An Alternative Pathway for Meiotic Chromosome Segregation in Yeast

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In meiosis I of most organisms, homologous chromosomes pair, recombine, and then segregate to opposite poles of the cell. Crossing-over is normally necessary to ensure the proper segregation of the homologs. Recently developed techniques have made it possible to study meiosis with highly defined artificial chromosomes. These techniques were used to demonstrate the existence of a system capable of segregating pairs of nonrecombined artificial chromosomes, regardless of the extent of their sequence homology. This system may contribute to the high fidelity of meiosis by mediating the segregation of pairs of natural chromosomes that have failed to recombine.

THE SUCCESS OF SEXUAL REPRODUCTION DEPENDS UPON the faithful transmission of parental genetic information to the germ cells. Meiosis, the process of halving the diploid number of chromosomes to yield haploid germ cells, occurs in two steps. In meiosis I, homologous chromosomes, each composed of two sister chromatids, pair with each other, recombine, and then segregate to opposite poles of the spindle. The second meiotic division appears identical to mitosis: the sister chromatids of each chromosome separate from each other and segregate to opposite poles of the spindle (Fig. 1A). Meiotic chromosome segregation is extremely accurate; for yeast chromosome V errors occur at a frequency of less than 10^{-4} per meiosis (*1*). Improper meiotic segregation usually has severe consequences (Fig. 1, B and C). In humans, Down's syndrome occurs when a fetus carries an extra copy of chromosome 21, usually as a result of meiosis I nondisjunction in gametogenesis (*2*).

Previous studies of meiotic chromosome segregation have been complicated by two problems. First, the large size and undefined structural characteristics of natural chromosomes make it nearly impossible to determine which features are essential for proper segregation and which are dispensable. More important, aneuploid cells which result from segregation errors are often inviable and cannot be analyzed. We have been able to circumvent these difficulties by studying meiosis in yeast, where artificial chromosomes and small chromosome derivatives can be used as models for studying the meiotic behavior of natural chromosomes. These model chromosomes have defined structures that can easily be altered to meet the needs of a specific experiment. Furthermore, since they are not essential for viability, cells that have lost them are amenable to analysis.

Genetic studies in many organisms have shown that crossovers between homologous chromosomes are necessary to ensure that homologs segregate from each other at meiosis I. Mutations in *Drosophila melanogaster* females that decrease the number, or alter the distribution, of crossover events increase the level of meiosis I nondisjunction (*3*). In yeast, the recombination defective mutants *rad50* and *spo11* produce high levels of inviable spores; in the case of *spo11*, it has been shown that the rare viable spores have not recombined (*4, 5*). When either of these mutations is combined with the *spo13* mutation, which bypasses meiosis I, viable diploid spores are produced (*5*). These results suggest that homolog segregation in meiosis I requires recombination, and that the spore inviability observed in *rad50* and *spo11* mutants is due to increased meiosis I nondisjunction in the absence of reciprocal recombination.

These genetic studies are complemented by cytological observations. Crossovers can be viewed cytologically as chiasmata, the structures that appear in meiotic prophase I as the points of physical linkage between homologs (*6–10*). Chiasmata seem to serve two related functions. First, they keep homologs joined until all homolog pairs in the cell have become positioned at the metaphase plate. Second, chiasmata are probably part of the mechanism that signals that homologous centromeres are attached to spindle fibers that radiate from opposite poles rather than from the same pole (*11, 12*).

Although most chromosome segregation systems are recombination-dependent, there are exceptions. Female *Drosophila melanogaster* use a recombination-dependent system for the meiotic segregation of three of their four chromosomes, but they also use an additional recombination-independent segregation system (*13*). This segregation process, called the distributive system, is responsible for the correct segregation of chromosome 4, the shortest *Drosophila* chromosome (*14*), which does not undergo crossing-over in most meioses (*15*). The distributive system shows no dependence on sequence homology, but instead preferentially segregates noncrossover chromosomes of similar size (*16*). In *Drosophila*, the distributive system can successfully segregate only a small number of chromosomes. When meiotic crossing-over is completely blocked, high levels of aneuploidy are observed (*3, 17*).

We now present experiments that test the roles of sequence homology and crossing-over in meiosis I. Our results show that homologus artificial chromosomes composed mainly of bacteriophage lambda DNA exhibit unusually low levels of crossing-over and that this crossing-over is neither required for segregation, nor does it ensure segregation as it does for natural chromosomes. Instead, artificial chromosomes and small chromosome derivatives are segregated by a recombination and homology-independent system that may serve as a backup to the normal crossover-mediated pathway for meiosis I segregation.

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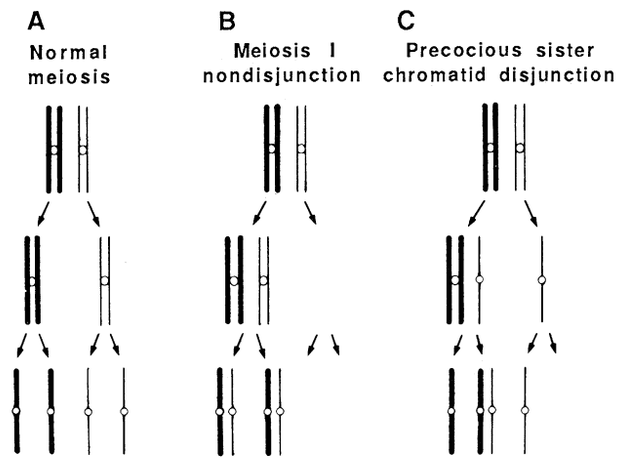

Fig. 1. (A) Normal meiotic chromosome segregation. In *Saccharomyces cerevisiae* the four haploid genomes are packaged into the four spores of an ascus. (B) Meiosis I nondisjunction, the segregation of a pair of homologs to the same pole in the first meiotic division, generates two genomes that include an extra copy of the nondisjunctional chromosome ($1n + 1$) and two genomes that are missing the nondisjunctional chromosome ($1n - 1$). (C) Precocious sister chromatid disjunction is the premature segregation of sister chromatids at meiosis I.

Segregation of homologous artificial chromosomes. We have tested whether the correct segregation of artificial chromosomes is dependent on crossing-over by constructing a pair of artificial homologs that allow us to monitor both segregation in meiosis I and crossing-over. The 61-kb artificial homologs constructed for this experiment are composed of a centrally located yeast centromere, an ARS element (a presumed replication origin), and four yeast genes embedded in a backbone of bacteriophage lambda DNA (Fig. 2A). These chromosomes and the other artificial chromosomes and chromosome III derivatives that we describe below are terminated with *Tetrahymena* telomeres that have been modified by the addition of yeast telomeric sequences (*18*). Two of the four genes in each chromosome have been mutated so that the two chromosomes have complementary genotypes. YLp45 is *LEU2 trp1 URA3 his3* and YLp48 is *leu2 TRP1 ura3 HIS3*. The positions of these genetic markers divide the chromosomes into three intervals and enable us to detect crossovers that occur anywhere along the length of the chromosomes except at the extreme ends.

We analyzed the meiotic behavior of these chromosomes by sporulating diploids that harbored both YLp45 and YLp48 and then scoring the segregation of the markers carried on the artificial chromosomes after tetrad dissection. Because there is some variation in the copy number of artificial chromosomes in vegetatively growing cells, individual cells may contain zero, one, or two copies of each of the artificial chromosomes at the time of meiosis (*19*). Only cells that showed $2^+:2^-$ segregation for each artificial chromosome, and therefore presumably contained one copy of each YLp at the time of entry into meiosis, were useful in our analysis. The artificial homologs, like natural homologs, segregated from each other at meiosis I. In 90 percent of the tetrads analyzed (107 of 119) YLp45 and YLp48 segregated to opposite poles at meiosis I (Fig. 2B). Meiosis I nondisjunction (Fig. 1B) occurred in 7 percent of the tetrads. Precocious sister chromatid disjunction (Fig. 1C) of one or the other artificial chromosome occurred in 3 percent of the tetrads.

Among the 107 tetrads with proper disjunction of YLp45 and YLp48, only three crossovers were observed between the artificial homologs. We conclude from these data that in the vast majority of meioses the artificial homologs segregate correctly in the absence of crossing-over. One objection to this conclusion is that nondisjunction of the artificial chromosomes might be a lethal event and therefore go unobserved. If this were true, tetrads arising from diploids carrying only a single copy of YLp45 or YLp48 would be overrepresented while diploids carrying both artificial chromosomes would give fewer than the expected numbers of viable tetrads. That this is not the case is shown in Fig. 3. Also, we cannot rule out the possibility of undetected crossovers in the telomeric sequences; however, even if these crossovers are occurring, it is unlikely they direct segregation since our results presented below demonstrate that crossovers between the artificial chromosomes do not direct their segregation.

Homology requirements for chromosome segregation. The recombination-independent segregation of artificial chromosomes that we observe in yeast is reminiscent of the distributive system in *Drosophila melanogaster* females, which functions independent of

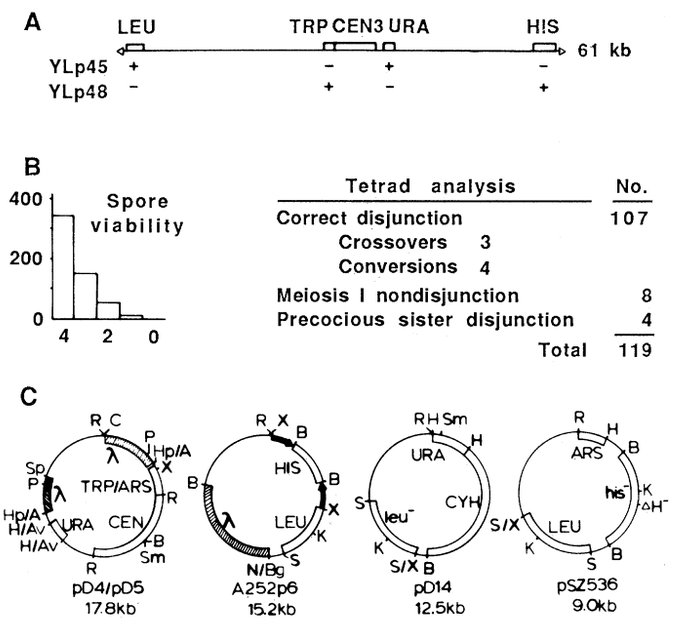

Fig. 2. The segregation of homologous artificial chromosomes. (A) YLp45 and YLp48 were constructed by stepwise modification of YLp21 (*19*) by means of the single-step gene replacement technique (*31*), except where noted. First the left end of YLp21 was replaced with the Bam HI telomere-*LEU2*/λ restriction fragment of A252p6. Then the centromeric region was replaced by the λ/*trp1*/*CEN3*/*URA3*/λ *Pvu* II fragment of pD5 in the construction of YLp45 or the λ/*TRP1*/*CEN3*/*ura3*/λ *Pvu* II fragment from pD4 in the construction of YLp48. YLp45 was generated by using cotransformation (*32*) to replace the functional *HIS3* gene with the *his3* gene of pSZ536 which had been inactivated by filling in a Hind III site (*33*). YLp48 was generated by replacing the functional *LEU2* gene with the *leu2* gene of pD14 which carries a 400-bp deletion. Kpn I cut pD14 was integrated at *LEU2* and leu$^-$ derivatives of these transformants were found by screening ura$^-$ revertants selected on medium containing 5-fluoroorotic acid (*34, 35*). Structures of the artificial chromosome were verified by Southern blots. (B) Meiotic behavior of YLp45 and YLp48 in strain Dd35. Segregation data are for those tetrads where both YLps segregate $2^+:2^-$. Additional data are shown in Fig. 3. Events identified genetically as gene conversions were analyzed with Southern blots. Standard procedures were used for tetrad analysis (*36*). Genotype of Dd35:*a*/α, *trp1*/*trp1*, *ura3*/*ura3*, *his3-11,15*/*his3-11,15*, *leu2-3,112*/*leu2-3,112*, *ade1*/+, *arg4-17*/+, *lys2*/+, YLp45 [*LEU2, trp1, URA3, his3*], YLp48 [*leu2, TRP1, ura3, HIS3*]. (C) Plasmids used in the construction of YLp45 and YLp48. Restriction enzyme sites: R, Eco RI; C, Cla I; B, Bam HI; X, Xho I; P, Pvu II; H, Hind III; K, Kpn I; S, Sal I; Sm, Sma I; Sp, Sph I; Hp/A, Hpa I–Aat II junction; H/Av, Hind III–Ava I junction; N/Bg, Nru I–Bgl II junction; S/X, Sal I–Xho I junction. The arrows on A252p6 represent *Tetrahymena* telomeric sequences.

sequence homology. To test whether the segregation of artificial chromosomes is dependent upon sequence homology, we have performed experiments similar to that described above except that the model chromosomes share only extremely limited DNA sequence homology (Fig. 4A). One of the chromosomes is a 72-kb chromosome III derivative (*20*). The mini III chromosome contains approximately 60 kb of contiguous sequences from chromosome III, including the centromere, and is flanked by the yeast selectable markers *URA3* and *TRP1*. The second chromosome, YLp54, carries *CEN4*, *ARS2*, and the selectable markers *LYS2* and *HIS3*. The only homologies between YLp54 and the mini III are the telomeres and the 275 bp between the Bam HI and Sal I sites of pBR322.

The meiotic segregation of the mini III/YLp54 pair (Fig. 4B) proved to be indistinguishable from the homologous pair described in Fig. 2. In both experiments the chromosome pairs disjoined in meiosis I in approximately 90 percent of the tetrads. These results suggest that neither reciprocal recombination nor sequence homology is essential for the proper meiotic segregation of short linear chromosomes. These experiments do not rule out the possibility that the 275-bp pBR322 homology or the homologies between the telomeres are sufficient for assuring disjunction. Telomeres cannot be essential for meiotic segregation, since pairs of circular minichromosomes, without telomeric sequences, show only low levels of nondisjunction (*21, 22*). Furthermore, since all or most natural chromosomes share the same telomeric sequences (*23*), it seems unlikely that telomeric homologies are responsible for directing partner recognition or the segregation of natural chromosomes.

The effect of homology on segregation partner selection. The experiments described above show that extensive sequence homology is not a prerequisite for the disjunction of artificial chromosomes. It seemed appropriate to ascertain whether such homology, when available, might influence segregation. A direct test of the role of homology in segregation is a competition experiment in which the meiotic behavior of two homologous chromosomes is examined in a cell that contains a third nonhomologous chromosome. We constructed a diploid strain that contains a mini III chromosome and two homologous artificial chromosomes, YLp45 and YLp53 (Fig. 5A). Two models for the meiotic pairing and segregation of these chromosomes are shown in Fig. 6. If homology does direct the segregation of YLp45 and YLp53, then the two artificial chromosomes will always segregate from each other at meiosis I, and the nonhomologous mini III chromosome will segregate randomly. This homology-dependent segregation would yield two types of tetrads that would occur with equal frequency (Fig. 6A). Alternatively, if segregation of the three chromosomes is independent of sequence homology, then there are three possible segregation patterns for the three chromosomes (Fig. 6B). These models can be distinguished because homology-independent pairing predicts that in 33 percent of the tetrads, the homologous artificial chromosomes (YLp45 and YLp53) would have segregated to the same pole at meiosis I and consequently would be found in the same sister spores (class C in Fig. 6B). This class would not be expected by the homology-dependent model.

The results of tetrad analysis on strains containing YLp45, YLp53, and the mini III chromosome are shown in Fig. 5B. The observed patterns of marker segregation closely match those predicted by the homology-independent segregation model.

The genetic markers on YLp45 and YLp53 allow determination of whether crossing-over between them directs their segregation from each other. Crossovers between YLp45 and YLp53 were observed in 14 tetrads. In about one-third (4 of 14) of these recombinant tetrads the artificial chromosomes segregated to the same pole at meiosis I. This is the same frequency with which nonrecombinant YLp45 and YLp53 chromosomes segregate to the

YLp48 \ YLp45	4:0	3:1	2:2	1:3	0:4	Total
4:0	8 .025 (.025)	3 .009 (.006)	21 .063 (.064)	0 .000 (.002)	5 .015 (.015)	37 .111
3:1	4 .012 (.007)	0 .000 (.002)	4 .012 (.017)	0 .000 (.001)	2 .006 (.004)	10 .030
2:2	40 .120 (.137)	9 .027 (.031)	127 .381 (.348)	5 .015 (.009)	21 .063 (.082)	202 .607
1:3	3 .009 (.003)	1 .003 (.001)	0 .000 (.009)	0 .000 (.000)	1 .003 (.002)	5 .015
0:4	20 .060 (.053)	4 .012 (.012)	39 .117 (.136)	0 .000 (.004)	16 .048 (.032)	79 .237
Total	75 .225	17 .051	191 .574	5 .015	45 .135	333 1.00

Fig. 3. The segregation patterns of YLp45 and YLp48 in Dd35 tetrads with four viable spores. The five rows from top to bottom contain data for tetrads showing 4+:0−, 3+:1−, 2+:2−, 1+:3−, and 0+:4− segregation of YLp48, while columns from left to right contain the analogous data for YLp45. Each sector contains three values; the top one is the number of tetrads observed in that class, the middle one is the observed frequency, and the bottom one (in parentheses) is the frequency of tetrads expected to fall in this class. The expected number for each class was calculated (*37*) by multiplying the number of tetrads for which YLp45 gave the segregation pattern characteristic of that class by the number of tetrads for which YLp48 gave the segregation pattern characteristic of that class and then dividing by the total number of tetrads (333). For example, the expected value for the upper left sector (4:0 for both YLp45 and YLp48) is (75 × 37)/333 = 8.3; 8.3/333 = .025, the expected frequency. A contingency χ^2 test of observed compared to expected gives $P > .01$ (*37*).

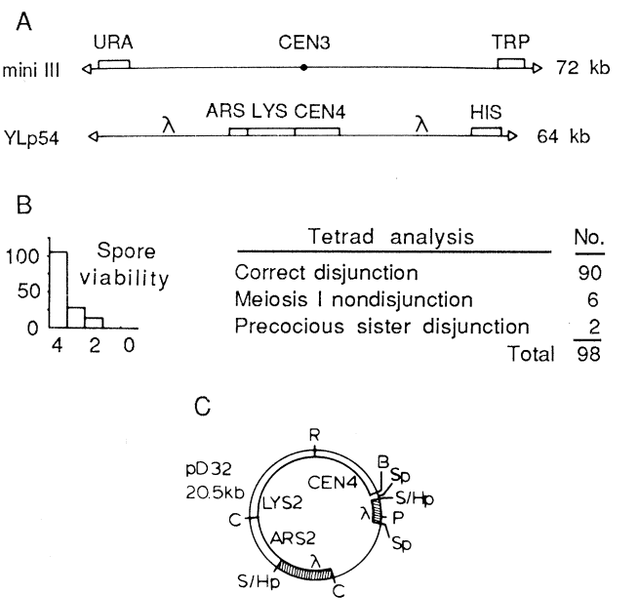

Fig. 4. The segregation of the nonhomologous YLp54 and mini III chromosomes. (A) The construction of the mini III has been described (*20*). YLp54 was constructed by replacing the centromeric region of YLp22 (*19*) with the λ/ARS2/LYS2/CEN4/λ Cla I–Sph I partial digest fragment of pD32 (*31*). (B) Tetrad analysis was used to examine the meiotic segregation of the *HIS3* and *LYS2* markers on YLp54 and the *TRP1* and *URA3* markers on the mini III in strains Dd80, 81, 82, and 83. In all four strains the YLp54/mini III pair showed similar frequencies of meiosis I disjunction. Strain genotypes: Dd80, Dd81, Dd82, and Dd83, *a*/α, *trp1*/*trp1*, *ura3*/*ura3*, *leu2-3,112*/*leu2-3,112*, *his3-11, 15*/*his3-11,13*, *lys2*/+, *ade1*/+, *arg4-17*/+, YLp54 [*HIS3, LYS2*], mini III [*URA3, leu2, TRP1*]. (C) Map of pD32. Restriction sites: Cla I; Sph I; Eco RI; Bam HI; Pvu II; S/Hp, Sal I–Hpa I junction.

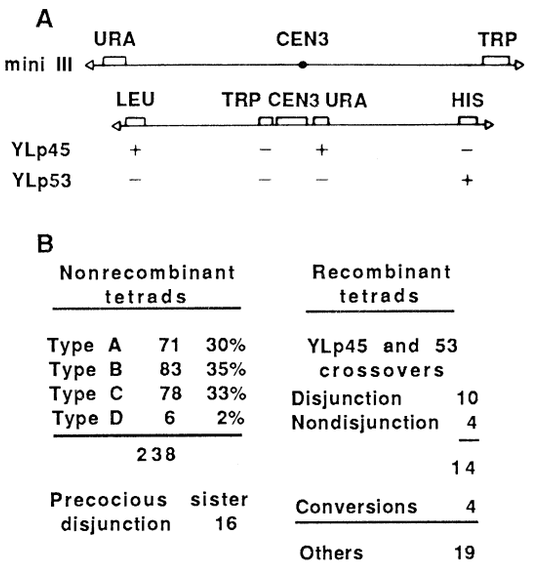

Fig. 5. Competition between homologous and nonhomologous chromosomes. (A) The structures of the mini III and YLp45 were described above. YLp53 is a derivative of YLp48 in which *TRP1* was gene-converted to *trp1*. (B) The segregation patterns of YLp45, YLp53, and the mini III were examined by following the segregation of *LEU2, HIS3,* and *TRP1*, respectively. Type A, B, and C segregation patterns are shown in Fig. 6. Type D tetrads are those in which the markers from YLp45, YLp53, and the mini III were all found in the same two sister spores. The predicted linkage of markers on the artificial chromosomes in the type C, recombinant, and "others" tetrads was verified by taking advantage of the mitotic instability of the artificial chromosomes (*19*). Spore colonies were grown nonselectively for about ten generations and then tested to confirm that the markers that we presumed were physically linked were always lost simultaneously in mitotic loss events. The "others" tetrads were also analyzed by Southern blots. These unusual marker patterns could be due to, for example, mitotic events, multiple recombination events or artificial chromosome segregation errors, intramolecular recombination between repeated sequences on the artificial chromosomes, or loss of the terminal genes on the artificial chromosome. The proportion of tetrads with four viable spores, and the number of tetrads contributed to the pooled data were: Dd57, 33 percent, 44; Dd62, 86 percent, 9; Dd64, 57 percent, 139; Dd65, 58 percent, 5; Dd68, 64 percent, 88; and Dd69, 67 percent, 6. A χ^2 analysis of the data from Dd57, 64, and 68 showed that the frequencies with which these three strains gave type A, B, and C tetrads did not differ significantly from each other, with $P = 0.1$. Strain genotypes: Dd64, Dd65, Dd68, and Dd69 *a*/α, *trp1/trp1, ura3/ura3, leu2-3,112/leu2-3,112, his3-11,15/his3-11,15, lys2/lys2, ade1/+, arg4-17/+, CYH2/CYH2, TCM1/TCM1, CAN1/CAN1*, YLp45 [*LEU2, trp1, URA3, his3*], YLp53 [*leu2, trp1, ura3, HIS3*], mini III [*URA3, leu2, TRP1*]. Dd57 and Dd62 had identical genotypes except that Dd57 is *lys2/+, can1/+, tcm1/+, cyh2/+*, and Dd62 is *lys2/+*.

same pole at meiosis I (class C), demonstrating that crossovers between the two artificial chromosomes do not direct them to segregate from each other.

Homology-independent chromosome segregation. Previous examinations of the meiotic behavior of both circular and linear artificial homologs have shown that these molecules disjoin properly in meiosis I about 90 percent of the time (*19, 21, 22*). These observations were provocative since the meiosis I disjunction of natural chromosomes is dependent on crossing-over, yet these model chromosomes were small enough that they would not be expected to recombine in most meioses. By building linear artificial chromosomes with the appropriate genetic markers, we have been able to rigorously test the requirements for the meiosis I disjunction of small model chromosomes. Our results show that the artificial chromosomes and the mini III chromosome, unlike natural chromosomes, are segregated by a system that is not dependent on crossing-over or sequence homology. We also find that our artificial chromosomes differ significantly from natural chromosomes in both the frequency with which crossovers occur and the effect of crossing-over on segregation.

The presence of genetic markers at the extreme ends of the homologous artificial chromosomes YLp45 and YLp48 enabled us to measure the frequency of reciprocal crossovers in the intervening 60 kb. The observed frequency of three crossovers in 119 tetrads, or about 1 centimorgan (cM), is substantially lower than would be expected over a similarly sized interval within a yeast chromosome. For example, the 50-kb interval from *LEU2* to *PGK1* which, like the artificial chromosomes, includes *CEN3*, has a genetic length of approximately 10 cM (*24*). We propose two possible explanations for this discrepancy. One is that the artificial chromosomes might be too short to recombine efficiently. If the establishment of synaptonemal complex requires extensive stretches of homology, then short artificial chromosomes might form this structure inefficiently. Second, because the artificial chromosomes are composed mainly of bacteriophage lambda DNA, they may be lacking sequences that are required for homologous pairing or recombination. Genetic evidence suggests that specialized sequences may mediate these processes in yeast. For example, a site near the *ARG4* locus seems to increase recombination in nearby sequences (*25*), and the insertion of certain foreign DNA sequences into the yeast genome has been shown to increase the local frequency of crossing-over (*26*). The low levels of exchange exhibited by artificial chromosomes provide us with an assay for investigating the sequence requirements for chromosome pairing and recombination.

Although several experiments demonstrate that crossing-over is essential for meiosis I disjunction of natural chromosomes, the rare crossovers between YLp45 and YLp53 did not ensure disjunction of these two artificial chromosomes. Since crossovers appear to mediate segregation by physically linking homologs, we suggest that the linkage between the recombined artificial chromosomes dissolves prior to anaphase (the time at which homologs segregate from each other), releasing the recombinant homologs to segregate by the same homology-independent system as nonrecombinant artificial chromosomes. It has been argued that chiasmata can link homologs only because the nonsister chromatids that become covalently joined by crossing-over are also physically connected to their sister chromatids distal to the point of the crossover (*27, 28*). The maintenance of the association of sister chromatids distal to the chiasma would

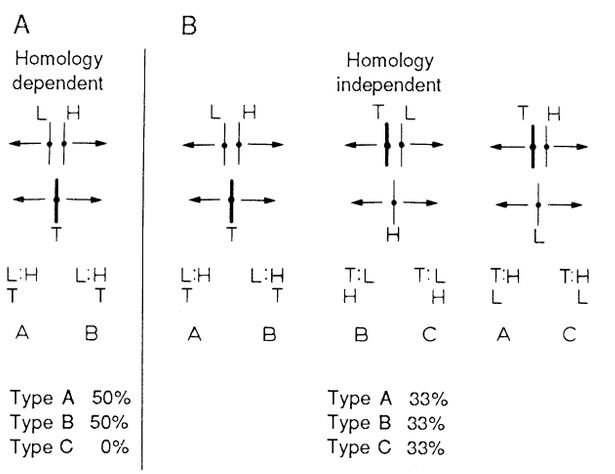

Fig. 6. Models for the segregation of two homologous artificial chromosomes and a nonhomologous mini III chromosome. (A) and (B) show the meiosis I segregation and consequent patterns of genetic markers predicted for two homologous and one nonhomologous chromosome by (A) homology-dependent or (B) homology-independent segregation pathways. YLp45 and YLp53 are represented by thin lines and are labeled L and H, respectively, to indicate their genetic markers *LEU2* and *HIS3*. The mini III chromosome is represented by a thick line and is labeled T for its unique genetic marker *TRP1*.

prevent the terminalization of the chiasma and the consequent dissolution of the linkage of homologs. The physical basis for the cohesion of sister chromatids remains a mystery, but it might be dependent on chromosome length or specialized sequences. The artificial chromosomes differ from natural chromosomes in both length and sequence composition. The phage lambda DNA would not be expected to contain specialized "chiasma binder" sequences which have been hypothesized to stabilize or maintain the association of sister chromatids (*27, 28*). Alternatively, chiasmata on the artificial chromosomes may be too near the termini to be maintained since the arms of the artificial chromosomes are much shorter than the arms of even the shortest yeast chromosomes (about 30 kb compared to about 150 kb for chromosome I). Experiments in *Drosophila* females have shown that crossovers near the chromosome ends are less likely to ensure proper segregation than more proximal ones (*29*). The catenation of sister chromatids has been invoked to explain the length dependence of the fidelity of sister chromatid disjunction in mitosis (*20*). Catenation may play a similar role in meiosis I as the "glue" that holds sister chromatids together and thereby stabilizes chiasmata.

What is the role of the homology-independent system that segregates the artificial chromosomes? We suggest that yeast employs a backup chromosome segregation system to increase the fidelity with which genetic information is transmitted. The biological strategy of using multiple independent systems to achieve high fidelity has been observed in other processes. For example, the process of DNA replication includes a replication function, an editing function, and a mismatch repair function to ensure faithful replication of genetic material (*30*). We suggest that yeast, like *Drosophila* females, has an exchange-dependent system to segregate most homolog pairs and a homology-independent process to segregate chromosomes that have not undergone exchange or have undergone exchange and subsequently lost their chiasmata. In yeast it is difficult to calculate the frequency of meioses with a nonexchange chromosome pair since the effect of chiasma interference on the extent of crossing-over is not completely understood. However, a simple approximation of the frequency at which a given chromosome has not undergone exchange (E_0) can be made with the use of the zero term of the Poisson distribution ($E_0 = 1/e^x$, where x is the average number of exchanges expected on that chromosome per meiosis). On the basis of this calculation, the shorter chromosomes (I, III, V, VIII, X, XII, XIV, XVI) each would be nonexchanged in 1 to 5 percent of the meioses, and the extent of meioses with at least one nonexchange chromosome pair would be about 10 percent. The backup system we have described would reduce this number by a factor of 10 and might be more efficient for full-length natural chromosomes than the model chromosomes we have tested.

The presence of similar homology-independent segregation pathways in organisms as phylogenetically distant as yeast and *Drosophila* suggests that the use of backup segregation systems may be a common biological strategy. The demonstration that such a system exists in yeast, and the availability of artificial chromosomes whose structures can be easily manipulated, should facilitate our attempts to uncover the mechanism of homology-independent segregation.

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37. A contingency χ^2 test was performed on the data observed (compared to the expected) presented in Fig. 3. The data in the $3^+:1^-$ and $1+:3^-$ columns and rows were pooled to reduce the number of classes where E was less than 5. $\chi^2 = \Sigma (O - E)^2/E = 14.26$. With df = 9, this gives a value of $P \simeq 0.1$. There is therefore no reason to reject the hypothesis that misbehavior of YLp45 and YLp48 are independent.

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