini III chromosomes, pooled tetrads from strains DD97 and DL648 were examined. Among the 1733 dissected tetrads, 1291 had 4 viable spores. In addition to the tetrad classes indicated in the figure, we also identified 103 tetrads with PSSC of one of the mini III chromosomes, 69 tetrads that had 2 or 0 copies of 1 of the mini III chromosomes, 36 tetrads with an apparent gene conversion of a mini III chromosome marker (half of these were 1:3 for the Ura^+ phenotype and could indicate a gene conversion, a loss, or a meiosis II nondisjunction), 12 tetrads in which 1 of the mini III chromosomes had experienced a modification in mitosis, and 36 tetrads that had experienced multiple events (about half of these were most easily explained by double crossovers or crossovers with a gene conversion).

sequences, from *LEU2* to *CEN3*, and interval 3 extends from the centromere to a *TRP1* gene inserted at the *PGK* locus of chromosome III (8, 17).

As with the YLps, mini III chromosomes that experienced exchange showed significantly lower levels of nondisjunction than those that did not (6.5% nondisjunction versus 15.4%; $G = 7.5$, $P < 0.01$; Fig. 4*c*). Exchanges in interval 1 were correlated with significantly higher levels of meiosis I nondisjunction (29%) than exchanges in the adjacent interval 2 (0%) (Fig. 4*b*).

DISCUSSION

The differential abilities of exchanges to enhance meiotic chromosome segregation is probably not unique to yeast. Studies of *Drosophila melanogaster nod*, *Dub*, and *ord* mutants, all with defects in meiotic chromosome segregation, have suggested that not all exchanges are equally able to enhance segregation. The meiotic apparatus of *Drosophila* females includes an achiasmate segregation system that can properly partition one or two pairs of nonexchange chromosomes in meiosis I (22, 23). *nod* mutants are specifically defective in the achiasmate pathway, and exhibit greatly elevated levels of nondisjunction of nonexchange chromosomes (24, 25). *Dub* mutants are defective in achiasmate segregation as well as other aspects of meiotic chromosome partitioning (26). Both of these mutants experience slightly elevated levels of nondisjunction of chromosomes that have single, centromere distal exchanges (exchanges near their telomeres). Observations of the *nod* mutants led to the suggestion that in wild-type *Drosophila*, the achiasmate system is occasionally employed to partition chromosomes with exchanges near their telomeres that have failed to ensure disjunction (24, 25). Finally, the product of the *ord* gene is important for sister chromatid cohesion and is a good candidate for contributing to chiasma binder function (27, 28). In *Drosophila* with mildly defective *ord* alleles, exchanges in intervals near the telomeres have reduced ability to enhance disjunction (27).

Also, in humans, there is evidence that not all crossovers contribute equally to enhancing chromosome segregation. Recent studies of human meiotic chromosome segregation have shown that among nondisjoined copies of chromosome 21 that have experienced exchange, the distribution of exchanges is skewed toward the telomere as compared to the exchange distribution of properly segregated copies of chromosome 21 (2).

Why don't all exchanges have the same ability to enhance chromosome segregation? It has been suggested that distal exchanges in *Drosophila* may be unable to orient the attachment of kinetochores to fibers from opposite spindle poles because the exchanges are too far from the centromeres (25). For the yeast model chromosomes, even the exchanges that are farthest from the centromeres (30 kb) would be considered reasonably close to the centromere were they on a natural chromosome. In most yeast meioses there are probably several chromosomes with no exchanges located within 30 kb of the centromere, yet these chromosomes segregate properly. This suggests that this degree of centromere-exchange proximity is not needed to orient the kinetochores in yeast, and that the failure of distal exchanges on the model chromosomes is not because they are too far from the centromere. An alternative explanation for the failure of these exchanges to enhance disjunction is that they are too close to the ends of the chromosomes. The failure of exchanges adjacent to telomeres to ensure disjunction may be attributable to the inability of chiasma binder to secure them in place such that the homologs lose their linkage and segregate independently of each other at anaphase I (Fig. 1*b*). Chiasma binder might be imagined to fail to secure chiasmata that are near telomeres for one of three reasons. First, it may be that chiasma binder is not efficiently

established in telomeric regions because of the DNA sequences or chromatin structures found in these regions. Second, if chiasma binder strength reflects the combined contributions of multiple weak associations between sister chromatids distal to the exchange, the effectiveness of chiasma binder would decrease with the reduced length of the telomere-to-exchange interval. Similarly, if a single sister chromatid association provides full chiasma binder activity, and if these associations are infrequent, then as exchanges approach telomeres there is a decreasing probability that there will be an association distal to the exchange. Our observation that the ability of an exchange to enhance segregation fidelity appears to be proportional to its distance from the telomere is consistent with the latter two models.

Model chromosomes with centromere-proximal exchanges occasionally nondisjoined in our experiments (about 1% for intervals 2, 3, and 4 on the YLps). Whether the failure rate of these exchanges is the same as would be seen for single exchanges on natural chromosomes or is higher because of inadequacies of the model chromosomes is unclear. If exchanges on natural chromosomes fail at this frequency, then it may be that the high fidelity of yeast chromosome segregation is attributable to the additive ability of several exchanges, each with a modest failure rate, to ensure disjunction.

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