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Meiotic Disjunction of Homologs in *Saccharomyces cerevisiae* Is Directed by Pairing and Recombination of the Chromosome Arms but Not by Pairing of the Centromeres

Richard T. Surosky[1] and Bik-Kwoon Tye

Section of Biochemistry, Molecular and Cell Biology, Cornell University, Ithaca, New York 14853

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ABSTRACT

We explored the behavior of meiotic chromosomes in *Saccharomyces cerevisiae* by examining the effects of chromosomal rearrangements on the pattern of disjunction and recombination of chromosome *III* during meiosis. The segregation of deletion chromosomes lacking part or all (telocentric) of one arm was analyzed in the presence of one or two copies of a normal chromosome *III*. In strains containing one normal and any one deletion chromosome, the two chromosomes disjoined in most meioses. In strains with one normal chromosome and both a left and right arm telocentric chromosome, the two telocentrics preferentially disjoined from the normal chromosome. Homology on one arm was sufficient to direct chromosome disjunction, and two chromosomes could be directed to disjoin from a third. In strains containing one deletion chromosome and two normal chromosomes, the two normal chromosomes preferentially disjoined, but in 4–7% of the tetrads the normal chromosomes cosegregated, disjoining from the deletion chromosome. Recombination between the two normal chromosomes or between the deletion chromosome and a normal chromosome increased the probability that these chromosomes would disjoin, although cosegregation of recombinants was observed. Finally, we observed that a derivative of chromosome *III* in which the centromeric region was deleted and *CEN5* was integrated at another site on the chromosome disjoined from a normal chromosome *III* with fidelity. These studies demonstrate that it is not pairing of the centromeres, but pairing and recombination along the arms of the homologs, that directs meiotic chromosome segregation.

IN meiosis the chromosomes pair, recombine and then segregate, resulting in the reduction of the chromosome number by half. It is critical that the chromosomes segregate in an orderly manner such that each meiotic product receives a single copy of each chromosome. Aberrant segregation of the chromosomes produces aneuploid cells that may be inviable. In addition to a reduction in chromosome number, meiosis also produces genetic diversity as a result of recombination and segregation. In the yeast *Saccharomyces cerevisiae*, meiosis is part of a process termed sporulation (reviewed by ESPOSITO and KLAPHOLZ 1981). Diploid yeast cells containing the *MAT***a** and *MAT*α mating type alleles, when placed in the appropriate media, undergo meiosis and spore formation to yield four haploid spores (ROMAN and SANDS 1953). Analysis of meiosis in *S. cerevisiae* indicates that it is fundamentally similar to meiosis in other eukaryotes (HOOPER *et al.* 1974; ZICKLER and OLSON 1975).

The behavior of chromosomes in meiosis has been extensively examined by cytological analysis. Early in meiosis, the chromosomes condense and homologous chromosomes approach each other. When the homologs are sufficiently close, formation of the synaptonemal complex is initiated, often at the ends of the chromosome (MOENS 1969a). The complex then spreads along the length of the chromosome until the homologs are intimately paired. The synaptonemal complex, present in nearly all eukaryotic cells, plays a central role in chromosome pairing by physically holding the homologs in close proximity (reviewed by MOSES 1968; WESTERGAARD and VON WETTSTEIN 1972). In most species, formation of this complex is required for meiotic recombination to occur (GILLIES 1975).

As meiosis proceeds, the attraction between the homologs suddenly ends, the synaptonemal complex disappears and homologs separate, except in regions containing chiasmata (WESTERGAARD and VON WETTSTEIN 1970). These chiasmata, seen as physical attachments between homologs, result from crossing over between chromatids (MATHER 1933; WHITE 1954). The bivalent, held together at the chiasmata, migrates to the metaphase plate where it often assumes a bipolar orientation. One kinetochore is oriented toward one spindle pole and the other kine-

[1] Present address: Department of Molecular Genetics and Cell Biology, University of Chicago, 1103 East 57th Street, Chicago, Illinois 60637.

tochore toward the other spindle pole (OSTERGREN 1951; NICKLAS 1967). This orientation determines which kinetochore will be attached to which spindle pole, and therefore how the chromosomes will segregate (NICKLAS 1967). In instances where homologs become attached to the same spindle pole, the chromosomes will be reoriented until proper attachment is achieved (HUGHES-SCHRADER 1943). Studies have demonstrated that it is the tension on the homologs generated by their attachment to opposite spindle poles that stabilizes the attachment of the microtubules (NICKLAS and KOCH 1969; BUSS and HENDERSON 1971; NICKLAS 1974). To segregate the chromosomes, the chiasmata are released, allowing the chromosomes to move toward the spindle poles. Following this first meiotic division, a second occurs; this latter division is similar to mitosis (BOSTOCK and SUMNER 1978).

Genetic analysis using meiotic mutants has also suggested a relationship between pairing, recombination and segregation. In the female Drosophila, mutations that reduce meiotic recombination by affecting either the preconditions for exchange (such as pairing) or the exchange process itself have been identified (reviewed by BAKER and HALL 1976). The frequency of chromosome nondisjunction is also increased in nearly all of these mutants. Other mutations that do not affect exchange yet increase chromosome nondisjunction may specify functions involved in segregation itself (DAVIS 1969). In yeast, both the *spo11* and *rad50* mutations decrease or abolish meiotic recombination (KLAPHOLZ, WADDELL and ESPOSITO 1985; GAME *et al.* 1980). In these mutants, there is a high frequency of nondisjunction and mostly aneuploid spores are produced. Strains containing a *spo12* or *spo13* mutation bypass the first meiotic division and proceed directly to a second meiotic division following recombination (KLAPHOLZ and ESPOSITO 1980a,b). Mutants containing both the *spo11* and the *spo13* mutations produce two nonrecombinant diploid spores (MALONE and ESPOSITO 1981; KLAPHOLZ, WADDELL and ESPOSITO 1985).

Both cytological and genetic studies indicate that pairing, recombination and segregation are required to insure that each meiotic product receives the proper chromosome complement. Although the processes may occur independently, success of one process is dependent on the proper execution of the others. Without pairing, recombination cannot occur, and without the chiasmata that result from recombination, chromosomes cannot disjoin properly.

We have previously described the construction of a series of deletion derivatives of chromosome *III* in *S. cerevisiae* and the mitotic stability of these chromosomes (SUROSKY and TYE 1985a; SUROSKY, NEWLON and TYE 1986). In this paper, we investigate the sequence of events that lead to the meiotic segregation of chromosomes by examining the meiotic behavior of several of these deletion chromosomes in the presence of one or two copies of the normal chromosome *III*. Our studies confirm prior cytological and genetic observations that pairing and recombination are important for the proper segregation of homologs. Proper segregation, however, does not require the pairing of the centromeres of the homologs.

MATERIALS AND METHODS

Strains: *Escherichia coli* strain HB101 (F^- *proA2 leuB6 thi-1 hsdS20 recA13 rpsL20 lacY1 galK-2 ara-14 xyl-5 mtl-1 supE44*) (BOYER and ROULLAND-DUSSOIX 1969) was used as the host for plasmid amplification. The yeast strains used in this study are listed in Table 1. The crosses are listed in Table 2. The construction of some of the yeast strains is described below.

Strains in crosses 3 to 11: The strains in which the original deletions were made are used. A description of the deletion chromosomes is presented in Figure 1 and RESULTS.

Strain 426-67A: The *URA3* gene was integrated at the *HIS4* locus on chromosome *III*. The DNA fragment used in the replacement was constructed by digesting plasmid H4YRB (SUROSKY and TYE 1985b) containing the *HIS4* gene on a *Bam*HI/*Eco*RI fragment with *Hin*dIII. Four *Hin*dIII fragements, including most of the *HIS4* coding region, was excised and a 1.2-kb *Hin*dIII fragment containing *URA3* was inserted. The *Bam*HI/*Eco*RI yeast DNA fragment from this plasmid was used to transform strain 320-16C to Ura^+. The successful gene replacement was verified by hybridization analysis. This strain (SUH3) was crossed to strain 8534-8C, and spore 426-67A containing the gene replacement was obtained from this diploid.

Strain RS4-22: Strain 8534-8C was transformed to His^+ using *Sal*I DNA fragments from plasmid pYehis4 (HINNEN *et al.* 1979). This strain was crossed to strain SUH3 (see strain 426-67A). A Ura^+His^+ random spore, RS4-22, disomic for chromosome *III* was isolated. Genetic analysis of this spore using multiply marked tester strain F399 verified that it was disomic for chromosome *III*.

Strain 353-14ALU: In this Ura^-Leu^- derivative of strain 353-14A, one normal chromosome has replaced the dC_RT and dC_LT chromosomes as a result of a mitotic recombination event in the 1.5 kb of homology at the centromere. This strain was isolated on 5-fluoro-orotic acid media and replica plated onto Cm-leu media.

Strain in cross 22: In the haploid strain ICH2, a ring and linear derivative of chromosome *III* has replaced the normal chromosome. The ring consists of all sequences between the *LEU2* region on the left arm and the *PGK1* region on the right arm. The linear chromosome contains all sequences distal to *LEU2* and all sequences distal to *SUF16*. The linear chromosome also contains *CEN5* and *URA3* integrated at *HIS4*. This strain was crossed to strain 425-6D and a Leu^- derivative lacking the ring chromosome was isolated by replica plating onto Cm-leu media. Loss of the ring chromosome was verified by hybridization analysis.

Media and enzymes: YEPD and synthetic complete medium for yeast (SHERMAN, FINK and LAWRENCE 1974) and complete and minimal media for *E. coli* (MILLER 1972) have been described. Complete medium supplemented with 0.5 mg of 5-fluoro-orotic acid per milliliter was used for the positive selection of orotidylate decarboxylase-defective (*ura3*) strains (BOEKE, LACROUTE and FINK 1984).

TABLE 1

Yeast strains

Strain	Genotype	Ploidy and chromosome rearrangements	Source
8534-8C	*MATα his4 leu2 ura3*		G. Fink
320-13B	*MAT**a** ura3 met3 trp1 MAL2*		This study
320-16C	*MAT**a** leu2 ura3 met3 trp1 MAL2*		This study
DLT1	*his4 leu2 MATα mal2 ura3 MET3 TRP1* *[URA3] LEU2 MATa MAL2 ura3 met3 trp1*	Diploid with one normal chromosome *III* and one dLT chromosome	138C
DLT2	Same as DLT1		
DC$_{L}$T1	*his4 leu2 MATα mal2 ura3 MET3 TRP1* *[URA3] MAT**a** MAL2 ura3 met3 trp1*	Diploid with one normal chromosome *III* and one dC$_{L}$T chromosome	138C
DC$_{L}$T2	Same as DC$_{L}$T1		
DC$_{L}$T4	Same as DC$_{L}$T1		
DPT6M	*his4 leu2 MATα mal2 ura3* *HIS4 leu2 [LEU2] ura3* *::[URA3 MAT**a**] MET3 TRP1* *met3 trp1*	Diploid with one normal chromosome *III* and one dPT chromosome. *MATa* was integrated at *ura3*	168C
DPT11M	Same as DPT6		
DC$_{R}$T7M	*his4 leu2 MATα mal2 ura3* *HIS4 leu2 [LEU2]* *::[URA3 MAT**a**] MET3 TRP1* *ura3 met3 trp1*	Diploid with one normal chromosome *III* and one dC$_{R}$T chromosome. *MATa* was integrated at *ura3*	168C
DC$_{R}$T19M	Same as DC$_{R}$T7		
F399	*MAT**a** his4 leu2 thr4 ade6 trp1 lys2 tyr1 arg4 gal7 MAL2*		R. Esposito
F400	*MATα his4 leu2 thr4 ade6 trp1 lys2 tyr1 arg4 gal7 MAL2*		R. Esposito
369-5C	*MAT**a** thr4 cdc10 lys2 ade6 MAL2*		This study
369-21C	*MAT**a** leu2 thr4 cdc10 lys2 trp1 MAL2*		This study
338-15A	*his4 leu2 MATα ura3 met3* *HIS4 leu2 [LEU2]*	Strain contains one normal chromosome *III* and one dC$_{R}$T chromosome. Monosomic for other chromosomes	Cross 10
338-115B	*his4 leu2 MATα ura3 met3 trp1* *HIS4 leu2 [LEU2]*	Strain contains one normal chromosome *III* and one dC$_{R}$T chromosome. Monosomic for other chromosomes	Cross 10
RS2-145	*his4 leu2 MATα ura3 met3 trp1* *HIS4 leu2 [LEU2]*	Strain contains one normal chromosome *III* and one dPT chromosome. Monosomic for other chromosomes	Random spore from cross 8
RS3-3	*leu2 MATα ura3 met3 trp1* *leu2 [LEU2]*	Strain contains one normal chromosome *III* and one dC$_{R}$T chromosome. Monosomic for other chromosomes	Random spore from cross 10
RS4-22	*HIS4 leu2 MATα MAL2 ura3 met3 trp1* *his4 ::[URA3] leu2 MATα mal2*	Strain is disomic for chromosome *III*. Monosomic for other chromosomes	This study
AH2	*MAT**a** his4 leu2*		G. Fink
AGH5	*MATα leu2 ura3*		G. Fink
425-6D	*MATα leu2 his4 ura3 met3 MAL2*		This study
426-67A	*MATa leu2 his4::[URA3] ura3 MAL2*	The *URA3* gene replaced the *his4* gene	This study
DC$_{L}$T-C$_{R}$T3	*his4 leu2 [URA3] ura3 MET3 TRP1* *[LEU2] MATa MAL2 ura3 met3 trp1*	Diploid contains dC$_{R}$T and dC$_{L}$T chromosome	This study
353-14A	*his4 leu2 [URA3] ura3 met3 trp1* *[LEU2] MATa MAL2*	Strain contains dC$_{R}$T and dC$_{L}$T chromosome. Monosomic for other chromosomes	DC$_{L}$T-C$_{R}$T3
353-14ALU	*MATa his4 leu2 ura3 met3 trp1 MAL2*	Mitotic recombination between dC$_{R}$T and dC$_{L}$T chromosomes regenerated a normal chromosome	353-14A
357-201B	*his4 MATα mal2 ura3* *[URA3] MATα MAL2*	Strain contains dC$_{L}$T chromosome. Monosomic for other chromosomes	Cross 12
357-221B	*his4 leu2 MATa ura3 met3 trp1* *HIS4 leu2 [LEU2]*	Strain contains dC$_{R}$T chromosome. Monosomic for other chromosomes	Cross 12
ICH2	*MATα his4::[HIS4 URA3] trp1*	Strain contains ring and linear chromosome in place of normal chromosome *III*	Surosky and Tye (1985a)
TD5	*MATa his4 ura3*		T. Donahue

TABLE 2

Crosses used in this study

Cross	Diploid	Parents
1	138C	8534-8C × 320-13B
2	168C	8534-8C × 320-16C
3	DLT1	8534-8C × 320-13B
4	DLT2	8534-8C × 320-13B
5	DC_LT1	8534-8C × 320-13B
6	DC_LT2	8534-8C × 320-13B
7	DC_LT4	8534-8C × 320-13B
8	DPT6M	8534-8C × 320-16C
9	DPT11M	8534-8C × 320-16C
10	CD_RT7M	8534-8C × 320-16C
11	DC_RT19M	8534-8C × 320-16C
12		353-14A × AGH5
13		353-14ALUX × AGH5
14		338-115B × AH2
15		RS3-3 × AH2
16		338-15A × 426-67A
17		RS2-145 × AH2
18		RS2-145 × 426-67A
19		RS4-22 × AH2
20		RS4-22 × 426-67A
21		357-201B × 357-221B
22		ICH2 × 425-6D

DNA preparation, transformation and cell fusions: Purified plasmid DNA was prepared by the method described by BIRNBOIM and DOLY (1979). Rapid DNA minipreps were prepared by the boiling technique (HOLMES and QUIGLEY 1981). Total yeast DNA was isolated as described by CRYER, ECCLESSHALL and MARMUR (1975). Transformation of *E. coli* (MORRISON 1977) and yeast (HINNEN, HICKS and FINK 1979) were carried out as described. Yeast spheroplast fusions were performed using a method similar to the yeast transformation procedure. The step in which the DNA is added was omitted and at this point the two strains to be fused were mixed and incubated at room temperature for five minutes. The cells were plated on media selective for fused cells.

Hybridization analysis: The preparation of nick-translated ^{32}P-labeled DNA probes (RIGBY *et al.* 1977), transfer of DNA to nitrocellulose (SOUTHERN 1975) and hybridization conditions (CHAN and TYE 1980) have been described.

Genetic analysis: Diploids were sporulated in 1–5 ml of SM1 media (1% KOAc, pH 6.7) at a concentration of 5 × 10^7 cells per ml for 2–4 days at room temperature. Meiotic tetrad analysis was performed as described (SHERMAN, FINK and LAWRENCE 1974). Random spores were isolated by harvesting tetrads from 1 ml of SM1 media and resuspending in 1 ml 0.2 M KOAc, pH 5.5. Two milliliters of diethyl ether were added, and the mixture was shaken for 10 min (DAWES and HARDIE 1974). Approximately 45% of the spores and 0.01% of the vegetative cells survive this treatment. The tetrads were collected and washed two times in H_2O and resuspended in 0.2 ml of a 1/20 dilution of glusulase. After 1 hr of incubation at 30°, the tetrads were sonicated at 2 amp for 3 min resulting in the disruption of >90% of the tetrads. The spores were then plated on the appropriate media.

Verification of the presence of the deletion chromosome in Leu$^+$ or Ura$^+$ spores: Hybridization and genetic analysis was used to verify the presence of a normal and deletion chromosome in the Ura$^+$ or Leu$^+$ spores. DNA was prepared from each strain, digested with restriction enzymes, fractionated on an agarose gel, transferred to nitrocellulose and hybridized with a ^{32}P-labeled DNA probe. When DNA from strains containing a normal and deletion chromosome is digested with the appropriate restriction enzyme and hybridized with a probe from the region adjacent to the deletion, two bands, one from the normal chromosome and a second from the deletion chromosome, are obtained.

The spores were crossed to a tester strain with several markers on chromosome *III* as well as markers on other chromosomes. The testers used were F399, F400 369-5C or 369-21C depending on the mating type and the phenotype of the spore to be tested. To test the nonmating spores, the spores were fused to the tester strains. At least ten complete tetrads from each tester cross were analyzed. Deviations from the $2^+:2^-$ pattern of segregation for the markers indicated the strain contained a deletion chromosome or was disomic for other chromosomes.

RESULTS

Description of deletion chromosomes: We previously described the construction of a number of deletion derivatives of chromosome *III* (SUROSKY and TYE 1985a; b; SUROSKY, NEWLON and TYE 1986). Most of these deletion chromosomes were constructed by the replacement of a large chromosomal region with a small DNA fragment in DNA transformation. Four deletion chromosomes, ranging in size from 270 kb to 120 kb, were used in these experiments (Figure 1). The chromosomes dPT and dC_RT, contain right arm deletions marked by the insertion of the *LEU2* gene (SUROSKY, NEWLON and TYE 1986). The deletion in the dPT chromosome extends from the *PGK1* region to the telomeric *Y′* sequence (CHAN and TYE 1983). The deletion in the dC_RT chromosome extends from the right side of the centromere to the *Y′* sequence. In contrast, the dLT and dC_LT chromosomes contain left arm deletions marked by the insertion of the *URA3* gene (SUROSKY and TYE 1985a). The deletion in the dLT chromosome extends from the *LEU2* gene to the telomeric *Y′* sequence, and the deletion in the dC_LT chromosome extends from the left side of the centromere to the *Y′* sequence. We refer to the dC_LT and dC_RT chromosomes as telocentric although there is approximately 10 kb of DNA from the centromere to the end of the chromosome. The chromosome d315 contains a 66-kb deletion from the *LEU2* region to the *PGK1* region including *CEN3*. *CEN5* and *URA3* have been inserted at the *HIS4* region on the d315 chromosome (SUROSKY and TYE 1985b). Previous studies on these chromosomes have demonstrated that they are mitotically stable (SUROSKY and TYE 1985a; SUROSKY, NEWLON and TYE 1986), so it is unlikely that any aberrant meiotic segregation that is observed results from mitotic loss of these chromosomes.

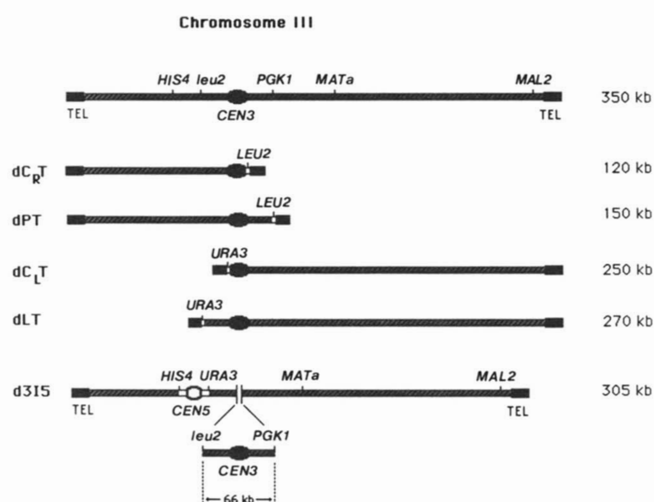


FIGURE 1.—Structure of the deletion chromosomes used in this study.

Homology in one of the chromosome arms is sufficient to foster faithful segregation of the homologs: In the first set of experiments, we examined whether deletion derivatives of chromosome *III* were capable of normal segregation from an unaltered chromosome *III*. These diploids produce at most two viable spores because of the extensive deletion on one chromosome. If the normal and deletion chromosomes disjoin at meiosis I, then the two deletion chromosomes and the markers linked to the deletions, either *URA3* or *LEU2*, will segregate to the inviable spores. If there is nondisjunction between the normal and deletion chromosomes at meiosis I, pairs of Ura$^+$ or Leu$^+$ spores will be obtained. To verify that these Ura$^+$ or Leu$^+$ spores contain a normal and deletion chromosome, they were subjected to hybridization and genetic analysis (see MATERIALS AND METHODS).

The behavior of four different deletion chromosomes (Figure 1), each in the presence of a single copy of the normal chromosome *III*, was analyzed. Strains DLT1 and DLT2 contain the deletion chromosome dLT. Strains DC$_L$T1, DC$_L$T2 and DC$_L$T4 contain a right arm telocentric chromosome, dC$_L$T. Strains DPT6 and DPT11 contain the deletion chromosome dPT. Strains DC$_R$T7 and DC$_R$T19 contain the left arm telocentric chromosome, dC$_R$T. The DLT and DC$_L$T strains are derived from diploid 138C, and the DPT and DC$_R$T strains are derived from diploid 168C. In the DPT and DC$_R$T strains, *MATa* has been lost as a result of the deletion. To sporulate these diploids, a plasmid containing *MATa* was integrated at *ura3*, creating the DPTM and DC$_R$TM strains.

Over 200 tetrads were analyzed from each set of diploid strains. In each case, more than 80% of the tetrads yielded 2 viable spores (Figure 2). Very few Leu$^+$ or Ura$^+$ spores were obtained. Six pairs of Leu$^+$ spores were produced by the DC$_R$TM diploids, but further analysis indicated that only four pairs contained a normal and deletion chromosome and were monosomic for other chromosomes. No other diploids produced pairs of spores containing the deletion chromosome. A few single Leu$^+$ or Ura$^+$ spores were obtained. Among these, only one Leu$^+$ spore from the DC$_R$TM strains and one Leu$^+$ spore from the DPTM strains actually contained a deletion chromosome *III*. Genetic analysis of these Leu$^+$ spores indicated they contained one normal chromosome and one deletion chromosome, so it is unlikely they resulted from nondisjunction of the normal and deletion chromosome at meiosis I, followed by nondisjunction of the sister chromatid of the deletion chromosome at meiosis II. These spores may have resulted from premature sister chromatid disjunction of the deletion chromosome or of the normal chromosome at meiosis I. In one tetrad, derived from a DPTM diploid, a single viable Leu$^+$ spore was observed. In the absence of the second viable spore, it cannot be determined whether this Leu$^+$ spore resulted from nondisjunction or some other event as described above. It is clear that in these diploids, the normal and deletion chromosomes segregate with fidelity and rarely is nondisjunction observed.

Meiotic recombination between the normal and deletion chromosome: We also examined the rates of recombination between the normal and deletion chromosomes in these crosses. Although no more than two viable spores were obtained, it was possible to determine if the centromere-linked chromosome *III* markers segregated in first or second division. The segregation of these markers was scored against two tightly linked centromere markers, *trp1* on chromosome *IV* and *met3* on chromosome *X*. Values obtained for the DLT strains, DPTM strains, DC$_L$T strains and DC$_R$TM strains have been combined in Table 3. As controls, recombination frequencies in the same intervals in the parental diploids 138C and 168C were determined. Recombination frequencies presented in MORTIMER and SCHILD (1980) are also given for comparison. The values shown in Table 3 are calculated as one-half of the second-division segregation frequencies. In the strains containing a deletion chromosome, recombination frequencies over intervals on chromosome *III* are increased or the same as compared to the control diploids. In the DLT strains, increases in recombination are observed on both the deleted and intact arm. Compared to the parental 138C strain, there is a twofold increase in the *leu2-cen3* interval and a 1.5-fold increase in the *MAT-cen3* interval. In the DPTM strains, there may be an increase in recombination in the *pgk11-cen3* interval. Recombination in the parental diploid can-

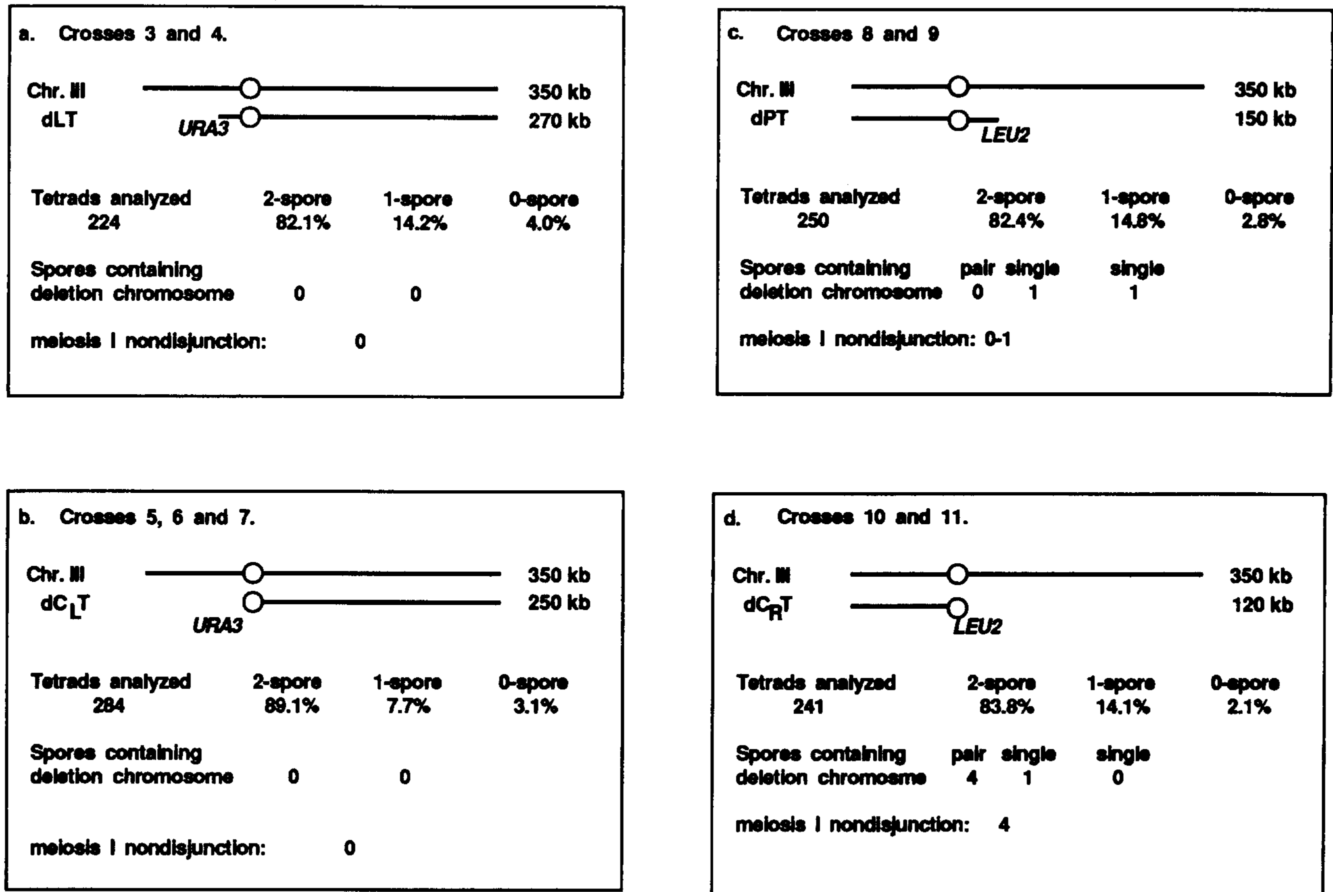


FIGURE 2.—Chromosome segregation in strains containing one normal chromosome *III* and one deletion chromosome. To simplify the data, the results from different crosses with identical chromosome configurations were combined. Presented are combined results of (a) DLT1 and DLT2. (b) DC_LT1, DC_LT2 and DC_LT4. (c) DPTM6 and DPTM11. (d) DC_RTM7 and DC_RTM11. The results from the individual crosses in any group were similar. Spores were initially identified as Ura^+ or Leu^+ by replica plating onto Cm-leu or Cm-ura media. The presence of the deletion chromosome in the Leu^+ or Ura^+ spores was verified by hybridization and genetic analysis as described in the text. In the figure, 2-, 1- and 0-spore indicate the number of viable spores per tetrad. Pair and single indicate the number of spores per tetrad containing the deletion chromosome.

TABLE 3

Centromere linkage of chromosome *III* markers in diploids containing one normal and one deletion derivative of chromosome *III*

	Centromere linkage						
Interval	DLT (224)	DC_LT (284)	DPTM (250)	DC_RTM (241)	138C (240)	168C (235)	MORTIMER and SCHILD (1980)
his4-cen3			28	23	18	22	18
leu2-cen3	13.6				6.9		4.8
pgk1-cen3			5				2.0
MAT-cen3	40	34			27	19	23

The gene-centromere distance was calculated as one-half of the frequency of second division segregants. The segregation of the chromosome *III* markers were scored against two tightly centromere-linked genes, *trp1* and *met3*. The values presented by MORTIMER and SCHILD (1980) are included for comparison. The numbers in parenthesis indicate the number of tetrads analyzed. Results from strains containing the same deletion chromosome have been pooled.

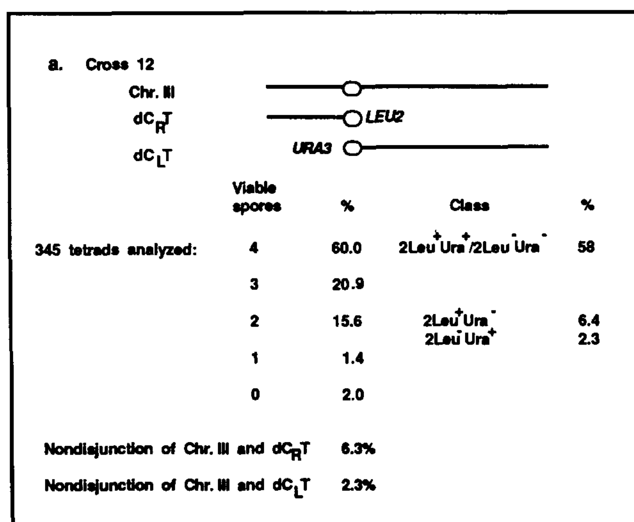
not be scored, but in comparison with values presented in MORTIMER and SCHILD, recombination in the *pgk1-cen3* interval in the DPTM strains is increased 2.5-fold. In the *his4-cen3* interval on the intact arm, recombination is increased by a factor of 1.3 over the frequency in parental strain 168C. In the DC_LT strains, recombination in the *MAT-cen3* interval was 1.3 times higher than in strain 138C. In the DC_RTM strains, the recombination frequency was similar to that in strain 168C. All differences in recombination frequencies between the strains containing a deletion chromosome and the parental

strains are significant ($P < 0.01$) based upon chi-square analysis. It is not known if recombination in centromere-distal regions of chromosome *III* or on other chromosomes is also affected. These results support those obtained from the segregation analysis, that there is no impairment of normal meiotic behavior due to the absence of sequences from one arm.

Segregation pattern of one normal and two telocentric chromosomes containing opposite arms: The results from crosses involving one normal chromosome *III* and either a left arm or a right arm telocentric demonstrated that the normal and deletion chromosomes disjoin with fidelity. In a diploid containing a normal chromosome and both a left arm (marked by *LEU2*) and right arm (marked by *URA3*) telocentric, would the two telocentrics preferentially cosegregate and disjoin from the normal chromosome? If so, then tetrads produced from this diploid should contain four viable spores, two Leu$^+$Ura$^+$ and two Leu$^-$Ura$^-$. Alternatively, the normal chromosome may always disjoin from one of the deletion chromosomes with the second telocentric chromosome segregating at random. A third possibility is that each chromosome may have an equal probability of disjunction with the third chromosome segregating at random. These three alternatives can be distinguished by the pattern of spore viability and the phenotypes of the viable spores.

In cross 12, a strain containing the two telocentric chromosomes and one normal chromosome was constructed and sporulated. Sixty percent of the tetrads (207 of 345) yielded four viable spores, and 20.9% of the tetrads (71 of 345) yielded three viable spores. The large percentage of three-viable-spore tetrads makes the analysis of this cross difficult. This inviability does not appear to result from aberrant segregation of the two telocentrics since Leu$^+$ and Ura$^+$ cosegregated in all but two of the 71 three-viable-spore tetrads, and Leu$^+$ and Ura$^+$ segregated in first division in all tetrads. This suggests that in the three-viable-spore tetrads the two telocentric disjoined from the normal chromosome, and the inviable spore arose as a result of some other property of the strain (see below).

As a control, we repeated tetrad analysis using an isogenic strain (353-14ALU) in which a normal chromosome had replaced the two telocentric chromosomes. Even with two normal chromosomes, this strain, isogenic to the strain used in cross 12, produced similar percentages of three-viable (19%) and two-viable-spore (13%) tetrads (Figure 3b, cross 13). This is further evidence that the poor spore viability observed in cross 12 did not result from the presence of the telocentric chromosomes. If we combine the four and three spore tetrads, then it appears that two telocentrics paired with and disjoined from the single normal chromosome *III* in at least 80% of the first meiotic divisions.



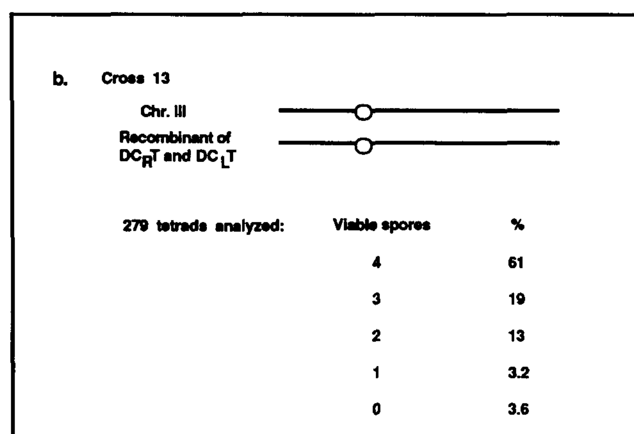
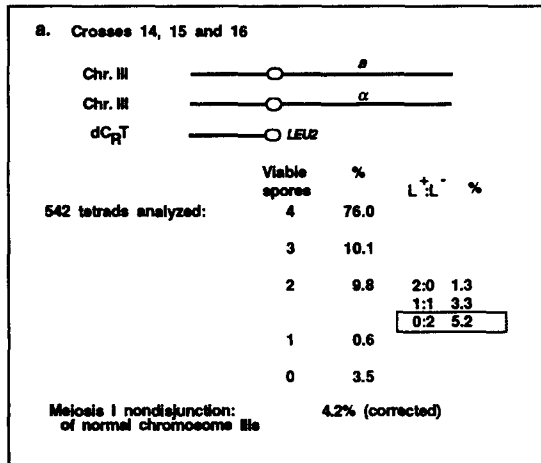
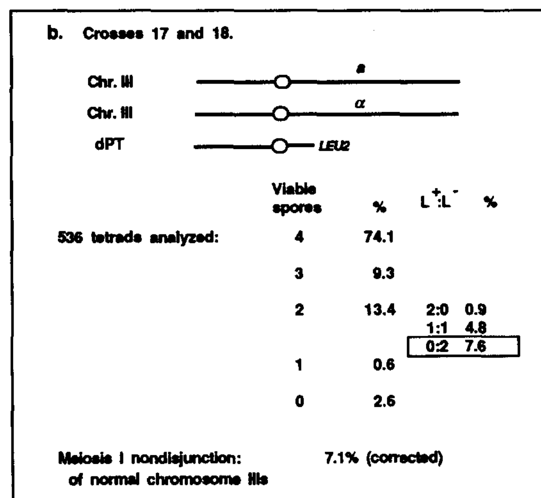


FIGURE 3.—Analysis of tetrads derived from a strain containing one normal chromosome *III*, one right arm telocentric and one left arm telocentric chromosome. Only the percentages of those classes of interest in this study are shown. The 2$^+$:2$^-$ class of four-viable-spore tetrads contains those tetrads in which both tetrads have disjoined from the normal chromosome. The 2$^+$:0$^-$ class of two-viable-spore tetrads contains those tetrads in which one of the telocentric chromosomes has cosegregated with the normal chromosome.

As expected, most (200 of 207) of the four-spore tetrads contained two Leu$^+$Ura$^+$ spores (Figure 3), indicating cosegregation of the telocentric chromosomes. We further analyzed the segregation pattern of the telocentric chromosomes in the two-viable-spore tetrads in cross 12. In this class, 6.4% (22 of 345) contain two Leu$^+$Ura$^-$ spores and 2.3% (8 of 345) contain two Leu$^-$Ura$^+$. These most likely resulted from cosegregation of the dC_RT or dC_LT chromosome, respectively, with the normal chromosome. Hybridization analysis of four pairs of spores





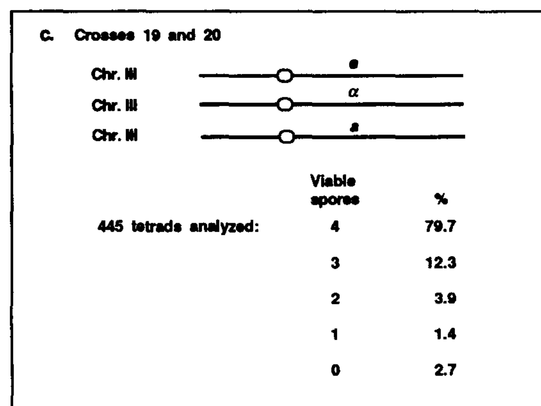


FIGURE 4.—Analysis of tetrads derived from strains containing two normal and one deletion chromosome. Results from different strains with identical chromosome configurations were combined. The percentages of spore viability and *leu2* segregation are calculated from the total tetrads analyzed. The corrected nondisjunction frequency was determined as described in the text.

from each of these two classes of tetrads verified that each spore contained one normal and one deletion chromosome. The other predominant class (5.8%) among two-viable-spore tetrads contains one Leu^+Ura^+ and one Leu^-Ura^- spore. The small number of two-viable-spore tetrads in which a telocentric and normal chromosome cosegregate indicate that in most meioses the two telocentrics cosegregate and disjoin from the normal chromosome. Over twice as many pairs of Leu^+Ura^- spores as Leu^-Ura^+ spores were obtained, indicating that the smaller telocentric, the left arm telocentric, was more likely to cosegregate with the normal chromosome.

Chromosome segregation patterns in diploids containing two normal and one deletion chromosome: The deletion derivatives segregate with fidelity from a normal chromosome *III*. We can now examine the segregation pattern in a strain containing two normal chromosome *III*s and one deletion chromosome. If we assume that in any meiosis only two chromosomes can disjoin with the third segregating at random, then there is a competition between the normal and deletion chromosome. If the deletion chromosome competes as well as the normal chromosome so any two chromosomes have equal probability of disjunction, then we predict that in one-third of the tetrads there should be only two viable spores both of which lack the deletion chromosome and are therefore Leu^-. If the deletion chromosome cannot compete with the normal chromosomes so the two normal chromosomes always disjoin, then all tetrads should contain four viable spores.

Tetrads from diploids containing either the left arm telocentric, dC_RT, or the deletion chromosome dPT, were analyzed. The results of the analysis are shown in Figure 4a and b. We observe more four-viable-spore tetrads and fewer two-viable-spore tetrads than would be expected if there was an equal probability of disjunction between any two chromosomes. We also obtain tetrads with three viable spores at a high frequency. Analysis of the phenotype of the spores from the three-viable-spore tetrads indicates that no phenotypic pattern predominates, suggesting that a particular type of chromosome segregation did not produce the inviability. Control crosses (see below and Figure 4c) also suggest that the poor spore viability is inherent in these strains and does not result from the presence of the deletion chromosomes. Likewise, some of the two-viable-spore tetrads may result from poor spore viability and not cosegregation of the two normal copies of chromosome *III*.

In crosses 14, 15 and 16 involving the left arm telocentric, 76.0% (412 of 542) of the tetrads contained four viable spores and 9.8% (53 of 542) contained two viable spores. A good indication of the

frequency of cosegregation of the two normal chromosome *III*s is the number of tetrads with two viable spores, both of which are Leu$^-$. In crosses 14, 15 and 16 involving the left arm telocentric chromosome, tetrads of this type are obtained at frequencies of 5.2% (28 of 542). Sixty percent of these tetrads contain two nonmating spores, and the remainder contain one *MAT*α, one *MAT***a** spore. To confirm that these spores contain two normal copies of chromosome *III*, we mated or fused some of these spores to tester strains containing multiple markers on chromosome *III* as well as markers on other chromosomes (see MATERIALS AND METHODS). Results from the tester crosses indicated that all of the nonmating spores and approximately half of the mating spores were disomic for chromosome *III*. Adjusting our frequencies for these results, we obtain the value of 4.2% (23 of 542) for normal chromosome *III* nondisjunction in crosses involving one left arm telocentric and two normal chromosomes *III* (Figure 4a). If, in half of the cases, the randomly segregating chromosome *III* cosegregated with the other normal chromosome *III*, then random segregation of the two normal chromosomes occurred in 8.4% of the diploids.

The chromosome segregation pattern in tetrads derived from crosses 17 and 18, containing the dPT chromosome, was also analyzed (Figure 4b). In these crosses, 74.1% (397 of 536) of the tetrads contained four viable spores and 13.4% (72 of 536) contained two viable spores. Some of the tetrads containing two viable spores, both Leu$^-$, were further tested by mating or fusing the spores to the tester strains. Most, but not all, of the spores appeared disomic for chromosome *III* and monosomic for the other chromosomes. The adjusted percentage of two-viable-spore tetrads resulting from meiosis I nondisjunction is 7.1% (38 or 536) (Figure 4b). In these crosses the two normal chromosomes segregate at random with respect to each other in 14.2% of the meiotic cells.

In crosses 17 and 18, nondisjunction events between the normal chromosomes would be obscured if recombination occurred between the normal and deletion chromosome in the *PGK1-cen3* region. This event would produce a three-viable-spore tetrad with *leu2* segregating $1^+:2^-$. Examination of the three-viable-spore tetrads shows there is no excess of this class ($2^+:1^-$ compared to $1^+:2^-$) (data not shown).

As controls, strains containing three copies of chromosome *III* were sporulated and spore viability was examined. In crosses 19 and 20, the spore viability in tetrads derived from strains trisomic for chromosome *III* was examined (Figure 3c). The strain in cross 19 has a similar genetic background to those used in crosses 14, 15 and 17, and the strain in cross 20 has a similar genetic background to those used in crosses 16 and 18 (see MATERIALS AND METHODS and Table 2). Again a comparable fraction of tetrads yielded three viable spores but the number of two-viable-spore tetrads was diminished, suggesting that in crosses involving a deletion chromosome, a fraction of the two-viable-spore tetrads resulted from nondisjunction of the normal chromosomes. These results support the idea that some of the spore inviability observed in these crosses results from properties of the strain and not the presence of the deletion chromosome. Spore viability was also examined in a diploid that had lost the dPT chromosome but otherwise was isogenic to the diploid in cross 18. Even in the absence of the deletion chromosome, similar spore viability was observed (data not shown).

The results from these crosses suggest that the two normal chromosomes preferentially disjoin, but in some meiosis the two normal chromosomes cosegregate, disjoining from the deletion chromosome. This appears to occur in approximately 4.2% of the meiosis in diploids containing a left arm telocentric chromosome. In crosses involving the deletion chromosome dPT, the two normal chromosomes cosegregate at an increased frequency of 7.1%. The addition of 20 kb of homologous sequence on the right arm of the deletion chromosome may allow the deletion chromosome to compete better with the normal chromosome. Although chi-square analysis indicates that the difference observed between strains containing dPT and dC_RT chromosomes is significant ($P < 0.01$), the strains containing the dC_RT and dPT chromosomes are not isogenic so other factors may contribute to this difference. Both of these frequencies are well below the 33% expected if random segregation of the chromosomes occurred.

Recombination between the normal and deletion chromosomes: In the pairs of spores in which the two normal chromosomes cosegregate, several contain one *MAT*α and one *MAT***a** spore. Genetic analysis of these pairs verified that one spore contained two *MAT*α loci and the other two *MAT***a** loci. These spores resulted from a recombination event between the two normal chromosomes, indicating that recombination between the two normal chromosomes on the right arm does not insure their disjunction. However, the fact that nonrecombinants in this interval are more frequent among the normal chromosome *III*s that do not disjoin (81.2%) than among the chromosomes that do disjoin (46.2%), suggests that recombination on the right arm increases the probability of disjunction.

The relationship between recombination and segregation on the left arm of chromosome *III* in the *his4-cen3* interval was also examined. In crosses 16 and 18, the left arm of the deletion chromosome is marked by *HIS4*, the left arm of one normal chromosome is marked by *URA3*, and the left arm of the

TABLE 4

Relationship between recombination and chromosome segregation

Cross	Class 1: Nonrecombinant — A: Normal chromosome *III* disjunction[a]	Class 1: Nonrecombinant — B: Normal chromosome *III* nondisjunction	Class 2: Recombination between two normal chromosome *III*s — A: Normal chromosome *III* disjunction	Class 2: Recombination between two normal chromosome *III*s — B: Normal chromosome *III* nondisjunction[b]	Class 3: Recombination between deletion chromosome and one normal chromosome *III*—the recombinants disjoin — A: Normal chromosome *III* disjunction	Class 3 — B: Normal chromosome *III* nondisjunction	Class 4: Recombination between deletion chromosome and one normal chromosome *III*—the recombinants cosegregate — A: Normal chromosome *III* disjunction[c]	Class 4 — B: Normal chromosome *III* nondisjunction
16	38.0–39.7% (72–75/189)	58.3% (7/12)	39.1% (74/189)	0 (0/12)	17.9% (34/189)	41.6% (5/12)	0–3.2% (0–6/189)	0
18	44.0–46.7% (81–86/184)	11.1% (1/9)	36.4% (67/184)	0 (0/9)	13.0% (26/184)	88.8% (8/9)	1.1–5.4% (2–10/184)	0

In crosses 16 and 18, recombination in the his4-cen3 interval on the left arm of chromosome *III* was determined. In the strains used, the deletion chromosome and the two normal chromosomes each contain a different allele at his4, so that four classes of recombinants can be distinguished. These four classes were then divided based upon segregation of the normal chromosomes. Only tetrads in which no more than one recombination event occurred in this interval were scored. Tetrads with segregation patterns that may have resulted from multiple crossovers, may also have been produced by several other types of events and, due to this ambiguity, were not scored.

[a] The range of percentages reflects the fact that some spores classified as type 1A may result from a class 4A event.

[b] Only one-half of the events of this type can be identified. The other half will produce spores with the same phenotype as in type 1B.

[c] This range results from the fact that only one-half of the events of this type can be identified. The other half will produce spores with the same phenotypes as those resulting from a type 1A event.

other normal chromosome contains *his4*. Thus, recombination between any two chromosomes can be detected as second division segregation of markers associated with those chromosomes (Table 4). Among the tetrads in which the normal chromosome *III*s have disjoined, most are either nonrecombinant (type 1A, 38.0–39.7% cross 16; 44.0–46.7% cross 18) or contain recombinants between the two normal chromosomes (type 2A, 39.1% cross 16; 36.4% cross 18). The range of percentages results from ambiguities in classifying some of the tetrads. In the remaining tetrads, a recombination event between the deletion chromosome and one normal chromosome had occurred. Interestingly, among this latter class, in most cases the deletion chromosome disjoined from the normal chromosome that it had recombined with (type 3A versus type 4A). This suggests that even in the meioses in which the two normal chromosomes disjoin, the segregation of the deletion chromosome is directed by recombination.

Among the tetrads in which the two normal chromosomes have cosegregated, the frequency that some classes are obtained are altered dramatically, although it should be noted that the sample size is much smaller. In a much higher percentage of tetrads, a recombination event has occurred between the deletion chromosome and one normal chromosome. In crosses 16 and 18, 41.6% and 88.8% of the tetrads, respectively, show this type of recombination (type 3B). In none of these two-viable-spore tetrads is recombination between the two normal chromosomes observed (type 2B). Chi-square analysis indicates that the differences in frequency with which type 3A and 3B tetrads are obtained is significant ($P < 0.01$).

Together these results suggest a correlation between recombination and segregation. In meioses in which the two normal chromosomes have recombined either on the right arm or the left arm, the probability that they will segregate is improved. In most meioses, the deletion chromosome disjoins from the normal chromosome it recombined with even when the two normal chromosomes disjoin. In tetrads in which the deletion and one normal chromosome recombine, the chance that the two normal chromosomes will cosegregate is increased.

Segregation pattern of two normal and two deletion derivatives of chromosome *III*: The cosegregation of the two telocentric chromosomes in cross 12 suggested that the two telocentrics act as though they were a single homolog in their pairing and disjunction from the normal chromosome *III*. In cross 21, we constructed a diploid containing two normal chromosome *III*s, one left arm telocentric and one right arm telocentric. We could then examine whether the left and right arm telocentrics together were able to compete with a normal chromosome for disjunction (Figure 5). In analyzing this cross, only four-viable-spore tetrads were examined. In ignoring the tetrads with less than four viable spores, several types of segregation events would go undetected. In this cross, however, we wanted to examine whether the preferential cosegregation of the two telocentric chromosomes was maintained. This would be reflected in an excess of pairs of $Leu^+ Ura^+$ spores among the four-viable-spore tetrads. As in previous crosses, sporulation of the diploid produced a high percentage of three-viable-spore (30.8%) and two-viable-spore (8.8%) tetrads. Among the four-viable-spore tetrads, we obtained 50 tetrads in which the

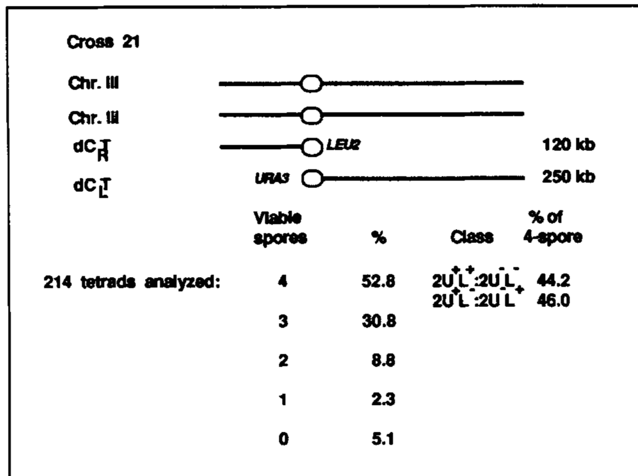


FIGURE 5.—Analysis of tetrads derived from a strain containing two normal chromosome *III*s, one right arm telocentric and one left arm telocentric chromosome. Percentages for spore viability and the segregation of the *leu2* and *ura3* markers in tetrads containing four viable spores are shown.

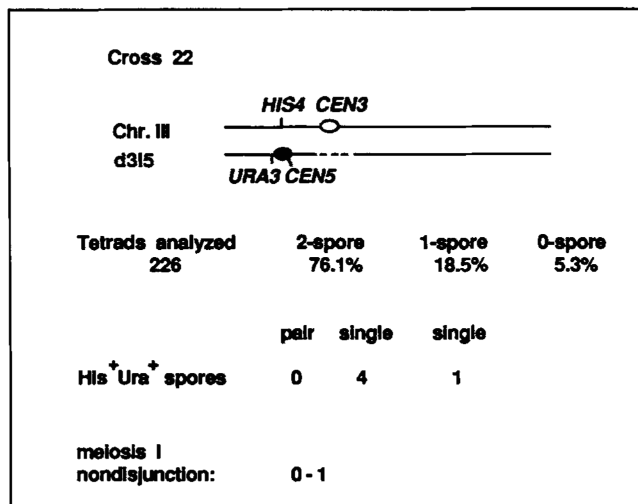


FIGURE 6.—Analysis of tetrads derived from a strain containing one normal chromosome *III* and one centromeric deletion chromosome. Pair and single indicate the number of spores per tetrad that were $His^+ Ura^+$

two telocentrics cosegregated producing 2 $Leu^+ Ura^+/2\ Leu^- Ura^-$ spores and 52 tetrads in which the telocentrics segregate producing 2 $Leu^+ Ura^-/2\ Leu^- Ura^+$ spores. Thus, there appeared to be no preference for either cosegregation or disjunction of the two telocentric chromosomes in this case. The addition of a second normal chromosome disrupted the preferential cosegregation of the two telocentrics observed in the earlier cross.

Pairing of the centromeric region is not required for the faithful disjunction of homologs in meiosis I: To examine the importance of centromeric pairing, we examined chromosome segregation in a diploid containing one normal chromosome *III* and a derivative of chromosome *III* in which the centromeric region including *CEN3* has been deleted and *CEN5* has been integrated at the *HIS4* region (Figure 1). Approximately 30 kb on either side of *CEN3* on the normal chromosome and 7 kb around *CEN5* on the deletion chromosome would be unpaired. This diploid was sporulated, and tetrads were dissected and analyzed (Figure 6, cross 22). Due to the deletion on the rearranged chromosome, at most two viable spores per tetrad were obtained. Improper segregation of the normal and deleted chromosome *III* would be indicated by pairs of $His^+ Ura^+$ spores. Two viable spores were obtained from 76.1% (172 of 226) tetrads analyzed. All but four of these tetrads gave rise to pairs of $His^+ Ura^-$ spores, indicating that these two homologs carrying nonhomologous centromeres disjoin from each other faithfully during meiosis I. Four of the two-viable-spore tetrads yielded one $His^+ Ura^-$ and one $His^+ Ura^+$ spore (Figure 6). In addition, one spore among the one-viable-spore tetrads was $His^+ Ura^+$. Hybridization analysis of DNA isolated from these five $His^+ Ura^+$ spores verified that a normal and deletion chromosome *III* were present. The $His^+ Ura^+$ spore among the one-viable-spore tetrads may have resulted from nondisjunction or other types of aberrant segregation (discussed below).

Single, rather than pairs, of $His^+ Ura^+$ spores may have resulted from nondisjunction of the normal and deletion chromosome in meiosis I and nondisjunction of the sister chromatids of the deletion chromosome in meiosis II. This would produce one spore with one normal chromosome *III*, and one spore with one normal chromosome *III* and two deletion chromosomes. Alternatively, the sister chromatids of the deletion chromosome or the normal chromosome may have segregated prematurely in meiosis I producing one spore with a normal chromosome *III* and the other with one normal and one deletion chromosome. A third possibility, is that these $His^+ Ura^+$ spores may have resulted from gene conversion or multiple exchange events between the two chromosomes. The five $His^+ Ura^+$ spores were crossed (or fused in the case of the nonmaters) to tester strain TD5, and the diploids were sporulated and analyzed. At least eight complete tetrads were analyzed, and in all cases the *his4* and *ura3* alleles segregated $2^+:2^-$ indicating a single *HIS4* and *URA3* gene was present in each of the original spores ruling out the first possibility. Whatever their cause, these spores represent an infrequent event and in most cases the two chromosomes disjoin properly in meiosis I.

DISCUSSION

We examined the meiotic pairing and segregation in strains containing various combinations of normal and deletion derivatives of chromosome *III*. In strains

containing one normal copy of chromosome *III* and one deletion derivative in which all or part of one arm was removed, the chromosomes disjoined in nearly all tetrads examined. It should be noted, however, that if the frequency of meiotic nondisjunction of chromosome *III* is similar to that of chromosome *V* (6.5×10^{-5} per spore) (SORA, LUCCHINI and MAGNI 1982) then increases in nondisjunction by at least one order of magnitude would not be detected. Recombination frequencies between the normal and deletion chromosomes also indicate that interactions between the two chromosomes are not diminished due to the decrease in homology. In fact, recombination in the region adjacent to the deletion is slightly stimulated, perhaps due to the inability of a large portion of the chromosome to recombine, analogous to the interchromosomal effect in Drosophila (reviewed by LUCCHESI 1976). In a few percent of the diploids containing the dPT and dC_RT chromosomes, nondisjunction may have occurred. In these cases the chromosomes may not have paired, or may have paired without recombining due to decreases in the amount of homologous sequence. Alternatively, these spores many have resulted from increased copy number of the deletion chromosome. Previous studies have demonstrated that decreases in chromosome size may yield increases in chromosome copy number (MURRAY and SZOSTAK 1983; SUROSKY, NEWLON and TYE 1986). Results from other crosses involving these deletion chromosomes suggest that in rare cases the copy number of the deletion chromosome may be increased.

Pairing of the centromeres is not required for chromosome disjunction. The results from two sets of crosses demonstrate this point. In chromosome d315, the entire centromeric region is deleted and *CEN5* is integrated at the *HIS4* locus (SUROSKY and TYE 1985a). When this chromosome pairs with a normal chromosome in meiosis, approximately 30 kb on each side of *CEN3* and 7 kb on each side of *CEN5* remain unpaired, yet this chromosome still disjoins from a normal chromosome with fidelity. These results extend those of CLARKE and CARBON (1983) who demonstrated that a chromosome *III* in which *CEN11* had been substituted for *CEN3* still disjoined from a normal chromosome *III*. Thus, neither pairing of the regions immediately surrounding the centromeres or positioning the centromeres at equivalent positions on the homologs is required for proper disjunction.

The cosegregation of the right and left arm telocentrics and their disjunction from the normal chromosome also demonstrates that pairing of the centromeres is not required for disjunction. If pairing of the centromeres was the criteria used for segregation, then the presence of three centromeres would have resulted in the random segregation of the chromosomes. We observed that the two telocentrics disjoin from the normal chromosome in most meioses. The dC_RT and dC_LT chromosomes cosegregate with the normal chromosome in only 6.3% and 2.3% of the tetrads, respectively. Similar results were reported for a right and left arm telocentric derivative of chromosome *IV* which disjoined from a normal copy of the chromosome with fidelity (ZAKIAN *et al.*, 1986).

The ability of these deletion chromosomes to disjoin from a normal homolog can be explained in terms of both kinetochore orientation and opposing tension on the homologs. The homology between the normal and deletion chromosomes allows the chromosomes to recombine. The chiasmata that result hold the homologs together and orient the kinetochores toward opposite spindle poles. This bipolar orientation increases the probability that the homologous kinetochores become attached to opposite spindle poles (OSTERGREN 1951; NICKLAS 1967). At this time in meiosis, the chromosomes must have sufficient rigidity so that once oriented by the chiasmata, even on only one arm, this orientation is maintained at the kinetochores.

The attachment of the homologs at the chiasmata will also result in an opposing tension on the homologs when the kinetochores attach to opposite spindle poles. This tension stabilizes the microtubule attachment (NICKLAS and KOCH 1969; BUSS and HENDERSON 1971). In cases in which both homologs become attached to the same pole, reorientation will occur until the tension on the homologs become balanced. In the strain containing the d315 chromosomes and a normal chromosome (cross 22), both orientation and tension could be achieved when there is no pairing in the centromeric region and when nonhomologous centromeres are located at different positions on the homologs (Figure 7D). Thus, the kinetochore does not take an active role in chromosome segregation, but serves only as an apparatus for spindle attachment.

The pairing and recombination of the right and left arm telocentric with the normal chromosome could also direct the disjunction of the two telocentrics from the normal chromosome (cross 12) (Figure 7B). The chiasmata could orient the kinetochores of the two telocentrics in opposition to the kinetochore of the normal chromosome. Attachment of the microtubules from one spindle pole to the telocentrics and from the other pole to the normal chromosome would provide the opposing force. Several explanations could account for the cosegregation of one of the telocentrics with the normal chromosome observed in two to six percent of the tetrads. The simplest would be that the normal and the deletion chromosomes did not pair or did not recombine due to decreases in the amount of homologous sequence.

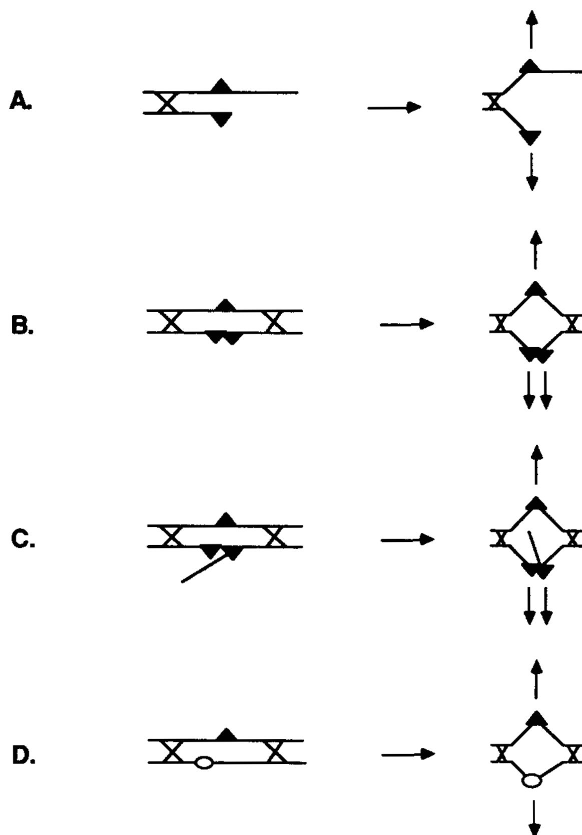


FIGURE 7.—Pairing and recombination direct meiotic chromosome segregation. (A) One normal and one deletion chromosome. (B) One normal and two telocentric chromosomes. (C) Two normal and one deletion chromosome. (D) One normal chromosome and one chromosome with a displaced centromere.

This would explain why the smaller dC_RT chromosome cosegregated with the normal chromosome more frequently than the dC_LT chromosome. The normal and deletion chromosomes that cosegregate appear to recombine less frequently than than those that disjoin, but there are too few cosegregants to draw any significant conclusions. The second explanation is that the presence of three kinetochores may result in the displacement of one of the kinetochores and aberrant microtubule attachment. When one of the telocentrics becomes attached to the same spindle pole as the normal chromosome, an opposing tension would still be generated between the normal chromosome and the other telocentric, so that the aberrantly attached telocentric might not reorient.

In diploids containing one deletion and two normal chromosomes, the two normal chromosomes disjoin at a higher frequency than if segregation occurred at random. Nondisjunction of the two normal chromosomes was observed in only 4% of the tetrads derived from a strain containing the right arm telocentric chromosome and at slightly higher frequency of 7% of the tetrads derived from a strain containing the dPT chromosome. In these crosses, the relationship between recombination and segregation was examined, but two shortcomings to this analysis must be noted. Only recombination events in the centromeric regions were examined and the effects of recombination in the centromere distal regions could not be determined. Secondly, only tetrads which had at most one recombination in the *his4-cen3* interval were examined. Those tetrads in which it appeared that all three chromosomes had recombined in this interval were eliminated due to ambiguity in interpreting the results.

Again the pairing and recombination between the two normal and one deletion chromosome can explain the segregation patterns that were observed (Figure 7C). Trivalent pairing of trisomes in yeast has been demonstrated by genetic and cytological studies. Strains trisomic for chromosome *III* form trivalents in almost all meioses (SHAFFER *et al.* 1971); strains trisomic for chromosome *XI* formed trivalents in 86% of the meioses (CULBERTSON and HENRY 1973) and strains trisomic for chromosome *XIV* form trivalents in at least 50% of the meiotic cells (KLAPHOLZ and ESPOSITO 1982). Studies on synaptonemal complex formation in triploid cells have shown that all three homologs are closely associated, with two participating in synaptonemal complex formation (MOENS 1969b). Along the length of the complex, partners are continuously switched so that the unpaired homolog may form a complex with one of the homologs that was previously paired.

Even in the presence of a deletion chromosome, the two normal chromosomes are able to pair and recombine on the right (noncompetitive) arm. Recombination in the *MAT-cen3* interval on the right arm between the two normal chromosomes increases the probability that the two chromosomes will disjoin, but does not insure this segregation. When the two normal chromosomes recombined in the *his4-cen3* interval on the left arm, disjunction was always observed. Recombination in the centromeric region may produce a tighter alignment of the kinetochores of the normal chromosomes and prevent displacement by the kinetochore of the deletion chromosome. The possibility of displacement was eliminated when the recombination occurs on the competitive arm. Recombination also insures that an opposing tension will be generated by attachment of microtubules from opposite spindle poles to the two normal chromosomes.

In the tetrads in which the two normal chromosomes disjoined, the deletion chromosome did not segregate at random. When the deletion chromosome recombined with one of the normal chromosomes, in most cases they disjoined. As in the previous cross, recombination could direct the disjunction of two chromosomes against a third. An increase in the nondisjunction of the normal chromosomes was observed when the deletion chromosome recombined

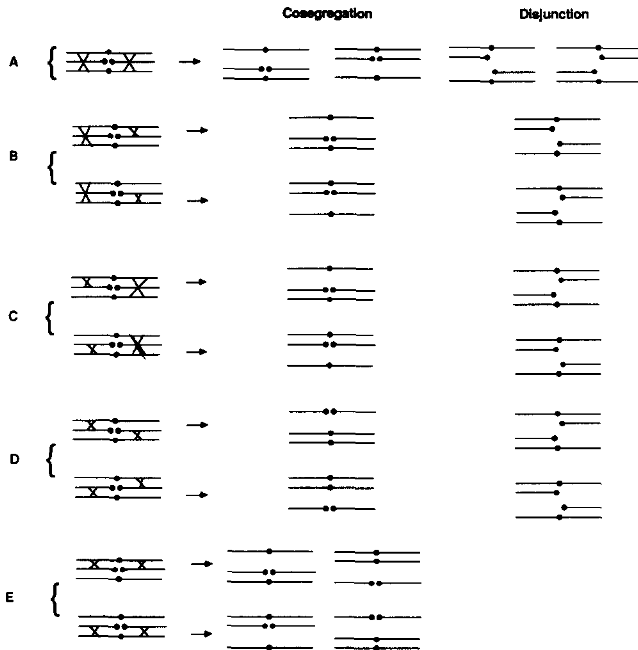


FIGURE 8.—Chromosome segregation in strains containing two normal and two deletion chromosomes. We assume that all chromosomes have an equal probability of recombining. The crossover closest to the centromere will determine which chromosomes will disjoin. Eleven segregation patterns produce cosegregation of the telocentric and seven segregation patterns yield disjunction. The frequency of cosegregation is 0.61.

with one of the normal chromosomes. In this case, the alignment of the kinetochores of the normal and deletion chromosome that had recombined was strengthened and the kinetochore of the other normal chromosome was displaced. Opposing tension would be generated when microtubules from opposite spindle poles are attached to the recombinants even if the two normal chromosomes become attached to the same pole.

In the strain containing two normal and two telocentric chromosomes, we assume all chromosomes will be involved in pairing and the disjunction will be determined by recombination between the chromosomes. The deletion chromosomes can disjoin from either normal chromosome or segregate randomly, so the strong preference for cosegregation of the two telocentrics observed in cross 12 is not observed in this cross. In Figure 8, all possible chromosome segregation patterns have been shown. Each pattern would produce a tetrad with four viable spores. If all segregation patterns have an equal probability, then tetrads in which the two telocentrics cosegregate should be in slight excess. In fact, we observe an equal probability of cosegregants and disjunctants. This discrepancy may result from the fact that not all segregation patterns are equally likely. For example, the two normal chromosomes, contiguous in the centromeric region, may have a greater probability of recombining on both sides of the centromere. Thus, some segregation patterns may be more likely than others. Aberrant microtubule attachment may also alter segregation patterns.

Our results indicate pairing and recombination play an important role in directing the disjunction of homologs in meiosis I. Other factors may also affect segregation and other interpretations of our results are possible. Certain types of pairing, such as that occuring at the ends of the chromosomes or at specific regions along the arms may be critical. Competition for pairing between the chromosomes may also occur, with the most stable interactions being maintained. Studies on the behavior of artifical chromosomes in meiosis suggest that other systems may also govern chromosome segregation (DAWSON, MURRAY and SZOSTAK 1986). Further studies using other chromosome alterations as well as meiotic mutants are now required to better understand meiotic chromosome segregation.

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LITERATURE CITED

BAKER, B. S., and J. C. HALL, 1976 Meiotic mutants: genic control of meiotic recombination and chromosome segregation. pp. 352–434. In: *The Genetics and Biology of Drosophila*, Vol. 1a, Edited by M. ASHBURNER and E. NOVITSKI. Acdemic Press, New York.

BIRNBOIM, H. C., and J. DOLY, 1979 A rapid alkaline extraction procedure for screening recombinant plasmid DNA. Nucleic Acids Res. **7:** 1513–1523.

BOEKE, J. D., F. LACROUTE and G. R. KINK, 1984 A positive selection for mutants lacking orotidine-5′-phosphate decarboxylase activity in yeast: 5-fluoro-orotic acid resistance. Mol. Gen. Genet. **197:** 345–346.

BOSTOCK, C. J., and A. T. SUMNER, 1978 *The Eukaryotic Chromosome*. North-Holland, Amsterdam.

BOYER, H. W., and D. ROULLAND-DUSSOIX, 1969 A complementation analysis of the restriction and modification of DNA in *E. coli*. J. Mol. Biol. **41:** 459–472.

BUSS, M. E., and S. A. HENDERSON, 1971 The induction of orientation instability and bivalent interlocking at meiosis. Chromosoma **35:** 153–183.

CHAN, C. S. M., and B.-K. TYE, 1980 Autonomously replicating sequences in *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA **77:** 6329–6333.

CHAN, C. S. M., and B.-K. TYE, 1983 Organization of DNA sequences and replication origins at yeast telomeres. Cell **33:** 563–573.

CLARKE, L., and J. CARBON, 1983 Genomic substitutions of centromeres in *Saccharomyces cerevisiae*. Nature **305:** 23–28.

CRYER, D. R., R. ECCLESSHALL and J. MARMUR, 1975 Isolation of yeast DNA. Methods Cell Biol. **12:** 39–44.

CULBERTSON, M. R., and S. A. HENRY, 1973 Genetic analysis of hybrid strains trisomic for the chromosome containing a fatty acid synthetase gene complex (*fas1*) in yeast. Genetics **75:** 444–458.

DAVIS, D. G., 1969 Chromosome behavior under the influence

of claret-nondisjunctional in *Drosophila melanogaster*. Genetics **61:** 577–594.

DAWES, I. W., and I. D. HARDIE, 1974 Selective killing of vegetative cells in sporulated yeast cultures by exposure to diethyl ether. Mol. Gen. Genet. **131:** 281–289.

DAWSON, D. S., A. W. MURRAY and J. W. SZOSTAK, 1986 An alternative pathway for meiotic chromosome segregation in yeast. Science **234:** 713–717.

ESPOSITO, R. E., and S. KLAPHOLZ, 1981 Meiosis and ascospore development. pp. 211–287. In: *The Molecular Biology of the Yeast Saccharomyces: Life Cyle and Inheritance*, Edited by J. STRATHERN, E. JONES and J. BROACH. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

GAME, J. C., T. J. ZAMB, R. J. BRAUN, M. RESNICK and R. M. ROTH, 1980 The role of radiation (*rad*) genes in meiotic recombination in yeast. Genetics **94:** 51–68.

GILLIES, C. B., 1975 Synaptonemal complex and chromosome structure. Annu. Rev. Genet. **9:** 91–109.

HINNEN, A., J. B. HICKS and G. R. FINK, 1978 Transformation of yeast. Proc. Natl. Acad. Sci. USA **75:** 1929–1933.

HINNEN, A., P. FARABAUGH, C. ILGEN and G. R. FINK, 1979 Isolation of a yeast gen (*his4*) by transformation of yeast. ICN-UCLA Symp. **14:** 43–50.

HOLMES, D. S., and M. QUIGLEY, 1981 A rapid boiling method for the preparation of bacterial plasmids. Anal. Biochem. **114:** 193–197.

HOPPER, A. K., P. T. MAGEE, S. K. WELCH, M. FRIEDMAN and B. D. HALL, 1974 Macromolecule synthesis and breakdown in relation to sporulation and meiosis in yeast. J. Bacteriol. **119:** 619–628.

HUGHES-SCHRADER, S., 1943 Polarization, kinetochore movement and bivalent structure in the meiosis of male mantids. Biol. Bull. **85:** 265–300.

KLAPHOLZ, S., and R. E. ESPOSITO, 1980a Isolation of *spo12-1* and *spo13-1* from a natural variant of yeast that undergoes a single meiotic division. Genetics **96:** 567–588.

KLAPHOLZ, S., and R. E. ESPOSITO, 1980b Recombination and chromosome segregation during the single division meiosis in *spo12-1* and *spo13-1* dipoloids. Genetics **96:** 589–611.

KLAPHOLZ, S., and R. E. ESPOSITO, 1982 Chromosomes *XIV* and *XVII* of *Saccharomyces cerevisiae* constitute a single linkage group. Mol. Cell. Biol. **2:** 1399–1409.

KLAPHOLZ, S., C. S. WADDELL and R. E. ESPOSITO, 1985 The role of the *SPO11* gene in meiotic recombination in yeast. Genetics **110:** 187–216.

LUCCHESI, J. C., 1976 Interchromosomal effects. pp. 315–329. In: *The Genetics and Biology of Drosophila*, Vol 1a, Edited by M. ASHBURNER and E. NOVITSKI. Academic Press, New York.

MALONE, R. E., and R. E. ESPOSITO, 1981 Recombinationless meiosis in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **1:** 891–901.

MATHER, K., 1933 Interlocking as a demonstration of the occurrence of genetical crossing-over during chiasma formation. Am. Nat. **67:** 476–479.

MILLER, J. H., 1972 *Experiments in Molecular Genetics*. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

MOENS, P. B., 1969a The fine structure of meiotic chromosome polarization and pairing in *Locusta migratoria* spermatocytes. Chromosoma **28:** 1–2.

MOENS, P. B., 1969b Fine structure of meiotic chromosome pairing in the triploid, *Lilium tigrinium*. J. Cell. Biol. **40:** 273–279.

MORRISON, D. A., 1977 Transformation in *Escherichia coli*: cryogenic preservation of competent cells. J. Bacteriol. **132:** 349–351.

MORTIMER, R. K., and D. SCHILD, 1980 Genetic map of *Saccharomyces cerevisiae*. Microbiol. Rev. **44:** 519–571.

MOSES, M. J., 1968 Synaptonemal complex. Annu. Rev. Genet. **2:** 363–412.

MURRAY, A. W., and J. W. SZOSTAK, 1983 Construction of artificial chromosomes in yeast. Nature **305:** 189–193.

NICKLAS, R. B., 1967 Chromosome micromanipulation. II. Induced reorientation and the experimental control of segregation in meiosis. Chromosoma **21:** 17–50.

NICKLAS, R. B., 1974 Chromosome segregation mechananisms. Genetics **78:** 205–213.

NICKLAS, R. B., and C. A. KOCK, 1969 Chromosome micromanipulation. III. Spindle fiber tension and the reorientation of mal-oriented chromosomes. J. Cell Biol. **43:** 40–50.

OSTERGREN, G., 1951 The mechanism of co-orientation in bivalents and multivalents. Heredetis **37:** 85–156.

RIGBY, P. W. J., M. DIECKMANN, C. RHODES and P. BERG, 1977 Labelling deoxyribonucleic acid to high specific activity *in vitro* by nick translation with DNA polymerase I. J. Mol. Biol. **113:** 237–251.

ROMAN, H., and S. M. SANDS, 1953 Heterogeneity of clones of *Saccharomyces* derived from haploid ascospores. Proc. Natl. Acad. Sci. USA **39:** 171–179.

SHAFFER, B., I. BREARLEY, R. LITTLEWOOD and G. R. FINK, 1971 A stable aneuploid of *Saccharomyces cerevisiae*. Genetics **67:** 483–495.

SHERMAN, F., G. R. FINK and C. W. LAWRENCE, 1974 *Methods in Yeast Genetics*. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

SORA, S., G. LUCCHINI and G. E. MAGNI, 1982 Meiotic diploid progeny and meiotic non-disjunction in *Saccharomyces cerevisiae*. Genetics **101:** 17–33.

SOUTHERN, E. M., 1975 Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **98:** 503–517.

SUROSKY, R. T., and B.-K. TYE, 1985a Resolution of dicentric chromosomes by Ty-mediated recombination in yeast. Genetics **110:** 397–419.

SUROSKY, R. T., and B.-K. TYE, 1985b Construction of telocentric chromosomes in *S. cerevisiae*. Proc. Natl. Acad. Sci. USA **82:** 2106–2110.

SUROSKY, R. T., C. S. NEWLON and B.-K. TYE, 1986 The mitotic stability of deletion derivatives of chromosome *III* in yeast. Proc. Natl. Acad. Sci. USA **83:** 414–418.

WESTERGAARD, M., and D. VON WETTSTEIN, 1970 Studies on the mechanism of crossing over. IV. The molecular organization of the synaptinemal complex in *Neottiella* (Cooke) Saccardo (Ascomyates). C. R. Trav. Lab. Carlsberg. **37:** 239–268.

WESTERGAARD, M. and D. VON WETTSTEIN, 1972 The synaptinemal complex. Annu. Rev. Genet. **6:** 71–110.

WHITE, M. J. D., 1954 *Animal Cytology and Evolution*. University Press, Cambridge, Great Britain.

ZAKIAN, V. A., H. M. BLANTON, L. WETZEL and G. M. DANI, 1986 Size threshold for *Saccharomyces cerevisiae* chromosomes: generation of telocentric chromosomes from an unstable minichromosome. Mol. Cel. Biol. **6:** 925–932.

ZICKLER, D., and L. W. OLSON, 1975 The synaptonemal complex and the spindle plaque during meiosis in yeast. Chromosoma **50:** 1–23.

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