Mixed Segregation and Recombination of Chromosomes and YACs During Single-Division Meiosis in *spo13* Strains of *Saccharomyces cerevisiae*

Yasser Hugerat and Giora Simchen

Department of Genetics, The Hebrew University of Jerusalem, Jerusalem 91904, Israel

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ABSTRACT

Diploid yeast strains, homozygous for the mutation *spo13*, undergo a single-division meiosis and form dyads (two spores held together in one ascus). Dyad analysis of *spo13/spo13* strains with centromere-linked markers on five different chromosomes and on a pair of human DNA YACs shows that: (a) in *spo13* meiosis, chromosomes undergo mixed segregation, namely some chromosomes segregate reductionally whereas others, in the same cell, segregate equationally; (b) different chromosomes exhibit different segregation tendencies; (c) recombination between homologous chromosomes might not determine that a bivalent undergoes reductional rather than equational segregation.

MEIOSIS is a special kind of cell division, in which the chromosome complement is precisely halved. Two nuclear divisions occur during meiosis. At meiosis I, each pair of homologous chromosomes segregates, one chromosome to each of the two nuclei. At meiosis II, the sister chromatids segregate from each other. A single diploid cell of the yeast *Saccharomyces cerevisiae* undergoing meiosis produces four haploid cells, which are held together in one ascus, called a tetrad. However, certain mutants complete only one of the two meiotic divisions, and therefore form unreduced, diploid products. Diploid strains homozygous for either of the temperature-sensitive mutations *cdc5* or *cdc14*, when sporulated at permissive or semipermissive temperatures, yield a high proportion of 2-spored asci (dyads), which have undergone only meiosis I (SCHILD and BYERS 1980). The late release of a *cdc5* or *cdc14* homozygous strain from restrictive to permissive conditions leads to a single-division meiosis, in which some of the centromeres segregate reductionally whereas others segregate equationally (SHARON and SIMCHEN 1990a); thus the chromosomes undergo a division that is a mixture of meiosis I and meiosis II. Strains that are homozygous for the mutation *spo13* also undergo an atypical meiosis, consisting of one rather than two divisions (KLAPHOLZ and ESPOSITO 1980a). Genetic analysis of *spo13* homozygous diploids showed that the centromeres underwent a second-division segregation (KLAPHOLZ and ESPOSITO 1980b). During single-division meiosis in *spo13*, *cdc5* or *cdc14* homozygotes, the early landmark events of meiosis take place, including premeiotic DNA synthesis, chromosome pairing, synaptonemal complex formation and meiotic recombination (SCHILD and BYERS 1980; Klapholz and ESPOSITO 1980b). Surprisingly, analyses of single-division meiosis in haploid *spo13* strains that were disomic for chromosome *III* showed that the chromosomes *III* commonly underwent a reductional segregation (WAGSTAFF, KLAPHOLZ and ESPOSITO 1982; HOLLINGSWORTH and BYERS 1989; ENGEBRECHT and ROEDER 1989). The other, haploid chromosomes, could of course divide only equationally.

To gain a better understanding of chromosome segregation in single-division meiosis, we have undertaken an analysis of different centromere-linked markers in *spo13* strains. We report here that diploid *spo13* strains also undergo mixed segregation in meiosis, in which some chromosomes segregate reductionally like in meiosis I whereas others, in the same cell, undergo meiosis II segregation (Figure 1). The choice of segregation pattern by individual chromosomes may not depend on whether they have recombined, but rather on the centromeres they carry.

MATERIALS AND METHODS

Strains: The strains used in this study, with their genotypes and origins, are listed in Table 1. The construction of the yeast strains is described below.

Strains 2271 and 2281: These strains were derived from crosses among laboratory strains of various origins. They contain the following centromere-linked markers: *trp1* is linked to CEN4, *leu1* is linked to CEN7 and *met14* is linked to CEN11.

Strains 2346, 2350 and 2351: These strains were derived from strains YPH4, 2281, and 2271, respectively, in which the *SPO13* gene was disrupted, using the plasmid PNKY58, as described below.

Strain 2362: Strain YPH603, in which only the selectable markers on the YAC arms were replaced, by *LYS2* on the short arm and *HIS3* on the long arm.

Strain 2388: Strain YPH603, in which the prototrophic markers on the short and long arms were replaced by *URA3* and *ADE2*, respectively.

Strains 2390 and 2393: These strains were derived from

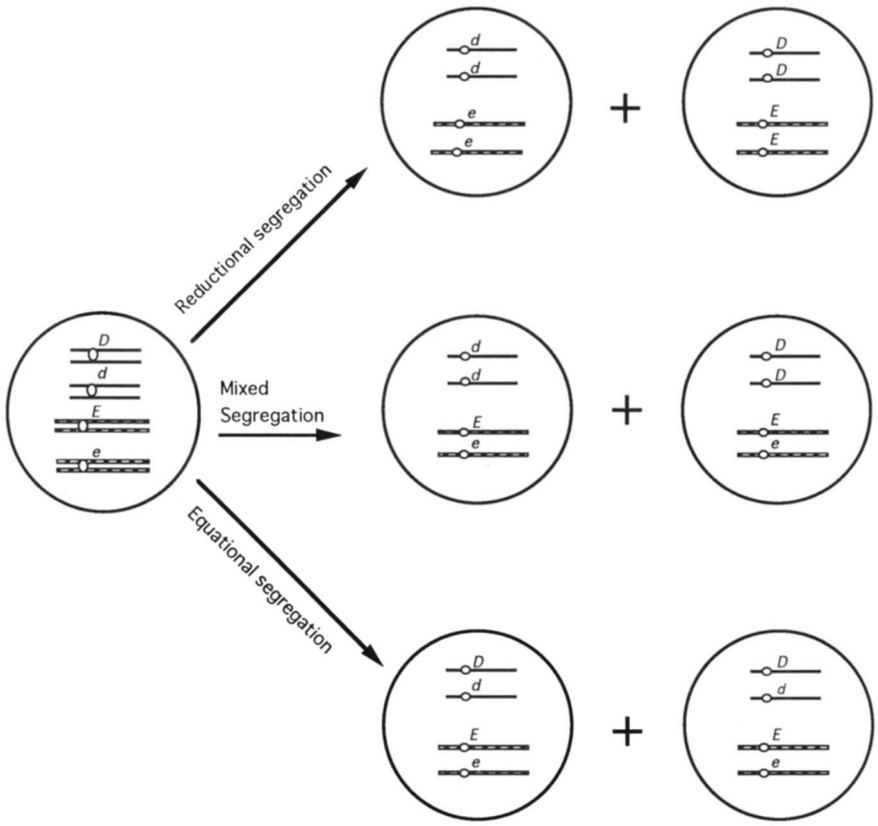

FIGURE 1.—Patterns of chromosome segregation in single-division meiosis. Two diploid spores (a dyad) are produced by each meiosis. Homologous chromosomes are drawn similar in length and shape. *D* and *E* indicate dominant alleles of centromere-linked markers on different chromosomes. *d* and *e* indicate the corresponding recessive alleles. Small open circles indicate centromeres. No recombination events are shown.

strains 2350 and 2351, respectively, in which the gene *RME1* was disrupted using the plasmid PHH1-2. Disruption of the gene *RME1* is useful in that homozygous *rme1/rme1* **a/a** or α/α may undergo sporulation (KASSIR and SIMCHEN 1976).

Strain 2406: Strain 2393, into which YAC-A was transferred by Kar$^-$ mating (see below) from strain 2388.

Strain 2414: Lys$^-$ derivative of strain 2406, selected by plating cells on α-aminoadipate medium. The new allele is designated *lys2-214* (this mutation does not complement *lys2-801*).

Strain 2418: Strain 2414, into which YAC-B was also transferred by Kar$^-$ mating, as described below, from strain 2362. Strain 2418 contains both YAC-A and YAC-B.

Strain 2419: Strain 2390 was transformed to Cdc$^+$ (changing *cdc14* to *CDC14*) using the *Eco*RI-*Sal*I fragment, from plasmid pCH1103 (obtained from CONNIE HOLM).

Strain 2421: This strain was derived from strain 2393, in which the gene *URA3* was inserted near CEN6, using pGS131 as described below.

Strain 2441: Strain 2421, in which the gene *ADE2* was inserted near CEN3, using the pYMC3 plasmid as described below.

Substitution of the prototrophic markers at both ends of the YACs and transfer of YACs: Every YAC used in this study is derived from the previously characterized YAC12 (PAVAN, HIETER and REEVES 1990), which contains a 360-kb human genomic DNA fragment (Figure 3). Prototrophic markers and the centromere of the YAC were modified by gene replacement with a set of marker-exchange plasmids (SHERO *et al.* 1990). One replacement plasmid was constructed in this work, to replace the *TRP1* gene at the end of the long arm with *ADE2*. The 3.6-kb *Bam*HI *ADE2* fragment was removed from the plasmid R-854 (obtained from G. S. ROEDER) and cloned between the *Bgl*II sites of the gene *HIS3* in pJS102 (obtained from P. HIETER). The new plasmid, called pHH2, has unique restriction sites for *Sac*I and *Not*I, which bracket the replacement fragment. The replacements of the selectable markers on the YAC were done in the original YAC12 in the strain YPH603. Replacements of marker and centromere sequences were verified by contour-clamped homogeneous electric field (CHEF) gels, followed by chromosome blot analysis (SCHWARTZ and CANTOR 1984) and Southern blot analysis.

The YACs were transferred from the substitution strains to the appropriate strains either by spheroplast transformation, according to CONNELLY *et al.* (1991), or by chromoduction in Kar$^-$ matings (DUTCHER 1981). For the latter, a *kar1* mutation (*kar1Δ15*) was introduced into the substitution strains (2388 and 2362) by transformation with plasmids pMR1593 (M. ROSE, personal communication) and pHk-9 (Y. HUGERAT, unpublished). Cells of the Kar$^-$ substitution strain and of the recipient strain were incubated together for 5–8 hr in liquid YEPD medium. Cells were then plated on a selective medium that contained cycloheximide and had the deficiencies that were selective for the markers on the YACs, for instance Ura$^-$ or Lys$^-$. The presence of the YAC in the recipient strain was verified by pulse-field gel electrophoresis (CHEF) of chromosome-size DNA.

Disruption of *SPO13*: To disrupt the gene *SPO13* we used the plasmid pNKY58 (obtained from N. KLECKNER). This plasmid contains in the *SPO13* gene the "Gene Blaster" cassette, which is made of *URA3* placed between tandem repeats of the Salmonella gene *hisG* (ALANI, CAO and KLECKNER 1987), inserted at the *Bst*EII site of *SPO13*. The plasmid was digested with *Bam*HI and then transformed into the yeast strain by the lithium acetate procedure (ITO *et al.* 1983), targeting the integration to *SPO13*. Ura$^+$ transformants were tested by mating to *spo13* testers and the diploids were examined on sporulation medium for tetrad or dyad formation. The transformants were converted to Ura$^-$ by

TABLE 1

Genotypes and origins of strains

Strain	Genotype	Origin
YPH4	*MAT***a** *ura3-52 ade2-101 his3Δ200 lys2-801*	PHIL HIETER
YPH54	*MATα ura3-52 l his3Δ200 lys2-801 trp1Δ1 ade2-101*	PHIL HIETER
YPH603	*MATα ura3-52 lys2-801 ade2-101 his3 Δ200 trp1Δ1 leu2Δ1* [ΔCEN6::*LEU2* CEN11] + YAC12 [*HIS3* CEN4 D8B human insert *TRP1*]	PHIL HIETER
2188	*MATα ura3-52 trp1 leu2-3,112 ade2-101 his6-1 can1 rme1::LEU2 spo13::URA3*	This laboratory
2271	*MATα ura3-52 his3Δ200 ade2-101 trp1 lys2-801 can1 cdc14*	This study
2281	*MAT***a** *ura3-52 his3Δ200 ade2-101 leu1 met14 cdc14 cyh2*	This study
2343	*MATα ade1 his2 ura3 trp1 met14 leu1 spo13::URA3*	This study
2346	Isogenic to strain YPH4, *spo13::hisG*	This study
2350	Isogenic to strain 2271, *spo13::URA3*	This study
2351	Isogenic to strain 2281, *spo13::URA3*	This study
2362	Isogenic to YPH603, structure of YAC12-B:[*LYS2* CEN4 D8B human insert *HIS3*]	This study
2388	Isogenic to YPH603, structure of YAC12-A: [*URA3* CEN4 D8B human insert *ADE2*]	This study
2390	Isogenic to 2350, *rme1::hisG spo13::hisG*	This study
2393	*MAT***a** *ura3-52 ade2-101 his3Δ200 leu1 met14 cdc14 cyh2 spo13::hisG rme1::hisG*	This study
2406	Isogenic to strain 2393, but containing YAC12-A from strain 2388	This study
2414	Isogenic to strain 2406, also containing *lys2-214*	This study
2418	Isogenic to strain 2414 + YAC12-B: [*LYS2* CEN4 D8B human insert *HIS3*] (this strain contains two YACs)	This study
2419	Isogenic to strain 2390 converted to Cdc^+ by plasmid pCH1103	This study
2421	Isogenic to strain 2393, in which CEN6 is marked with *URA3*	This study
2441	Isogenic to strain 2421, in which CEN3 is marked with *ADE2*	This study
YH20	*MATα/MAT***a** *ura3-52/ura3-52 his3Δ200/his3Δ200 ade2-101/ade2-101 trp1/TRP1 lys2-801/LYS2 can1/CAN1 LEU1/leu1 MET14/met14 rme1::hisG/rme1::hisG spo13::hisG/spo13::hisG CYH2/cyh2 CDC14/cdc14* CEN6/CEN6::*URA3*	This study (2419 × 2421)
YH21	*MATα/MAT***a** *ura3-52/ura3-52 his3Δ200/his3Δ200 ade2-101/ade2-101 trp1/TRP1 lys2-801/lys2-214 can1/CAN1 LEU1/leu1 MET14/met14 rme1::hisG/rme1::hisG spo13::hisG/spo13::hisG CYH2/cyh2 CDC14/cdc14* YAC12-A(*URA3* CEN4 D8B human insert *ADE2*)/YAC12-B(*LYS2* CEN4 D8B human insert *HIS3*)	This study (2419 × 2418)
YH22	*MATα/MAT***a** *ura3-52/ura3-52 his3Δ200/his3Δ200 ade2-101/ade2-101 trp1/TRP1 lys2-801/lys2-214 LEU1/leu1 MET14/met14 RME1/rme1::hisG SPO13/spo13::hisG CYH2/cyh2 CDC14/cdc14* YAC12-A(*URA3* CEN4 D8B human insert *ADE2*)/YAC12-B(*LYS2* CEN4 D8B human insert *HIS3*)	This study (YPH54 × 2418)
YH23	*MATα/MAT***a** *ura3-52/ura3-52 HIS3/his3Δ200 ade2-101/ade2-101 trp1/TRP1 can1/CAN1 LEU1/leu1 leu2-3,112/LEU2 MET14/met14 rme1::LEU2/rme1::hisG his6-1/HIS6 spo13::URA3/spo13::hisG CYH2/cyh2 CDC14/cdc14*	This study (2188 × 2393)
YH24	*MATα/MAT***a** *ura3/ura3-52 HIS3/his3Δ200 ADE2/ade2-101 trp1/TRP1 leu1/LEU1 his2/HIS2 ade1/ADE1 met14/MET14 spo13::URA3/spo13::hisG LYS2/lys2-801*	This study (2343 × 2346)
YH25	*MATα/MAT***a** *ura3-52/ura3-52 his3Δ200/his3Δ200 ade2-101/ade2-101 trp1/TRP1 lys2-801/LYS2 can1/CAN1 LEU1/leu1 MET14/met14 rme1::hisG/rme1::hisG spo13::hisG/spo13::hisG CYH2/cyh2 CDC14/cdc14* CEN6/CEN6::*URA3* CEN3/CEN3::*ADE2*	This study (2419 × 2441)
YH7s1	*MATα/MAT***a** *ura3-52/ura3-52 his3Δ200/his3Δ200 ade2-101/ade2-101 trp1/TRP1 lys2-801/LYS2 can1/CAN1 LEU1/leu1 MET14/met14 spo13::hisG/spo13::hisG CYH2/cyh2 cdc14/cdc14*	This study (2390 × 2393)

plating the strains on 5-fluoroorotic acid (5-FOA). Papillae on this medium had lost the gene *URA3* by recombination between the *hisG* repeats, leaving behind one copy of the Salmonella *hisG* sequence inserted in *SPO13*. The insertions in the transformants and loop-out derivatives were verified by Southern blot analysis.

Disruption of *RME1*: To enable *MAT***a**/**a** or *MATα/α* homozygotes to sporulate, we disrupted the gene *RME1*. A 1.2-kb *Bgl*II RME1 fragment (COVITZ *et al.* 1991) from the plasmid pJM249 (J. P. MARGOLSKEE, unpublished) was replaced by the 3.8-kb *Bam*HI-*Bgl*II "Gene Blaster" fragment from the plasmid pNKY51 (ALANI, CAO and KLECKNER 1987). The new plasmid, called pHH1–2 (Figure 2A), had two *Pvu*II sites, one 181 bp from the *RME1* insert, in the vector portion of the plasmid, and the other in the *RME1* gene, 2.4 kb beyond the "Gene Blaster" insert. The plasmid pHH1–2 was digested with *Pvu*II and then transformed into yeast by lithium acetate, targeting the integration to the gene *RME1*. Transformants with the "Gene Blaster" insert which were Rme^- were converted to Ura^- as described above. The insertion of *hisG* in *RME1* in the transformants was then verified by Southern blot analysis.

Insertion of URA3 near CEN6: We used the plasmid pGS131 to mark CEN6 with *URA3*. A 1.1-kb *Xho*I fragment containing the gene *URA3* was excised from plasmid B1056 (obtained from G. R. FINK), and inserted between the *Xho*I sites of plasmid pLA276; the latter was obtained from P. PHILIPPSEN and consists of the *Bam*HI fragment carrying δ228 cloned into plasmid YRp7 (see Figure 1 of PANZERI *et al.* 1985). The new plasmid was called pGS131 (Figure 2B). A 4.8-kb *Hin*dIII-*Hin*dIII fragment was used to transform yeast cells, thus inserting *URA3* approximately 10 kb from CEN6, on the right arm of chromosome *VI*.

Insertion of *ADE2* near CEN3: To mark CEN3 with *ADE2*, we constructed the plasmid pYMC3 (Figure 2C). A 5.5-kb *Bgl*II fragment from plasmid pYe(CDC10)1 (CLARKE

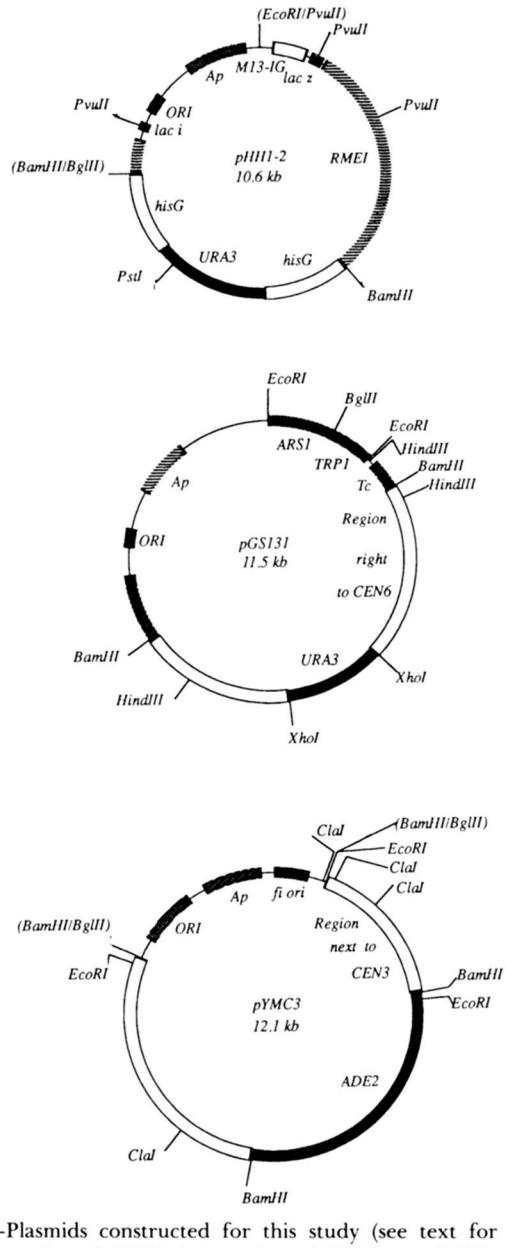

FIGURE 2.—Plasmids constructed for this study (see text for details). A, Disruption of *RME1*; B, insertion of *URA3* near CEN6; C, insertion of *ADE2* near CEN3.

and CARBON 1980), which contains a DNA fragment that is located between the *CDC10* and *LEU2* genes, was cloned into *Bam*HI site of pBluescript SK$^+$ (Stratagene). The 3.6-kb *Bam*HI ADE2 fragment was removed from the plasmid R-854 (obtained from G. S. ROEDER) and cloned into the *Bam*HI site of the chromosomal fragment in the pBluescript-derived plasmid. The new plasmid, with *ADE2* inserted near CEN3, was called pYMC3. This plasmid was cleaved by *Cla*I within the chromosomal insert, and the fragment was used to transform yeast cells. This resulted in insertion of *ADE2* close to CEN3, on the left arm of chromosome *III*. The correct insertion was verified by Southern blot analysis.

Genetic procedures: Liquid culture media, PSP2 and SPM, and SPO solid sporulation medium were prepared according to KASSIR and SIMCHEN (1991). Other solid media were prepared as described previously (SHILO, SIMCHEN and SHILO 1978). 5-FOA medium was prepared as described by BOEKE, LACROUTE and FINK 1984). α-Aminoadipate (αAA) medium was prepared according to CHATTOO *et al.* (1979). Yeast manipulations and yeast transformation were carried out according to ROSE, WINSTON and HIETER (1990). All transformants were verified by SOUTHERN (1975) blot analysis.

Sporulation and dyad analysis: Yeast strains were sporulated on plates as described by KASSIR and SIMCHEN (1991). Tetrads and dyads were dissected as described by KLAPHOLZ and ESPOSITO (1980b). Mating ability was tested by mating with *MAT***a** and *MAT*α auxotrophic tester strains. Phenotypes of dissected spores were determined following replica plating to selective dropout media.

RESULTS

Analysis of chromosome segregation in *spo13* dyads: In diploid strains homozygous for *spo13*, a single-division meiosis takes place. Sporulation in *spo13/spo13* mutants leads to more than 99% dyads (KLAPHOLZ and ESPOSITO 1980a), in which only single-division meiosis has occurred. To study chromosome segregation in single-division meiosis, we constructed various diploid strains that are homozygous for *spo13* and heterozygous for up to five centromere-linked markers (Table 2): *trp1* next to CEN4, *leu1* next to CEN7, *met14* near CEN11, *URA3* inserted next to CEN6, and *ADE2* inserted near CEN3. Although the sporulation frequency of the *spo13* homozygotes was relatively high, spore viability varied (Table 2), but this was probably not related to the *spo13* mutation. Following dyad dissection, segregation patterns of the centromere-linked markers on the five chromosomes were determined by replica plating of the spore colonies on selective media. A high proportion of the dyads showed mixed segregation (Table 2), in which some chromosomes segregated reductionally, while others segregated equationally (Figure 1). It should be noted that the equational segregation frequencies (Table 3) were calculated for the samples of meioses that actually showed mixed segregation, which were the majority of dyads (Table 2). Had we marked all 16 pairs of chromosomes, we believe that all dyads would have exhibited mixed segregation. To be on the safe side, however, we calculated frequencies only for the *proven* cases of mixed meioses. Analysis of the segregation patterns of the different chromosomes (Table 3) shows that CEN3 and CEN11 tend to segregate reductionally whereas CEN4 and CEN6 tend to segregate equationally. CEN7 has an intermediate segregation tendency, although it varies between different diploids. The segregation tendencies of CEN11, CEN7, CEN4 and CEN3 are like their tendencies in single-division meiosis in *cdc5* or *cdc14* homozygotes (SHARON and SIMCHEN 1990a), and *spo13* homozygotes (HOLLINGSWORTH and BYERS 1989), respectively.

Diploids homozygous for *spo13* have been reported to have an increased rate of aberrant segregation in single-division meiosis (KLAPHOLZ and ESPOSITO

TABLE 2

Segregation patterns in *spo13* dyads with two viable spores: first division, second division, or mixed segregation

Strain	Total[a]	First division	Second division	Mixed segregation (%)	Centromere marked chromosome	Percent sporulation	Percent spore viability
YH20[b]	42	4	3	35 (83)	*IV VI VII XI*	65	40
YH21[b]	136	11	27	98 (72)	*IV VII XI* YAC	41	44
YH23	39	2	21	16 (41)	*IV VII XI*	72	71
YH7s1[b]	26	6	2	18 (69)	*IV VII XI*	70	54
YH24	35	0	5	30 (86)	*IV VII XI*	78	73
YH25[b]	161	24	6	131 (81)	*III IV VI VII XI*	66	69

[a] Dyads with two viable spores.
[b] Isogenic strains.

TABLE 3

Equational segregation of individual centromere-linked markers in mixed-meiosis dyads

Chromosome	Centromere-linked marker	Strain		
		YH20	YH21	YH25
III	*ADE2*	—	—	50 (38%)
IV	*trp1*	19 (54%)	58 (59%)	86 (66%)
VI	*URA3*	21 (60%)	—	75 (57%)
VII	*leu1*	14 (40%)	55 (56%)	84 (64%)
XI	*met14*	11 (31%)	27 (28%)	37 (28%)
YAC (A/B)	*LYS2/URA3*	—	89 (91%)	—
Mixed-segregation dyads/total dyads		35/42	98/137	131/162

1980b), resulting in products that are trisomic and monosomic for one or some chromosomes. We asked whether aberrant segregation could explain why some centromere-linked markers show more reductional segregation than equational segregation. To examine aberrant segregation that does not affect spore viability, we introduced a differentially marked pair of yeast artificial chromosomes (YACs; see Figure 3) into the *spo13* homozygotes. The YACs contain all the known elements of a yeast chromosome, namely ARSs, CENs and telomeres. The YACs recombine regularly and segregate with high fidelity in normal meiosis (SEARS, HEGEMANN and HIETER 1992). The two YACs used, YAC-A and YAC-B, consist of the same 360 kb of human DNA sequence and yeast telomeres. In addition, both YACs have the centromere from chromosome *IV* (CEN4). YAC-A has the selectable markers, *URA3* and *ADE2*, integrated in the short arm next to the centromere and the long arm next to the telomere, respectively. YAC-B has the selectable markers *LYS2*, integrated in the short arm next to the centromere, and *HIS3* on the long arm next the telomere (Figure 3). The segregation of the YACs could be followed unambiguously, and aberrant segregation could be fully recorded. Moreover, co-dominance of *MAT***a** and *MATα* allowed us to recognize many cases of aberrant segregation of chromosomes *III*.

Another chromosome for which aberrant segregation could be observed was chromosome *VII*, because it contained the heterozygous marker *CYH2/cyh2*, which allowed us to distinguish heterozygotes from homozygotes.

As shown in Table 4, aberrant segregation occurred at elevated levels for chromosomes *VII*, *III* and the YACs (6–12%). This elevated level of aberrant segregation, however, is not sufficient to explain the high levels of reductional segregation observed for chromosomes *III*, *VI*, *VII* and *XI*, which may reach 60% (Table 4).

A previous study of meiotic chromosome segregation in *cdc5/cdc5* strains, in which CEN11 was replaced by CEN4 (SHARON and SIMCHEN 1990b), showed that the centromere was responsible for the choice between the two segregation patterns, reductional or equational. Here we reexamined the effect of the centromere on the segregation of the YACs.

The segregation behavior of the two YACs (YAC-A/YAC-B) (see Figure 4) was scored in 136 dyads with two viable spores. Both YACs (YAC-A/YAC-B), when in the same cell, exhibited equational segregation in 90% of the mixed meiosis cases (Table 3). This behavior parallels the behavior of chromosomes *IV*, which also show a high rate of equational segregation in single-division meiosis in the same diploid, as well as in *cdc5* and *cdc14* diploids (SHARON and SIMCHEN 1990a). This finding supports the notion that the centromere (here CEN4) may be responsible for the segregation tendency of the YACs in *spo13* meiosis. It

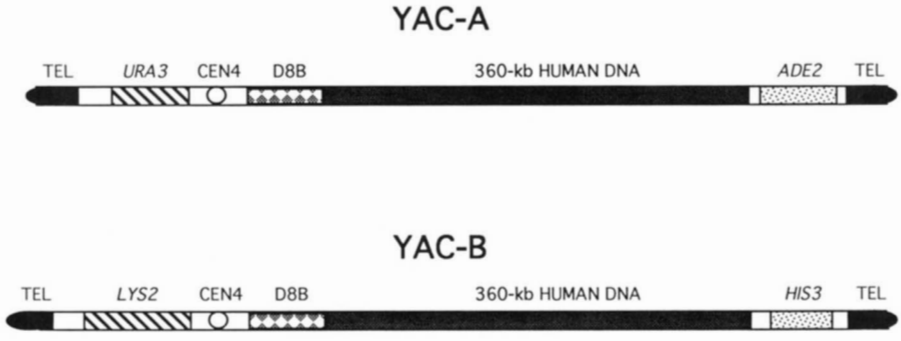

FIGURE 3.—Two differenitially marked YACs that contain an identical 360-kb human DNA segment. YAC-A is marked with *URA3* (on the short arm) and *ADE2* (on the long arm). YAC-B is similarly marked with *LYS2* (on the short arm) and *HIS3* (on the long arm). Total length of each of the YACs is approximately 370 kb.

TABLE 4

Aberrant chromosome segregation in mixed-meiosis dyads in strain YH21 (*spo13/spo13*)

Chromosome	No. of aberrant dyads
III	15[a]
VII	8[b]
YAC	8[c]
No. of mixed meiosis dyads	98

[a] Dyads with one nonmater and one mater spore.
[b] Dyads in which one spore colony was Cyh^s (*CYH2* monosome) and the other spore colony papillated on medium containing cycloheximide. In most cases the latter gave more extensive papillation on CYH medium than diploids heterozygous for *CYH2/cyh2*; therefore these colonies were assumed to be trisomic for chromosome *VII* (*CYH2/cyh2/cyh2*) and the dyad was scored as an aberrant segregation for this chromosome.
[c] Aberrant segregation of the YAC was recognized as shown in Figure 4B.

should be noted that the frequency of equational segregation of the YACs was considerably higher than that of chromosomes *IV*, suggesting that factors other than the centromere may also contribute to the segregation tendency (see DISCUSSION).

Chromosome segregation tendency is recombination-independent: One explanation for the heterogeneity in segregation patterns of individual pairs of chromosomes in single-division meiosis could be that the segregation depends on whether recombination has occurred between the two homologous chromosomes. According to this proposal, for any given pair of chromosomes, if recombination occurs, the chromosomes segregate reductionally, whereas if it does not occur the chromosomes segregate equationally. However, it is known that in *spo13* meiosis recombination occurs at levels close to those found in Spo$^+$ strains (KLAPHOLZ and ESPOSITO 1980b). Additional information pertinent to this issue is provided by the present experiments.

Recombination between the homologous YACs (YAC-A/YAC-B) was scored by the distribution of the four protrophic markers on the YACs among the diploid spore progeny in each dyad (Figure 4). In addition, recombination on a limited stretch of chromosome *VII* was measured by scoring cycloheximide resistance, which resulted from homozygosis of the recessive allele *cyh2*, for which the diploid was heterozygous (Figure 5). The recombination rate (in centimorgans) was calculated as shown in Table 5, based on the phenotypes and assumed genotypes given in Figures 4 and 5. In the *spo13* dyads, recombination values between the ends of the YAC (*URA3/LYS2* to *ADE2/HIS3*) and between *leu1* and *cyh2* on chromosome *VII* were 73 and 67 cM, respectively. These values were similar to values obtained in isogenic, Spo$^+$ strains (Table 5). In dyads that showed mixed chromosome segregation, recombination occurred on the YACs or chromosome *VII* pairs that segregated equationally, as well as on those that segregated reductionally (Table 6). It should be emphasized that only a limited stretch of chromosome *VII*, between *leu1* and *cyh2*, was tested for recombination. Recombination may, of course, have occurred in other parts of the apparently nonrecombinant chromosomes (the region between *leu1* and *cyh2* consists of 170 kb out of 1100 kb of the entire chromosome *VII*; ARBEL 1992). The recombination values obtained were similar to values expected from normal meiosis. These results show that in the map intervals we have tested, there is no correlation between recombination and the pattern of chromosome segregation in single-division meiosis.

DISCUSSION

Meiosis consisting of a single division was first reported for the *el* (elongate) mutant of maize (NEL 1975). Single-division meiosis was subsequently reported for the yeast *S. cerevisiae* mutants *cdc5/cdc5* (SCHILD and BYERS 1980) and *spo13/spo13* (KLAPHOLZ and ESPOSITO 1980a), both of which produce diploid meiotic products. Segregation of only one centromere-linked marker in the *cdc5* strains and of two such markers in *spo13* (KLAPHOLZ and ESPOSITO 1980b), suggested that the segregation patterns in these mutants were reductional and equational, respectively. It was further assumed that these segregation behaviors were shared by all the chromosomes in the cell, although KLAPHOLZ and ESPOSITO (1980b) state that "In some instances, segregational behavior does ap-

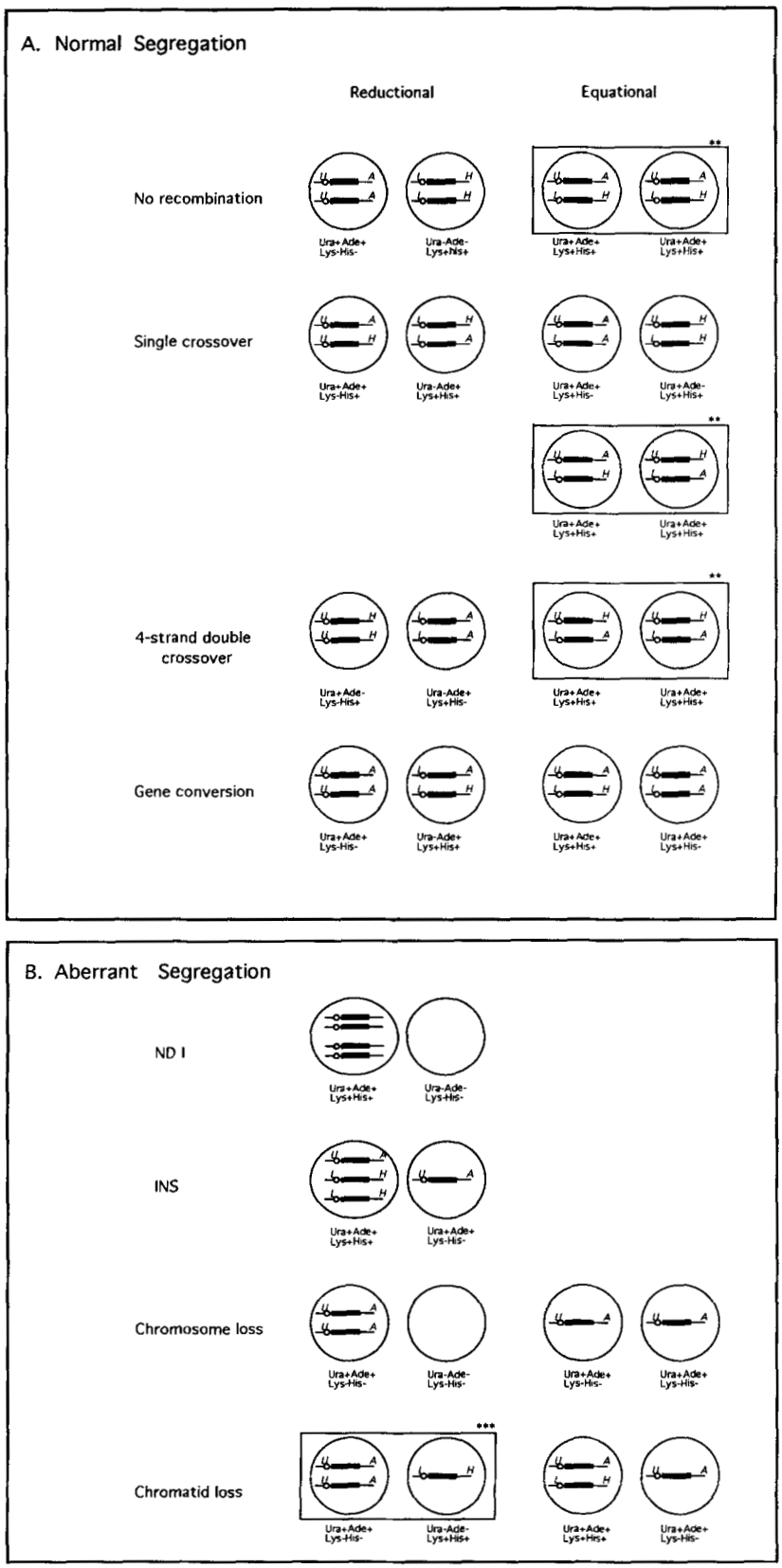

FIGURE 4.—Meiotic segregation patterns of the YAC pair in *spo13* dyads. The products of the YAC pair segregation in *spo13* dyads are shown in the large open circles, with markers indicated on the chromosomes. *U*, *A*, *L*, *H*–the markers *URA3*, *ADE2*, *LYS2*, *HIS3*, respectively. (A) Patterns of normal segregation of the YAC pair with, or without, recombination events. (B) Aberrant segregation of YACs in *spo13* dyads. In this figure abnormal segregation for the *URA3*-marked YAC is shown. Abnormal segregation of the *LYS2*-marked YAC and aberrant segregation with recombination events are not shown. INS, incoordinate segregation of the two YACs: one segregates reductionally and the other segregates equationally. ND I, nondisjunction of the YACs. **These pairs of spore colonies could not be distinguished from each other phenotypically. ***The phenotypes are the same as in a normal reductional (first division) segregation, without recombination.

pear to be regulated at the level of the individual chromosome" (p. 607). SHARON and SIMCHEN (1990a), using four marked centromeres, showed that in a *cdc5/cdc5* mutant strain the decision to divide reductionally or equationally is made in most cells at the level of the individual chromosome.

Genetic analysis of meiosis in diploids homozygous for the *spo13-1* allele (KLAPHOLZ and ESPOSITO 1980b)

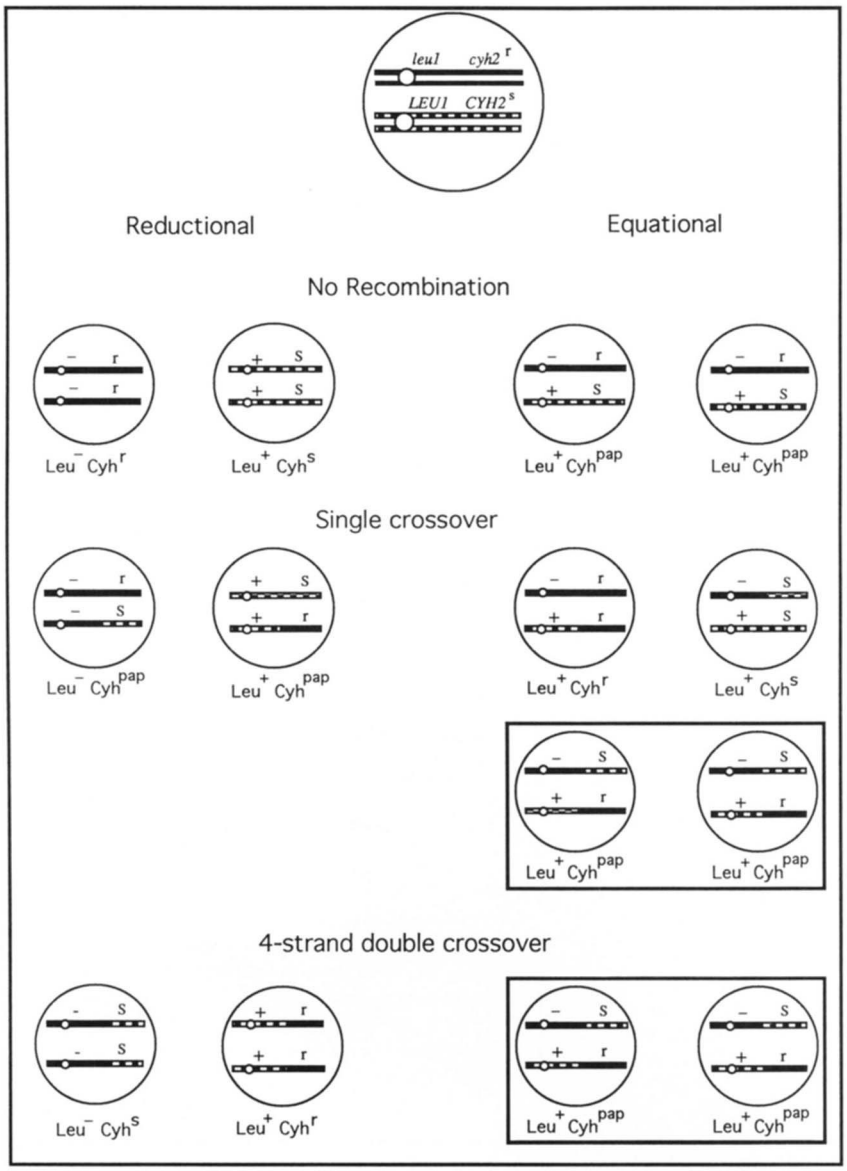

FIGURE 5.—Patterns of chromosome *VII* recombination and segregation in *spo13* dyads. Chromosomes *VII* are heterozygous for *cyh2* and *leu1*, as shown. The different homologs are designated by the solid and dashed lines. The centromeres are represented by open circles. The chromosomes shown at the top have completed premeiotic DNA replication, and each line represents a sister chromatid. The products of meiotic segregation in *spo13* dyads are shown in the large open circles, with phenotypes indicated. *LEU1* is very tightly linked to the centromere. Therefore, it is possible to determine which dyads have resulted from equational segregation. Furthermore, because the dominant *CYH2* allele confers sensitivity, and the recessive allele *cyh2* confers resistance to cycloheximide, it is possible to distinguish between spore colonies that are homozygous or heterozygous at *CYH2* by the papillation of the latter on medium containing cycloheximide (marked Cyh^{pap}). Due to mitotic recombination, heterozygotes give rise to Cyh^r papillae, whereas *CYH2* homozygotes do not. Reductional segregations without recombination generate Leu^- Cyh^r and Leu^+Cyh^s spore colonies. A recombination event followed by a reductional division produces Leu^- Cyh^{pap} and Leu^+ Cyh^{pap} spore colonies. A double crossover event followed by a reductional division produces Leu^- Cyh^s and Leu^+Cyh^r spore colonies. Equational segregation produces two Leu^+ Cyh^s spore colonies that papillate to Cyh^r. In half of the cases in which a recombination event is followed by equational segregation, Leu^+Cyh^r and Leu^+ Cyh^s spore colonies are formed. The other half gives rise to two Leu^+Cyh^{pap} spore colonies that are not detected as recombinants. The same pattern is generated if a four-strand double crossover has occurred and was then followed by an equational segregation. The ambiguous spore colonies are designated by large open boxes.

showed them to undergo predominantly equational segregation (however, some cases of reductional segregation, and others of abnormal segregation, were also observed). In the double-mutant diploids *rad50spo13* (MALONE and ESPOSITO 1981) and *spo11spo13* (KLAPHOLZ, WADDELL and ESPOSITO 1985), segregation was clearly equational and permitted the formation of viable two-spored asci. Strains homozygous for only *rad50* or *spo11* are deficient in the recombination process and therefore produce inviable spores. The *spo13* mutation rescues meioses that would have failed to produce viable spores because of other recombination-deficient mutations. This finding has led to the notion that the *spo13* mutation results in bypass of meiosis I, the reductional division. In the present study, however, we show that most of the diploid spores produced by *spo13* mutants result from single-division meioses with mixed segregation. In each of these meioses some chromosomes have segregated reductionally, whereas others have segregated equationally. Our study shows that in single-division meioses, chromosomes *III*, *XI*, *VI*, *VII* have high levels of reductional segregation. Similar reductional segregation behavior in *spo13* mutants was also observed for chromosomes *III* in homozygous *spo13* diploids and in haploid strains that were disomic for chromosome *III* (WAGSTAFF, KLAPHOLZ and ESPOSITO 1982; Hollingsworth and BYERS 1989; ENGEBRECHT and ROEDER 1989). We do not think that the high levels of reductional segregation observed for some chromosomes in these studies reflect aberrant segregation. We measured aberrant segregation for some chromosomes and found it to be relatively low and at a similar level for the different chromosomes examined (Table 4). The levels of aberrant segregation observed could not explain the high levels of reductional segregation of chromosomes *III*, *XI*, *VI* and *VII* in our study, or of chromosomes *III* in the above mentioned studies.

TABLE 5

Recombination of YACs and of chromosomes *VII* in *spo13/spo13* and *SPO13/spo13* strains

A. Strain YH21 (*spo13/spo13*)

Parental configuration of YACs:

URA3 —○— *ADE2*

LYS2 —○— *HIS3*

Dyad class[a]	Spore	Phenotype	No. of dyads	Distance (cM)[b]
1 c.o., reductional	A	$Ura^+ Ade^+ Lys^- His^+$	3	73
	B	$Ura^- Ade^+ Lys^+ His^+$		
1 c.o., equational	A	$Ura^+ Ade^+ Lys^+ His^-$	35	
	B	$Ura^+ Ade^- Lys^+ His^+$		
2 c.o., reductional	A	$Ura^+ Ade^- Lys^- His^+$	1	
	B	$Ura^- Ade^+ Lys^+ His^-$		
"NR," reductional	A	$Ura^+ Ade^+ Lys^- His^-$	4	
	B	$Ura^- Ade^- Lys^+ His^+$		
"NR," equational	A	$Ura^+ Ade^+ Lys^+ His^+$	60	
	B	$Ura^+ Ade^+ Lys^+ His^+$		
Aberrant segregation			34	
Total			137	

Parental configuration of chromosomes *VII*:

— — — // — ○— *LEU1* *CYH2*

— — — // — ○— *leu1* *cyh2*

Dyad class[a]	Spore	Phenotype	No. of dyads	Distance (cM)[b]
1 c.o., reductional	A	$Leu^- Cyh^{pap}$	38	67
	B	$Leu^+ Cyh^{pap}$		
1 c.o., equational	A	$Leu^+ Cyh^r$	31	
	B	$Leu^+ Cyh^s$		
2 c.o., reductional	A	$Leu^- Cyh^s$	5	
	B	$Leu^+ Cyh^r$		
"NR," reductional	A	$Leu^- Cyh^r$	10	
	B	$Leu^+ Cyh^s$		
"NR," equational	A	$Leu^+ Cyh^{pap}$	44	
	B	$Leu^+ Cyh^{pap}$		
Aberrant segregation			9	
Total			137	

B. Strain YH22 (*SPO13/spo13*)

	Ascus type				Distance (cM)	
Gene pair	PD	NPD	T	Total	This experiment	MORTIMER and SCHILD
leu1-cyh2	31	12	80	123	61.8	56
URA3/LYS2–ADE2/HIS3	30	15	50	95	73.8	

[a] "Reductional" and "equational" relate to the centromere-linked markers. The number of crossovers (c.o.) is the smallest number of events that can explain the phenotypes of the spore colonies. "NR," phenotypically nonrecombinant for the region flanked by the markers (However, 2-strand double crossovers, and crossovers that occur outside the marked region, are also included in this class).

[b] Recombination events for the *URA3/LYS2–ADE2/HIS3* and the *leu1-cyh2* intervals were identified among the dyads as illustrated in Figures 4 and 5, respectively. Map distance was calculated using PERKINS' (1949) formula as follows:

$$\text{Map distance} = 50 \times [\text{single crossovers} + 6\ (\text{4-strand double crossovers})]/\text{total}.$$

Of the single crossovers with equational segregation, only one half may be distinguished phenotypically (Figures 4 and 5). The observed frequency of dyads with single crossovers and equational segregation is therefore doubled, and added to the dyads with single crossovers and reductional segregation.

A four-strand double crossover could be detected only when followed by reductional division of these chromosomes (see Figures 4 and 5); therefore we assumed that double crossovers occurred at the same frequencies in chromosomes that segregated reductionally or equationally (first or second division segregation), respectively. Total numbers of 4-strand double crossovers were calculated as follows:

$$\text{Total 4-strand double crossovers} = dfd + dfd\ (sd/fd),$$

where *dfd* is the number of 4-strand double crossover events detected among reductional (first division) segregation dyads, *sd* is the number of dyads showing equational (second division) segregation, and *fd* is the number of dyads showing reductional (first division) segregation, for the chromosome under investigation.

TABLE 6

Recombination events in mixed-meiosis dyads in strain YH21 (*spo13/spo13*)

Chromosome	Reductional segregation		Equational segregation		Total[c]
	NR[a]	R[b]	NR[a]	R[b]	
VII (*leu1-cyh2*)	8	27	28	21	84
YAC (end markers)	5	2	48	22	77

[a] Non-recombinant (parental) arrangement of markers.
[b] Recombination events between the end markers on the YACs, or between *leu1* and *cyh2* on chromosomes *VII*, were recognized as shown in Figures 4 and 5, respectively.
[c] Included here are mixed-meiosis dyads in which the tested chromosome did not undergo aberrant segregation.

How does one reconcile the mixed-segregation behavior in *spo13* meiosis with the meiosis I bypass (equational segregation only) characteristic of the double mutants (*rad50spo13* or *spo11spo13*)? We suggest that the behavior of the double mutants stems from the pairing deficiency incurred by the mutations *rad50* and *spo11*, as well as other class-1 mutations (PETES, MALONE and SYMINGTON 1991), and that unpaired chromosomes tend to undergo equational segregation. Indeed, paired SCs were not observed in *rad50* (ALANI, PADMORE and KLECKNER 1990), in some *spo11* strains (GIROUX, DRESSER and TIANO 1989), and in *hop1* (HOLLINGTHWORTH and BYERS 1989), *red1* (ROCKMILL and ROEDER 1990) and *mer1* (ENGEBRECHT and ROEDER 1990) strains. The tendency of unpaired chromosomes to undergo equational rather than reductional segregation is seen as precocious sister chromatid separation (PSS) in meiosis I. This was observed, for instance, in the mutant *dy* of maize (MAGUIRE 1978) and in the unpaired univalents in wheat monosomics (MORRISON 1953), and can be inferred from the genetic segregation data obtained for the yeast mutants *red1* or *DIS1* (ROCKMILL and ROEDER 1988; ROCKMILL and FOGEL 1988). We suggest that spore lethality following *spo11* or *rad50* meiosis is largely due to PSS, which results from the failure to pair, and to its consequent aneuploidy. In the double mutants, the *spo13* mutation results in a single-division meiosis, in which the outcome of PSS of both homologs is equational segregation. This leads to viable spores. Thus the pairing deficiency converts the otherwise mixed segregation to mainly equational segregation. Hence, the double mutants *rad50spo13* and *spo11spo13* are rescued from the lethality conferred by the single mutations *rad50* or *spo11*.

The mixed segregation behavior of *spo13* meiotic cells suggests that the lesion caused by the mutation prevents the occurrence of two consecutive divisions, as in normal meiosis, resulting in the unusual, single-division meiosis. Thus the *spo13* lesion does not specifically lead to failure of the first, reductional division. It follows that the *SPO13* gene product is not responsible for reductional segregation. Rather, the product may be required for the occurrence of two consecutive meiotic divisions. In its absence there is only a single division, in which individual chromosomes can segregate either reductionally or equationally.

Recombination and chromosome pairing ensure the faithful reductional segregation of chromosomes in normal meiosis (for review, see ROEDER 1990). Does recombination also influence the choice between reductional and equational segregation in single-division meiosis? We know that meiotic recombination in *spo13* diploid strains occurs at normal levels (KLAPHOLZ and ESPOSITO 1980a) (see also Table 5). Furthermore, we find that in dyads in which some chromosomes have segregated equationally, these same chromosomes have previously recombined. These data show that meiotic levels of recombination in single-division meiosis do not ensure reductional segregation.

It has been demonstrated that in *cdc5/cdc5* mutants, which also undergo mixed meiosis, chromosomes *I*, *IV*, *VII* and *XI* have each a characteristic segregation tendency (SHARON and SIMCHEN 1990a). The centromeric region is responsible for the segregation tendency of a particular chromosome (SHARON and SIMCHEN 1990b). In the present study we add three chromosomes to the four examined by SHARON and SIMCHEN (1990a) in single-division meiosis, namely chromosomes *III* and *VI* and YAC12. The YACs show high levels of meiotic segregation fidelity, close to those of endogenous chromosomes (SEARS, HEGEMANN and HIETER 1992) and are not required for viability of the cell. We observe that a YAC containing the centromere from chromosome *IV* (CEN4) has a tendency to segregate equationally at high frequency, as does the native chromosome *IV*. This supports the notion that the centromeric regions are responsible for segregation tendencies in single division meiosis. However, the frequency of equational segregation of the YAC is considerably higher than that of chromosome *IV*. This suggests that other features of the YAC may contribute to its equational behavior, such as the proximity of the centromere to one of the telomeres (and/or to plasmid sequences from the YAC cloning vector). Another feature by which the YAC differs from yeast chromosomes is that it is made largely of human DNA. Either the presence of some sequences in human DNA, for instance repeats of *Alu* elements, or the absence of others that are normally necessary for reductional segregation, may affect the YACs' segregational behavior. Experiments to distinguish between these alternatives are in progress. It should be remembered, however, that in normal meiosis YAC12 segregates reductionally in meiosis I (SEARS, HEGEMANN and HIETER 1992). Its extreme equational behavior in mixed meiosis may mean that the YAC is

perhaps also handicapped when in competition with yeast chromosomes for a factor required for reductional segregation.

Our results show that different chromosomes have different tendencies to segregate equationally or reductionally. They suggest that the decision to divide reductionally or equationally is made at the level of the individual chromosome. This was also suggested by an earlier study (NICKLAS 1977), in which bivalents from the first-division spindle that were moved by a micromanipulator to a second-division spindle, continued their reductional separation. Similarly, meiosis II chromosomes placed in a meiosis I spindle divided equationally. The centromere is a good candidate for the structural element that mediates this chromosome-specific decision, because it is the region that attaches to the spindle microtubules. Indeed, in a mutational analysis of the centromere of yeast chromosome *III* (GAUDET and FITZGERALD-HAYES 1989), certain mutations had no effect on chromosome segregation in mitosis, but caused premature sister chromatid separation in meiosis. To characterize the centromeric sequences that are responsible for the different segregation tendencies in single-division meiosis, we are currently studying the segregation in single-division meiosis of YACs carrying various CENs and CEN mutations.

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