Meiotic recombination and segregation of human-derived artificial chromosomes in *Saccharomyces cerevisiae*

(meiosis/yeast artificial chromosomes/recombination/chromosome segregation)

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ABSTRACT We have developed a system that utilizes human DNA-derived yeast artificial chromosomes (YACs) as marker chromosomes to study factors that contribute to the fidelity of meiotic chromosome transmission. Since aneuploidy for the YACs does not affect spore viability, different classes of meiotic missegregation can be scored accurately in four-viable-spore tetrads including precocious sister separation, meiosis I nondisjunction, meiotic chromatid loss, and meiosis II nondisjunction. Segregation of the homologous pair of 360-kilobase marker YACs was shown to occur with high fidelity in the first meiotic division and was associated with a high frequency of recombination within the human DNA segment. By using this experimental system, a series of YAC deletion derivatives ranging in size from 50 to 225 kilobases was analyzed to directly assess the relationship between meiotic recombination and meiosis I disjunction in a genotypically wild-type background. The relationship between physical distance and recombination frequency within the human DNA segment was measured to be comparable to that of endogenous yeast chromosomal DNA—ranging from <2.0 to 7.7 kilobases/centimorgan. Physical analysis of recombinant chromosomes detected no unequal crossing-over at dispersed repetitive elements distributed along the YACs. Recombination between YACs containing unrelated DNA segments was not observed. Furthermore, the segregational data indicated that meioses in which YAC pairs failed to recombine exhibited dramatically increased levels of meiosis I missegregation, including both precocious sister chromatid separation and nondisjunction.

Meiotic chromosome segregation consists of two distinct types of chromosome disjunction and results in a reduction of genome content from a diploid to a haploid state. During meiosis I, homologous sister chromatid pairs are segregated to opposite poles; during meiosis II, sister chromatids are separated (in a manner analogous to mitotic chromosome segregation). New combinations of genetic information are generated by independent assortment of chromosomes and by recombination. The product of a meiosis event in *Saccharomyces cerevisiae* is a tetrad that is made up of four haploid spores enclosed in an ascus. Segregation of chromosomes through meiosis occurs with high fidelity to ensure the formation of four euploid spore products. The measured frequency of spontaneous chromosome V missegregation during meiosis I is less than one event in 10⁴ viable spores (1). Meiosis I errors involving chromosomes III and VII occur in ≈1 in 10³ and 1 in 10⁴ meioses, respectively (M. Goldway, T. Arbel, and G. Simchen, personal communication).

High fidelity of chromosome segregation during meiosis I is thought to be enhanced by recombination between non-sister chromatids and the subsequent formation of chiasmata between homologs. Evidence for a positive relationship

between meiotic recombination and meiosis I disjunction comes from cytological observation or genetic analysis of recombination-deficient mutants in several organisms (for review, see ref. 2). Recombination mutants exhibit increased levels of meiosis I nondisjunction and/or high levels of gamete inviability that are presumably caused by missegregation events that give rise to aneuploid meiotic products. Although these observations support the view that recombination directly affects the fidelity of chromosome segregation during meiosis I, no systematic analysis of this relationship has been undertaken in the context of a wild-type genetic background.

Because missegregation of chromosomes can give rise to inviable meiotic products that do not contain the full complement of a haploid genome, chromosome loss/gain in dead gametes must be inferred from the chromosome content of the viable gametes that remain. Therefore, the type or frequency of meiotic missegregation events cannot be accurately scored. We have developed a system that allows thorough examination of chromosome transmission during meiosis in *S. cerevisiae*. Meiotic chromosome recombination and segregation are analyzed using human DNA-derived yeast artificial chromosome (YAC) pairs. Unlike endogenous chromosome missegregation, aberrant segregation of these nonessential YACs can be scored easily because all four meiotic products are viable regardless of YAC copy number. Furthermore, the YACs and yeast host strain are suitably marked so that specific types of aberrant transmission can be distinguished and further analysis of each spore is possible. We find that homologous YACs disjoin from one another with high fidelity during meiosis I and that the frequency of meiotic recombination between human DNA-derived YAC homolog pairs is comparable to that of yeast endogenous chromosomes. By altering the structure of one member of the YAC homolog pair, we demonstrate directly that failure to recombine has a deleterious effect on meiosis I segregation fidelity causing high levels of precocious sister separation (PSS) and nondisjunction (NDI).

MATERIALS AND METHODS

Replacement of *CEN6* with $\Delta CEN6::LEU2-CEN11$. Two genomic *Hind*III–*Taq* I fragments, fragment A, 0.5 kilobase (kb) and fragment B, 0.6 kb (Fig. 1), which flank a 392-base-pair *Taq* I *CEN6* fragment were subcloned into pRS25 (R. Sikorski and P.H., unpublished data), a *LEU2*-containing derivative of pBluescript 1 (Stratagene), to give pJHH56. A 0.65-kb *CEN11* fragment (D. Jager and J.H.H., unpublished data) was ligated into pJHH56 to make pJHH57 (Fig. 1).

Abbreviations: YAC, yeast artificial chromosome; PSS, precocious sister separation; NDI and NDII, nondisjunction in meiosis I and II, respectively; PD, parental ditype; NPD, nonparental ditype; T, tetratype; CL, meiotic chromatid loss; cM, centimorgan(s).

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FIG. 1. Structure of the $\Delta CEN6::LEU2-CEN11$ deletion/insertion vector pJHH57 and replacement of *CEN6* with *LEU2-CEN11* on chromosome VI (CHR VI). H, *Hind*III; T, *Taq* I; A and B, fragments A and B, respectively.

pJHH57 was cleaved with *Hind*III and transformed into yeast strain YPH250 by the lithium acetate method (3) to select *Leu*⁺ transformants (Fig. 1). Replacement of the genomic 392-base-pair *CEN6* fragment by the *LEU2-CEN11* cassette on chromosome VI yielded YPH424 (*MATa ura3-52 ade2-101 trp1-Δ1 lys2-801 his3Δ200 leu2-Δ1 ΔCEN6::LEU2-CEN11*), which was verified by Southern blot analysis (data not shown). All derivatives of YPH424 were made using standard genetic methods (4).

Yeast Strains. All *S. cerevisiae* diploid strains used in this study are of the following genotype: *MATa/MATa ura3-52/ura3-52 ade2-101/ade2-101 trp1Δ1/trp1Δ1 lys2-801/lys2-801 his3Δ200/his3Δ200 leu2-Δ1/leu2-Δ1 CEN6/ΔCEN6::LEU2-CEN11*. Most YACs used in this study are derived from a characterized YAC (5) that is 360 kb long and contains a human genomic fragment. Prototrophic markers at the centromere-proximal and -distal ends of the YACs were modified by gene replacement with a set of marker change plasmids (6). YPH607 contains YAC pair A (Fig. 2a). YPH787, YPH788, and YPH789 carry YAC pairs B, C, and D, respectively (see Fig. 4). YPH785 and YPH880 contain YAC pairs E (which includes a 390-kb mouse DNA-derived YAC, ref. 7) and F (which includes a 300-kb human chromosome 21-derived YAC, ref. 7), respectively (see Fig. 4).

Sporulation and Tetrad Analysis. Yeast strains were sporulated on plates containing 1% potassium acetate, 0.05% Bacto yeast extract, 0.05% dextrose, and a full complement of nutritional supplements (4). Sporulating cultures were incubated at 30°C for 4 days. Tetrads were digested in 50 μ l of zymolyase (0.5 mg/ml)/1M sorbitol for 8 min, diluted with 500 μ l of 1 M sorbitol, and streaked onto YPD plates for dissection. To determine linkage of markers in spores containing two YACs, mitotic segregants lacking the *URA3*-marked YAC were selected on 5-fluoroorotic acid plates (8) and the remaining markers were scored.

RESULTS

An Experimental System for Analysis of Meiotic Chromosome Transmission Using Homologous YACs. To characterize the general meiotic segregation fidelity of YACs and their ability to participate in homologous recombination, a diploid strain was constructed that contains a pair of differentially marked homologous YACs (see YAC pair A, Fig. 2a). The *URA3-SUP11* and *LYS2* markers, as well as the *TRP1* and *HIS3* markers, can be scored as alleles since they are located at the same physical locations on the YACs.

FIG. 2. (a) YAC pair A. Each YAC is 360 kb long and has a centromere-linked marker (*URA3-SUP11* or *LYS2*), a distal marker (*TRP1* or *HIS3*), a centromere sequence derived from chromosome IV (*CEN4*), a human DNA insert, and two telomeres (*TEL*). (b) Possible tetrad configurations resulting from sporulation of a diploid strain containing YAC pair A. PD, parental ditype (distal markers associate with the same centromere markers as in the parent diploid; i.e., every spore is nonrecombinant for YAC markers); NPD, nonparental ditype (all four spores are recombinant for YAC markers, for example, as a result of a four-strand double recombination between distal markers; note that a two-strand double crossover between two nonsister chromatids results in a PD tetrad); T, tetra-type (two spores are recombinant and two spores are nonrecombinant for YAC markers; e.g., as a result of a single crossover).

The patterns for proper segregation of a YAC homolog pair with respect to a *LEU2* sister spore marker are shown in Fig. 3a. In our diploid strains, *CEN6* is replaced by a *LEU2-CEN11* cassette on one copy of chromosome VI (Fig. 1). Exchange between this region and the wild-type *CEN6* sequence by homologous recombination cannot occur. Therefore, the presence of *LEU2* will always denote a sister spore pair as it is perfectly *CEN*-linked. If the YACs segregate correctly, centromere-linked markers will segregate to sister spore pairs (Fig. 3a). If the YACs segregate incorrectly, the different types of aberrant segregation can be determined by scoring the centromere-linked markers with respect to the *LEU2* sister spore marker (described in Fig. 3b). In this system, meiotic loss that results in the loss of both sister chromatids (0:4 segregation of a *CEN*-linked marker) cannot be distinguished from premeiotic mitotic YAC loss. Therefore, tetrads exhibiting 0:4 segregation are excluded from the analysis.

To test the fidelity of meiotic segregation of YAC homolog pairs, the behavior of two homologous 360-kb YACs (Fig. 2a) was scored in a total of 387 tetrads. The homologous YACs exhibited normal meiosis I disjunction in 99.25% of the 387 tetrads scored. Only one PSS (0.25%) and two NDI (0.50%) missegregation events were observed (Table 1). In meiosis II, 96% of the tetrads exhibited normal meiosis II segregation of the YACs.

Meiotic Recombination Between Human DNA-Derived YAC Pairs. Recombination between the YAC homologs was measured by scoring the distribution of the four YAC prototrophic markers. During a recombination event, homologous nonsister chromatids exchange distal markers. Genetic

FIG. 3. Meiotic segregation patterns of YAC pairs as monitored by their centromere-linked markers. (a) Patterns of correct segregation of YACs into sister spore pairs marked by *LEU2*. (b) Possible aberrant segregation patterns for the *URA3*-marked YAC are diagrammed. Aberrant segregation of the *LYS2*-marked YAC is not shown. PSS, sister chromatids detach prematurely during meiosis I, distribute to opposite poles, and subsequently segregate to nonsister spores; NDI, nondisjunction in meiosis I (YACs are segregated to the same pole of the meiosis I spindle and both centromere-linked markers end up in the same sister spore pair); CL, meiotic chromatid loss; NDII, nondisjunction in meiosis II (both sister chromatids segregate to the same pole during the second meiotic division). CL and NDII were differentiated by mating spore clones to a haploid containing no YAC and performing tetrad analysis.

linkage of distal markers to centromere markers on the parental YACs was measured by scoring each tetrad type (described in Fig. 2b) and applying the mapping function of Perkins (9).

The 83 tetrads of YAC pair A were scored for recombination yielding a PD/NPD/T ratio of 14:6:63 (Table 2). These data indicated that the distal markers were only loosely linked to their centromeres. The recombination rate was, therefore, >50 centimorgans (cM), and the relationship between physical distance and recombination frequency (kb/cM) could not be deduced accurately for this interval of 360 kb but was <7 kb/cM. To obtain a more exact rate of

Table 1. Meiotic segregation of a 360-kb YAC

Type of segregation	Tetrads, no.		
	YAC pair A	YAC pair A*	Total
Meiosis I			
Normal	82	302	384 (99.25)
PSS	0	1	1 (0.25)
NDI	1	1	2 (0.50)
Meiosis II			
Normal	79	292	371 (96.0)
NDII	1	4	5 (1.3)
CL	3	8	11 (2.7)
Total tetrads	83	304	387

YAC pair A* is identical to YAC pair A except for lack of the *SUP11* gene. Numbers in parentheses are percent of total.

Table 2. Relationship between physical and genetic distance

YAC pair	Tetrads, no.			Recombination rate, cM	Length, kb	kb/cM
	PD	NPD	T			
A	14	6	63	>50	360	<7
B	21	10	55	>50	225	<4.5
C	58	0	28	16	75	4.7
D	80	0	12	6.5	50	7.7
E	98	0	0	—	360	—
F	111	0	0	—	300	—

Data include tetrads exhibiting correct and aberrant segregation.

recombination within this human insert, three YAC pairs containing smaller regions of homology within each pair were analyzed for recombination. The pairs were made using a full-length YAC of 360 kb and one of three deletion-derivative YACs—225, 75, or 50 kb long (Fig. 4). In YAC pair B, the 225-kb interval of homology between markers was still too large to accurately measure the genetic distance in cM but the kb/cM relationship was <4.5 kb/cM (Table 2). In YAC pair C, an interval of 75 kb exhibited a genetic distance of 16 cM, which corresponds to 4.7 kb/cM. Recombination occurring in the interval of 50 kb in YAC pair D was lower than in pair C and corresponds to 7.7 kb/cM. This 50-kb YAC may be unable to participate in recombination frequently because of its small physical size or because of negative centromere effects on recombination within the majority of the small DNA insert. DNA in close proximity to a centromere is known to be repressed for recombination (10, 11).

To determine whether this high frequency of recombination is a general characteristic of human DNA contained in a YAC, an additional pair of YACs was constructed in which the human-derived insert was different than the human insert common to the YAC pairs described above. The pair consisted of a 230-kb YAC and a homologous 100-kb YAC-deletion derivative. Out of 40 tetrads scored, the ratio of PD/NPD/T was 9:7:24 indicating a distance of >50 cM. The relationship between physical distance and recombination for this pair (<2 kb/cM) was, therefore, also comparable to the high yeast recombination frequency and suggests that high

FIG. 4. YAC pairs involving a deletion-derivative set and heterologous YACs. Each pair consists of one 360-kb YAC paired to a homologous deletion derivative of 225 kb (YAC pair B), 75 kb (YAC pair C), or 50 kb (YAC pair D) or to a heterologous YAC containing either a 390-kb mouse DNA insert (YAC pair E) or a 300-kb insert of human DNA unrelated to the 360-kb insert (YAC pair F).

levels of meiotic recombination is a general feature of exogenous DNA propagated in yeast as YACs.

Human DNA contains several types of repetitive elements. For example, *Alu* sequences occur in the human genome approximately once every 6 kb (12) and long interspersed repetitive DNA element (LINE) sequences occur approximately once every 30 kb (13). *Alu* profiles done on the YACs used in this study demonstrate the presence of *Alu* sequences along the lengths of the human inserts at the expected frequencies (ref. 13 and data not shown). Because these repeats might allow unequal recombination, 20 recombinant spores from the strain containing YAC pair B were analyzed on contour-clamped homogeneous electric field gels. Parental lengths of the YACs were retained in all cases, specifically 225 and 360 kb, and, thus, recombination between YACs occurred at equivalent sites along the insert; i.e., no unequal crossing-over was detected (data not shown).

Effect of Meiotic Recombination on Segregation of Homologous YAC Pairs. The large number of recombinant and nonrecombinant tetrad types scored above permitted direct evaluation of the segregation properties of homologous chromosome pairs with respect to recombination. The segregation data from all the YAC pairs was pooled and separated into two classes—recombinant (NPD + T) and nonrecombinant (PD) tetrads. Table 3 shows the segregation behavior of the 360-kb YAC (marked with *LYS2* at the centromere) that is common to all the YAC pairs analyzed. Tetrads that were scored as recombinant (174 total) exhibited normal YAC segregation 99.4% of the time in meiosis I. PSS was detected in 0.6% of tetrads, and no NDI of the homolog pairs was seen in the recombinant tetrad class. In the nonrecombinant class of tetrads (173 total), proper meiosis I segregation occurred in only 79.8% of the tetrads. Relative to the recombinant class, PSS events increased (from 0.6 to 2.9%), and nondisjunction events increased dramatically (from 0 to 17.3%). It is interesting to note that the frequencies of errors in the second meiotic division were approximately the same in the two tetrad classes. Thus, the absence of recombination does not seem to affect chromosome segregation during meiosis II.

Recombination and Segregation of Nonhomologous YAC Pairs. The class of "nonrecombinant" tetrads in Table 3 contains those configurations scored as PD. However, an even number of exchanges between two chromatids would give a parental-like arrangement of centromere and distal markers that would not be scored as recombinant and the frequency of errors due to the absence of recombination would be underestimated. Therefore, two additional YAC pairs were made that contain heterologous inserts, YAC pairs E and F (Fig. 4). For both the human-mouse and human-human heterologous YAC pairs, no recombination of YAC markers was observed in 98 and 111 tetrads, respectively (see Table 2).

Table 3. Effect of recombination on meiotic segregation of a 360-kb YAC

Type of segregation	Recombinant tetrads, no.	Nonrecombinant tetrads, no.
Meiosis I		
Normal	173 (99.4)	138 (79.8)
Abnormal		
PSS	1 (0.6)	5 (2.9)
NDI	0	30 (17.3)
Meiosis II		
Normal	162 (93.1)	167 (96.5)
Abnormal		
NDII	5 (2.9)	0
CL	7 (4.0)	6 (3.5)
Total tetrads	174	173

Numbers in parentheses are percent of total.

Table 4. Effect of homology on meiotic segregation of a 360-kb YAC

Type of segregation	Tetrads, no.		
	Heterologous		Homologous (pair A)
	Pair E (mouse)	Pair F (human)	
Meiosis I			
Normal	73 (75)	89 (80)	82 (99)
Abnormal			
PSS	5 (5)	11 (10)	0
NDI	20 (20)	11 (10)	1 (1)
Meiosis II			
Normal	94 (96)	109 (98)	79 (95)
Abnormal			
NDII	0	0	1 (1)
CL	4 (4)	2 (2)	3 (4)
Total tetrads	98	111	83

Numbers in parentheses are percent of total.

The segregation behaviors of YAC pairs E and F (Table 4) show that nonrecombinant YAC pairs exhibit high levels of PSS—5% and 10% of the meiosis I divisions scored. The absence of recombination also has a deleterious effect on the NDI of YACs from one another—20% and 10% NDI for pairs E and F, respectively. These nonrecombinant YAC pairs were properly disjoined in 75–80% of the tetrads scored. This result confirms previous evidence that a distributive disjunction mechanism exists in *S. cerevisiae* (see Discussion and refs. 14–16), and that nonrecombinant artificial chromosomes are incorporated into the distributive pool.

DISCUSSION

We describe a system in which recombination and segregation of artificial chromosomes through meiosis can be monitored by using telocentric YAC pairs that are differentially marked at their centromeres and distal ends. With exception of the yeast cis-acting elements required for chromosome maintenance, the YAC pairs are derived from exogenous DNA and are not essential for viability. In other systems of monitoring chromosome segregation, PSS and NDI cannot be accurately differentiated without using cytological analysis because both PSS and NDI give rise to disomic gametes in which the disomic chromosomes are nonsister chromatids. By using artificial chromosomes, aberrant meiotic segregation patterns can be assessed accurately because four viable meiotic products are generated and sister spores are clearly marked.

When homologous YAC pairs are present in a diploid during meiosis, the ratio of physical to genetic distance of their human-derived insert is measured in this system to be in the range of <2–7.7 kb/cM. This frequency of recombination is comparable to the amount of recombination measured between endogenous yeast chromosomes, which ranges from 0.5 to 12.5 kb/cM (10), and quite unlike the frequency of recombination between human homologous chromosomes, which is on the order of 1000 kb/cM. The high frequency of recombination between homologs was measured independently using two different human DNA inserts, arguing against the possibility that a hot spot for recombination contained in the first insert analyzed could solely account for the high levels of recombination. It is unlikely that any of the recombination events scored were due to exchange at human repetitive elements located along the YACs because no exchange was observed between two heterologous human YACs and because physical analysis showed only equivalent length exchange between homologous YACs. Dawson *et al.* (15) measured the rate of recombination between homologous λ -derived artificial chromosomes to be

about 44 kb/cM. The lower recombination frequency of the λ -derived YACs may be due to an effect of either the λ DNA itself or the fact that the λ -derived YACs were small and metacentric (containing chromosome arms of only 25 kb). The second possibility is consistent with repression of meiotic recombination in the vicinity of centromeres (10, 11).

Two factors are thought to contribute to the fidelity of homolog disjunction in meiosis I. The first is recombination between homologs that results in chiasma formation. Chiasmata are believed to hold homologous chromosomes together at the metaphase plate in meiosis I and balance the opposing poleward forces exerted on their kinetochores. The hypothesis that recombination and normal meiosis I disjunction are interrelated comes from previous studies of recombination mutants or aneuploids in several organisms (for review, see ref. 2). In these previous studies, it is possible that increased levels of meiotic missegregation were due to negative pleiotropic effects of the mutations or aneuploidies as opposed to simply the absence or reduced level of recombination. In our present work, we were able to alter the level of recombination between YACs segregating through meiosis by manipulating the length or homology of one of the YACs, without altering the wild-type diploid genotype of the strain. Nonrecombinant tetrads containing heterologous YACs (pairs E and F) showed a decrease in the percentage of normal meiosis I divisions from 99% to an average of 78% when compared to a homologous pair of YACs (pair A). PSS levels increased to an average of 8% of meioses in this nonrecombinant class and nondisjunction levels increased to an average of 15%. This provides compelling evidence that recombination of homologs is important to ensure their proper disjunction through the first meiotic division. These data show in a wild-type euploid genetic background that the absence of exchange between chromosomes is correlated with an increase of PSS during meiosis.

A second mechanism employed exclusively by meiotic cells to ensure proper segregation of chromosomes, specifically nonrecombinant chromosomes (homologous or heterologous), is distributive disjunction. "Distributive segregation" was first characterized in *Drosophila* females (for a review, see ref. 17) and has also been shown to exist in *S. cerevisiae* (15, 16, 18). Both biological systems use a distributive mechanism to ensure proper segregation of these non-exchange chromosomes. We provide additional evidence that the yeast *S. cerevisiae* has the capability of distributively segregating nonrecombinant chromosomes. Although segregation fidelity of chromosomes was decreased in the nonrecombinant nonhomologous pairs of YACs (pairs E and F), the percentage that disjoined properly in the first meiotic division was greater than expected for random segregation. Nevertheless, the distributive mechanism seems to be unable to ensure 100% disjunction of the chromosomes involved.

The results presented here are also relevant to the physical and functional analysis of large genes or DNA segments cloned as YACs. Several groups have taken advantage of the highly recombinant nature of human-insert-containing YACs to generate artificial chromosome clones that contain the entire sequence of a gene of interest (19, 20). The high recombination rate of human DNA during yeast meiosis indicates that relatively modest overlaps are useful for building recombinant YACs. For example, at 5 kb/cM, a 75-kb overlap would yield the desired recombinant in $\approx 15\%$ of random spores. Furthermore, absence of unequal crossing-over between homologous YACs and absence of recombination between heterologous YACs indicate that artifactual

recombinants involving dispersed repetitive elements will not be a problem.

The use of human-derived artificial chromosomes for the study of meiotic chromosome transmission can be used to facilitate the identification of meiosis-specific cis and trans elements required for proper chromosome segregation. YACs can be manipulated easily and their missegregation has no deleterious effect on the yeast strains that contain them. This system may be useful for assaying the meiosis segregation effects of various centromere DNA mutations, for characterizing mutants involved in the distributive disjunction system, for identifying or characterizing sites that are hyperactive in meiotic recombination, and for determining the effects that position and/or type of recombinational events have on meiotic chromosome segregation.

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