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Distributive Disjunction of Authentic Chromosomes in *Saccharomyces cerevisiae*

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ABSTRACT

Distributive disjunction is defined as the first division meiotic segregation of either nonhomologous chromosomes that lack homologs or homologous chromosomes that have not recombined. To determine if chromosomes from the yeast *Saccharomyces cerevisiae* were capable of distributive disjunction, we constructed a strain that was monosomic for both chromosome *I* and chromosome *III* and analyzed the meiotic segregation of the two monosomic chromosomes. In addition, we bisected chromosome *I* into two functional chromosome fragments, constructed strains that were monosomic for both chromosome fragments and examined meiotic segregation of the chromosome fragments in the monosomic strains. The two nonhomologous chromosomes or chromosome fragments appeared to segregate from each other in approximately 90% of the asci analyzed, indicating that yeast chromosomes were capable of distributive disjunction. We also examined the ability of a small nonhomologous centromere containing plasmid to participate in distributive disjunction with the two nonhomologous monosomic chromosomes. The plasmid appeared to efficiently participate with the two full length chromosomes suggesting that distributive disjunction in yeast is not dependent on chromosome size. Thus, distributive disjunction in *S. cerevisiae* appears to be different from *Drosophila melanogaster* where a different sized chromosome is excluded from distributive disjunction when two similar size nonhomologous chromosomes are present.

DURING meiosis I homologous chromosomes pair to form bivalents and segregate to opposite poles of a spindle. Homologous recombination is thought to be required for this process since mutants that reduce or eliminate recombination exhibit high levels of chromosome nondisjunction (reviewed in BAKER *et al.* 1976). However, chromosomes also are capable of segregating from each other in the absence of recombination. In *Drosophila melanogaster,* two nonhomologous chromosomes that lack homologs or two homologs that have failed to recombine, disjoin at meiosis I. This phenomenon has been termed distributive pairing or distributive disjunction and can result in chromosome disjunction 99.9% of the time when the chromosomes involved are approximately the same size. Distributive disjunction is thought to be essential for the segregation of Drosophila chromosome *IV* homologs, which rarely if ever recombine (GRELL 1976). Distributive disjunction also occurs when three nonrecombinant chromosomes are present. Two chromosomes cosegregate to one pole while the third segregates to the opposite pole. In Drosophila, when all three chromosomes are similar in size, each chromosome has the same probability of segregating away from the other two. In contrast, if the chromosomes are of different sizes, the two that are closest in size will preferentially disjoin while the third chromosome usually cosegregates with one of the other two chromosomes.

It has been suggested that distributive disjunction occurs in the yeast *Saccharomyces cerevisiae* (DAWSON, MURRAY and SZOSTAK 1986; MANN and DAVIS 1986; KABACK 1989). However, all evidence for it was based on the use of one or more synthetic chromosomes which do not always faithfully mimic the meiotic behavior of authentic chromosomes (CLARKE and CARBON 1980; FITZGERALD-HAYES, CLARKE and CARBON 1982; KOSHLAND, KENT and HARTWELL 1985; DAWSON, MURRAY and SZOSTAK 1986). Even though one synthetic and one authentic yeast chromosome undergo distributive disjunction (DAWSON, MURRAY and SZOSTAK 1986; KABACK 1989), their ability to segregate may still have been a property of the synthetic chromosome and not due to behavior normally exhibited by authentic chromosomes. Therefore, to determine whether distributive disjunction is actually a property of authentic chromosomes in *S. cerevisiae,* it is necessary to show that two authentic nonexchange chromosomes are capable of segregating from each other.

Distributive disjunction of synthetic chromosomes in *S. cerevisiae* was observed to occur only 63–90% of the time. Therefore, synthetic chromosomes failed to undergo distributive disjunction 100 times more fre-

quently in *S. cerevisiae* than authentic chromosomes in Drosophila. Since synthetic chromosomes are less efficiently transmitted during mitosis than authentic chromosomes (MURRAY and SZOSTAK 1983; KOSHLAND, KENT and HARTWELL 1985; HIETER *et al.* 1985a; HARTWELL and SMITH 1985), it is possible that the lower frequency of distributive disjunction observed in *S. cerevisiae* was due to inefficient segregation of synthetic chromosomes during meiosis. Therefore, if two authentic yeast chromosomes are capable of distributive disjunction, it is important to determine if they disjoin more frequently than synthetic chromosomes.

To determine if authentic chromosomes undergo distributive disjunction in *S. cerevisiae,* we investigated the meiotic segregation of two authentic nonhomologous chromosomes in a strain that was monosomic for both of these chromosomes. Our results suggested that the nonhomologous chromosomes segregated away from each other in most asci. Thus, authentic chromosomes are capable of distributive disjunction. Moreover, the frequency of distributive disjunction of authentic chromosomes was no better than observed for synthetic chromosomes suggesting that distributive disjunction is a less efficient process in yeast than in Drosophila. Also in contrast to Drosophila, we found that when three nonhomologous chromosomes were present, distributive disjunction was not size dependent.

MATERIALS AND METHODS

Growth and genetics of *S. cerevisiae:* Yeast strains used are listed in Table 1. All strains were grown, mated, sporulated and analyzed genetically as described (SHERMAN, FINK and HICKS 1986). Only asci containing four spores were analyzed. DNA transformation of yeast was performed by the method of ITO *et al.* (1983). Identification of sister spores and first division segregation were monitored using the heterozygous centromere-linked *TRP1* marker (MORTIMER and SCHILD 1980). All media not referenced in the text was prepared as described (SHERMAN, FINK and HICKS 1986).

Recombinant DNA procedures: Plasmids were constructed and amplified in *Escherichia coli* strain HB101 using standard procedures (MANIATIS, FRITSCH and SAMBROOK 1982). When appropriate, restriction fragment ends were dephosphorylated using Calf Intestine Alkaline Phosphatase (CIP) or 5′ overhanging ends were made into blunt ends using the Klenow fragment of DNA polymerase I. Enzymes were obtained from New England Biolabs or Boeringer Mannheim Biochemicals Inc. and used according to their specifications. DNA probes were ^{32}P-labeled using the Multiprime Kit from Amersham Inc.

Gel electrophoresis: Intact chromosomal DNA prepared by the method of DE JONGE *et al.* (1986) was separated on 1.0% (w/v) agarose gels using Transverse Alternating Field Electrophoresis (TAFE) (GARDINER and PATTERSON 1988). Electrophoresis was carried out using 170 milliamperes constant current, 220–250 volts at 13–15°. Pulses of 55 secs were used to generate complete electrophoretic karyotypes and 25-sec pulses were used to maximize separation of small chromosomes. Bacteriophage lambda DNA concatamers made by the method of SOUTHERN *et al.* (1987) were used as chromosomal size markers.

Yeast genomic DNA prepared by the method of HOFFMAN and WINSTON (1987) and digested with appropriate restriction enzymes was separated on conventional 0.8% (w/v) agarose gels using standard procedures.

Blot hybridization: DNA was transferred from ethidium bromide-stained gels to nylon membranes and hybridized with ^{32}P-labeled probes by the method of SOUTHERN (1975). Membranes were probed with either a 4.3-kb *Eco*RI fragment containing *CEN1* (STEENSMA, CROWLEY and KABACK 1987), a 1.1-kb *Hin*dIII fragment containing *URA3* (BACH, LACROUTE and BOTSTEIN 1979) or a 6.8-kb *Cla*I-*Sph*I fragment containing *MAK16* (WICKNER *et al.* 1987).

Insertion of a conditional centromere on chromosome *I*: To control the mitotic stability of chromosome *I, CEN1* was replaced with a DNA sequence containing *CEN3* with the *GAL1* promoter located immediately upstream (the conditional centromere). When galactose is added to the medium, transcription initiation at this promoter interferes with centromere function and the chromosome containing the conditional centromere becomes mitotically unstable (HILL and BLOOM 1987).

Plasmid pVG75 contains the conditional centromere on a 0.86-kb *Bam*HI fragment (from plasmid YEp13-865; HILL and BLOOM 1987) and *URA3* on a 1.1-kb *Hin*dIII fragment (BACH, LACROUTE and BOTSTEIN 1979) inserted at the *Bam*HI and *Xba*I sites, respectively, of a variant of plasmid pUC19. The pUC19 variant had a *Sal*I linker inserted at the *Eco*RI site enabling us to generate a 2.0-kb *Sal*I fragment containing both the conditional centromere and *URA3.* This *Sal*I fragment was inserted at the *Xho*I site of the *CEN1* replacement vector pVG73 (V. GUACCI and D. B. KABACK, submitted for publication) to give plasmid pVG92.

CEN1 was replaced by the conditional centromere using one-step gene replacement (ROTHSTEIN 1983). Haploid strain VG31-4B was transformed with 5.0 μg of *Eco*RI treated pVG92 DNA and stable Ura$^+$ transformants (VG-GALCEN) were selected. *Eco*RI digested DNA from the transformants was analyzed by blot hybridization using *CEN1* DNA as probe to confirm the gene replacement.

Isolation of monosomic strains: Diploid strain VG72 was constructed by interbreeding haploids VGGALCEN, YGALCEN3, R214-1C, VG36-11A, VG37-8B, VG31-4B, VG30-4A and VG27-31C (Table 1). In strain VG72, one copy of chromosome *I* contains both the conditional centromere and the *ade1::HIS3* allele (V. GUACCI and D. B. KABACK, submitted for publication), one copy of chromosome *III* contains both the conditional centromere and the *mat*Δ*::LEU2* allele, and one copy of chromosome *V* contains the *MAT***a** locus adjacent to *URA3* (Table 1). Strain VG72 was grown on YEPgalactose plates to induce chromosome loss as previously described (HILL and BLOOM 1987). Resultant colonies were dilution streaked on YEPD plates and replica plated to complete medium lacking either histidine or leucine (SHERMAN, FINK and HICKS 1986). Since chromosomes that contained a conditional centromere were marked with either *LEU2* or *HIS3,* Leu$^-$ colonies were picked as likely chromosome *III* monosomes, His$^-$ colonies were picked as likely chromosome *I* monosomes and Leu$^-$, His$^-$ colonies were picked as likely monosomes for both chromosomes (double monosomes). Putative monosomes were screened by TAFE to confirm chromosome loss.

Isolation of monosomic strains containing a bisected copy of chromosome *I*: Chromosome *I* was bisected using small linear plasmids by a modification of the method of ZAKIAN *et al.* (1986). To bisect chromosome *I* at *CEN1,* we first constructed circular plasmid pVG47, which is YIp5

TABLE 1

Strains

Strain	Genotype	Source/Cross/Ref.
VG37-8B	*MAT***a** *his3-11,15 leu2-3,112 trp1 ura3-1 arg4 met10*	This study
VG31-4B	*MATα ade1*::HIS3 his3-11,*15 leu2-3,112 ura3-1 arg4*	This study
YGALCEN3	*MAT***a** *cen3Δ::[GAL1p-CEN3-URA3]*[a] *leu2 ade1 met14 ura3 his3*	HILL and BLOOM (1987)
R214-1C	*MAT***a** *matΔ::LEU2 leu2 ade1 ura3-52-MAT***a***-URA3*[b]	J. HABER
VG30-4A	*MATα leu2-3,112 his 3-11,15 ura3-1 arg4*	This study
VG27-31C	*MATα ade1 ura3-1 arg1*	This study
VGGALCEN	*MATα cen1Δ::[GAL1p-CEN3-URA3]*[c] *ade1::HIS3 his3-11,15 leu2-3,112 ura3-1 arg4*	This study
VG37-8B:YLpVG47	*MAT***a** *fragments IL and IR*[d] *his3-11,15 leu2-3,112 trp1 ura3-1 arg4 met10*	This study
VG37-8B:YLpVG59	*MAT***a** *fragments IA and IB*[d] *his3-11,15 leu2-3,112 trp1 ura3-1 arg4 met10*	This study
VG72	*MATα cen1Δ::[GAL1p-CEN3-URA3]*[c] *ade1::HIS3 leu2-3,112* / *MAT***a** *CEN1 ADE1 leu2* *CEN3 MATα* / *cen3Δ::[GAL1p-CEN3-URA3]*[a] *matΔ::LEU2* *ura3-1 TRP1 MET10 his3-11,15* / *ura3-52-MAT***a***-URA3*[b] *trp1 met10 his3-11,15*	This study
VG79	*MATα cen1Δ::[GAL1p-CEN3-URA3]*[c] *ade1::HIS3 TRP1 MET10* / *MAT***a** *fragments IL and IR*[d] *ADE1 trp1 met10* *his3-11,15 leu2-3,112 ura3-1 arg4* / *his3-11,15 leu2-3,112 ura3-1 arg4*	VGGALCEN × VG37-8B:YLpVG47
VG80	*MATα cen1Δ::[GAL1p-CEN3-URA3]*[c] *ade1::HIS3 TRP1 MET10* / *MAT***a** *fragments IA and IB*[d] *ADE1 trp1 met10* *his3-11,15 leu2-3,112 ura3-1 arg4* / *his3-11,15 leu2-3,112 ura3-1 arg4*	VGGALCEN × VG37-8B:YLpVG59
VG82	*MATα ade1::HIS3 TRP1 MET10 his3-11,15* / *MAT***a** *fragments IL and IR*[d] *ADE1 trp1 met10 his3-11,15* *leu2-3,112 ura3-1 arg4* / *leu2-3,112 ura3-1 arg4*	VG31-4B × VG37-8B:YLpVG47
VG82-5A	*MATα fragments IL and IR[HIS]*[e] *his3-11,15 leu2-3,112 ura3-1 arg4*	This study
VG83	*MATα ade1::HIS3 TRP1 MET10 his3-11,15* / *MAT***a** *fragments IA and IB ADE1 trp1 met10 his3-11,15* *leu2-3,112 ura3-1 arg4* / *leu2-3,112 ura3-1 arg4*	VG31-4B × VG37-8B:YLpVG59
VG83-6B	*MATα fragments IA and IB[HIS]*[e] his3-11,*15 leu2-3,112 ura3-1 arg4*	This study
VG86	*MATα fragments IL and IR[HIS]*[e] *TRP1 MET10 his3-11,15* / *MAT***a** *fragments IL and IR*[d] *trp1 met10 his3-11,15* *leu2-3,112 ura3-1 arg4* / *leu2-3,112 ura3-1 arg4*	VG82-5A × VG37-8B:YLpVG47
VG87	*MATα fragments IA and IB[HIS]*[e] *TRP1 MET10 his3-11,15* / *MAT***a** *fragments IA and IB*[d] *trp1 met10 his3-11,15* *leu2-3,112 ura3-1 arg4* / *leu2-3,112 ura3-1 arg4*	VG83-6B × VG37-8B:YLpVG59
VG89-M3	*MATα leu2 ura3-1 TRP1 MET10* / *MAT***a** *0 ura3-52-MAT***a***-URA3*[b] *trp1 met10*	This study
VG90	*MATα cen1Δ::[GAL1p-CEN3-URA3]*[c] *ade1::HIS3 TRP1 MET10* / *MAT***a** *CEN1 ADE1 trp1 met10* *his3-11,15 leu2-3,112 ura3-1 arg4* / *his3-11,15 leu2-3,112 ura3-1 arg4*	VGGALCEN × VG37-8B

[a] *cen3Δ::[GAL1p-CEN3-URA3]* is the conditional centromere inserted in place of *CEN3* on chromsome *III*.
[b] *ura3-52-MAT***a***-URA3* is a copy of the *MAT***a** allele translocated to the *URA3* locus on chromosome *V*.
[c] *cen1Δ::[GAL1p-CEN3-URA3]* is the conditional centromere inserted in place of *CEN1* on chromosome *I*.
[d] Formation of chromosome *I* fragments *IA*, *IB*, *IL* and *IR* are described in MATERIALS AND METHODS.
[e] Fragments *IR[HIS]* and *IB[HIS]* are identical to fragments *IR* and *IB*, respectively, except that they contain the *ade1::HIS3* allele.

(STRUHL *et al.* 1979) containing both a 2.0-kb *Pvu*II fragment carrying *CEN1* inserted at the *Pvu*II site and a *Kpn*I linker inserted in the *Nru*I site. Plasmid pVG47 was linearized with *Kpn*I and its ends dephosphorylated. To form linear plasmid YLpVG47, the *Kpn*I cut plasmid was ligated to a four fold molar excess of *Tetrahymena* ribosomal DNA (rDNA) ends. The rDNA ends were on a 0.7-kb *Kpn*I-*Bam*HI fragment that was dephosphorylated at its *Bam*HI end (from plasmid pTC16-L, supplied by B. DUNN). These rDNA ends provide both *ARS* activity and telomere function (KISS, AMIN and PEARLMAN 1981; SZOSTAK and BLACKBURN 1982). The ligation mixture was analyzed by agarose gel electrophoresis to show that linear plasmid YLpVG47 was present (data not shown).

To bisect chromosome *I* at the *MAK16* region, we constructed circular plasmid pVG59 by inserting a 6.8 kb *Cla*I-*Sph*I fragment containing *MAK16* (isolated from plasmid YCp50 (*MAK16*)-1C; WICKNER *et al.* 1987) between the *Cla*I and *Sph*I sites of pVG47. Plasmid pVG59 was converted to linear plasmid YLpVG59 as described above for pVG47.

Ligation mixtures containing either linear plasmid YLpVG47 or YLpVG59 were transformed into haploid strain VG37–8B. Stable Ura$^+$ colonies were obtained after prolonged growth on liquid complete-minus uracil medium (SHERMAN, FINK and HICKS 1986) and screened by TAFE karyotyping. Blot hybridization using the *CEN1* probe confirmed the position of the chromosome *I* sequences on the TAFE gels. One or two colonies that lacked the full length copy of chromosome *I* and contained two chromosomal fragments of the predicted sizes were picked for each transformation. DNA from the transformants was examined by conventional agarose gel electrophoresis and blot hybridization to confirm the bisected chromosomes contained the predicted gene replacements. These strains were then crossed to strain VGGALCEN (contains the conditional *CEN* on chromosome *I*) yielding strains VG79 and VG80.

To obtain strains monosomic for the bisected copy of chromosome *I*, diploids VG79 and VG80 were grown on YEPgalactose plates and treated as described above for VG72. His$^-$ colonies were picked as likely candidates to be missing the full length copy of chromosome *I*. TAFE was used to confirm karyotypes.

Isolation of strains containing a reconstituted full length copy of chromosome *I*: After growth on YEPD medium, 1×10^6 cells from each bisected monosomic strain were plated on SD medium containing fluoro-orotic acid (FOA medium; BOEKE, LACROUTE and FINK 1984). Reconstitution of chromosome *I* occurs when there is a crossover between the 2- or 5-kb segment of homologous DNA shared by a set of chromosome fragments. The mitotically unstable linear plasmid is formed and then lost making cells Ura3$^-$ and able to grow in the presence of FOA. Ura$^-$ colonies were screened by blot hybridization of TAFE separated chromosomes to confirm the reconstitution. Approximately 90% of the Ura$^-$ colonies were eliminated because they contained both a full length copy of chromosome *I* and a copy of the chromosome *I* fragment lacking the *URA3* gene.

Analysis of monosome *I*-monosome *III* meiotic segregation: Two double monosomic strains were independently isolated from diploid strain VG72, sporulated, and the resultant asci dissected. Results from both isolates were almost identical and were combined. Asci containing four inviable spores were scored as showing disjunction of chromosome *I* and chromosome *III*. Two spores are inviable because they lack chromosome *I* and two spores are inviable because they lack chromosome *III*. Asci containing two viable and two inviable spores were scored as showing nondisjunction of the monosomic chromosomes. Two spores are viable because they have a complete set of chromosomes while two spores are inviable because they lack both chromosomes *I* and *III*. Asci containing only one viable spore and three inviable spores also were scored as showing nondisjunction of chromosome *I* and chromosome *III* since viable spores can only be produced if the monosomic chromosomes cosegregate.

Analysis of meiotic segregation of monosomic chromosome *I* fragments: One monosomic strain for each chromosome *I* bisection was analyzed in a similar fashion to what has been described for the double monosomic strain. Asci having four inviable spores were scored as showing disjunction of the chromosome fragments while asci having two or one viable spores were scored as showing nondisjunction. Each spore in an ascus with four inviable spores is missing a chromosome *I* fragment. In two viable-spored asci, the two viable spores each have both chromosome *I* fragments while the two inviable spores are missing both chromosome *I* fragments. Asci that had only one viable Ura$^+$ spore and three inviable spores also were scored as showing nondisjunction since Ura$^+$ viable spores are produced only when the monosomic chromosome fragments cosegregate. Asci containing one Ura$^-$ spore and three inviable spores were not included in the results and presumably arose due to recombination between the two chromosome fragments to reconstitute chromosome *I*.

Construction of an *ARG4* marked *CEN* plasmid: Plasmid pVG96 was constructed by removing the *Sma*I-*Nru*I fragment containing most of the *URA3* gene from *CEN* plasmid pWB2 (WANG and BRANDRISS 1986) and replacing it with a 2.0-kb *Hpa*I fragment containing the *ARG4* gene from plasmid pKML-1 (supplied by KAREN LUSNAK) (BEACHAM *et al.* 1984).

Isolation and meiotic analysis of double monosomic strains containing a centromere plasmid: Plasmid pVG96 was transformed into diploid strain VG72. Four transformants were grown on galactose, as described above to form double monosomics for further analysis. Plasmid pVG96 also was transformed directly into double monosomic strain VG72-DM and two transformants isolated. TAFE was used to confirm karyotypes while conventional agarose gel electrophoresis and blot hybridization were used to show that plasmid pVG96 had the expected size in all double monosomic isolates (data not shown). These plasmid containing double monosomic strains were sporulated and the resultant asci analyzed. Results from all isolates obtained by both methods were virtually identical and were combined.

Plasmid presence was determined by scoring viable ascospores for the plasmid borne *ARG4* gene. Asci containing one or two viable Arg$^-$ spores were scored as showing segregation of plasmid away from the two monosomic chromosomes (type 1). Asci containing four inviable spores were scored as showing segregation of plasmid and one monosomic chromosome away from the other monosomic chromosome (types 2 and 3). Asci containing one or two viable spores that were Arg$^+$ were scored as showing cosegregation of the plasmid with both monosomic chromosomes (type 4).

Microscopic analysis of meiotic nuclear division: Following sporulation, cells were stained with 4′,6-diamidino-2-phenylindole (DAPI) and examined by fluorescence microscopy as described previously (SHERMAN, FINK and HICKS 1986).

RESULTS

Formation of monosomic strains: Diploid strain VG72 contains conditional centromeres (HILL and

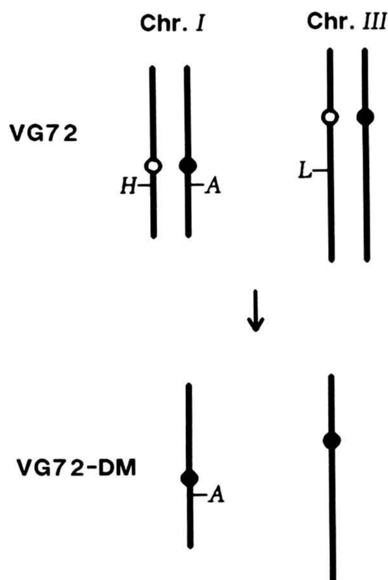


FIGURE 1.—Formation of a strain monosomic for chromosomes *I* and *III*. Diploid strain VG72 contains a conditional centromere on one copy of both chromosome *I* and chromosome *III*. Growth on galactose induces loss of chromosomes carrying conditional centromeres. (○) Conditional centromeres; (●) wild-type centromeres; A, *ADE1*; H, *ade1::HIS3*; L, *mat*Δ*::LEU2*.

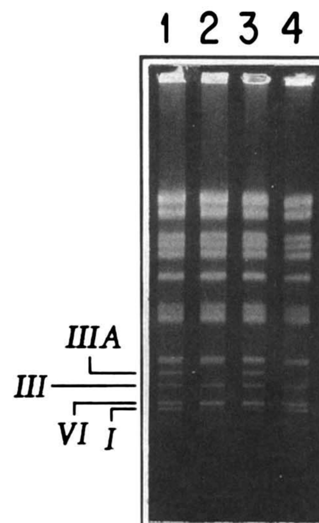


FIGURE 2.—Physical detection of chromosome loss. Electrophoretic karyotypes were analyzed using TAFE as described in MATERIALS AND METHODS. Electrophoresis was carried out for 20 hr using 55-sec pulses. Lane 1, diploid strain VG72; lane 2, double monosomic strain VG72-DM; lane 3, chromosome *I* monosomic strain VG72-M1; lane 4, chromosome *III* monosomic strain VG72-M3. The larger isoform (*IIIA*) is missing in strains monosomic for chromosome *III* and the relative intensity of chromosome *I* is approximately one-half that of chromosome *VI* in strains monosomic for chromosome *I*.

BLOOM 1987) on one copy of both chromosome *I* and chromosome *III*. This strain was grown on galactose as described in MATERIALS AND METHODS in order to produce isogenic strains that were monosomic for either chromosome *I* or chromosome *III* or monosomic for both chromosomes *I* and *III* (Figure 1). As predicted, the conditional centromeres became nonfunctional in the presence of galactose and the chromosomes on which they were located were lost at a high frequency. Two His$^-$, two Leu$^-$ and two His$^-$, Leu$^-$ colonies were picked and their electrophoretic karyotypes examined by TAFE (Figure 2). The His$^-$ colonies were monosomic for chromosome *I* as demonstrated by the decreased intensity of the chromosome *I* band relative to all the other bands on the TAFE gel. The Leu$^-$ colonies were monosomic for chromosome *III* as demonstrated by the loss of the upper polymorphic copy of chromosome *III* (*IIIA*). The His$^-$ Leu$^-$ colonies were monosomic for both chromosomes *I* and *III* as demonstrated by the decreased intensity of the chromosome *I* band and the loss of the chromosome *IIIA* band. The electrophoretic karyotypes also were consistent with these strains having two copies of all other chromosomes.

Spore viability in the parent diploid and isogenic strains monosomic for a single chromosome: Since the assay for distributive disjunction depended on the production of inviable spores, it was necessary to show that the genetic background of our strains did not contribute significantly to spore inviability. First, we examined spore viability in diploid strain VG72, the parent of all monosomic strains (Table 2). 90% of the 40 asci dissected contained four viable spores while the rest contained either three or two viable spores. In total, 96% of the spores were viable.

Second, isogenic derivatives of strain VG72 which were monosomic for either chromosome *I* (VG72-M1) or chromosome *III* (VG72-M3) were examined. First division segregation of the monosomic chromosome into only two of the four spores should give 2:2, viable:inviable asci (BRUENN and MORTIMER 1970). Spore viability was comparable to other single monosomic strains (BRUENN and MORTIMER 1970; KABACK 1989). 95% of the asci from VG72-M1 and 88% of the asci from VG72-M3 segregated spore viability 2:2 at the first meiotic division. Approximately 1% of the asci showed apparent second division segregation for spore viability. However, this level of second division segregation is consistent with the expected amount of recombination between *TRP1* and *CEN4*. 5% and 10% of the asci from VG72-M1 and VG72-M3, respectively, segregated spore viability 1:3 (viable:inviable). Only one ascus from strain VG72-M3 contained four inviable spores. The reasons for the 1:3 and 0:4 asci were not investigated. However, some spore inviability of unknown origin is common and these few asci do not affect our overall results. In summary, these results show that the genetic background of strain VG72 and its isogenic monosomic derivatives is not a significant source of unexpected spore inviability.

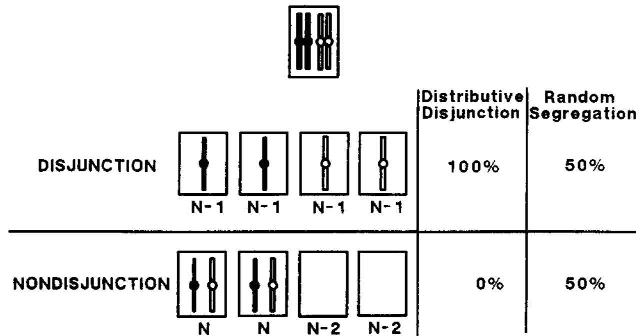


FIGURE 3.—Predicted consequences of segregation of two monosomic chromosomes in the double monosomic strain. Disjunction results in 0:4 (viable:inviable) asci; nondisjunction results in 2:2 (viable:inviable) asci. In some cases nondisjunction can also yield 1:3 (viable:inviable) asci due to spore inviability of unknown origin. Spore viability predicted for 100% distributive disjunction and random segregation is shown.

Distributive disjunction of chromosome *I* and chromosome *III*: To determine if authentic unpaired nonhomologous yeast chromosomes undergo distributive disjunction, we examined meiotic segregation in VG72-DM, the strain monosomic for both chromosome *I* and chromosome *III*. Asci were analyzed as described in MATERIALS AND METHODS. Disjunction of chromosomes *I* and *III* yields asci that contain four inviable spores (the 0:4 [viable:inviable] class) while nondisjunction of these two chromosomes yields asci with 2 viable and 2 inviable spores (the 2:2 class) (Figure 3). Sometimes nondisjunction of the monosomic chromosomes also can yield asci with only one viable and three inviable spores (the 1:3 class) due to spore inviability of unknown origin. If the two nonhomologous monosomic chromosomes always segregate by distributive disjunction, all asci will be in the 0:4 class. In contrast, if there is no distributive disjunction, the two monosomic chromosomes will segregate randomly and the number of asci in the 0:4 class should equal the total number of asci in the 2:2 and 1:3 classes.

The results showed that 89% of the 80 asci analyzed were in the 0:4 class while the rest were in the 2:2 or 1:3 class (Table 2). Chi-squared analysis indicated that these data were significantly different from those predicted for random segregation ($P < 10^{-6}$).

In the small number of asci in the 2:2 class, viable spores were always sisters indicating the unpaired chromosomes cosegregated at the first meiotic division.

To determine whether both meiotic nuclear divisions occurred normally in strain VG72-DM, sporulated cultures were treated with the DNA specific stain DAPI and examined by fluorescence microscopy. All four spored asci contained four normal appearing nuclei that fluoresced with approximately equal intensity, indicating that this strain undergoes two apparently normal meiotic divisions (data not shown). Thus, spore inviability was not caused by a failure to complete meiosis.

TABLE 2

Meiotic analysis of single and double monosomic strains

Strain	Ploidy[a]	No. of asci analyzed	Percentage of asci with ascus type (viable:inviable) 4:0	3:1	2:2	1:3	0:4
VG72	$2n$	40	90	3	7	0	0
VG72-M1	$2n - 1(I)$	40	0	0	95	5	0
VG72-M3	$2n - 1(III)$	40	0	0	88	10	2
VG72-DM	$2n - 2(I,III)$	80	0	0	9	2	89

The segregation of chromosomes in diploid strain VG72 and its monosomic derivatives were analyzed as described in MATERIALS AND METHODS.

[a] Monosomic chromosomes are noted in parentheses.

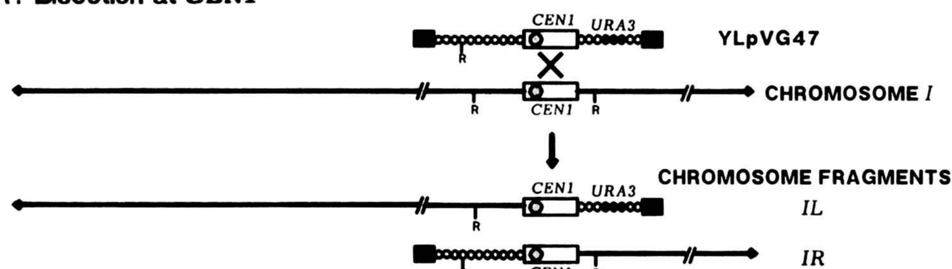
Taken together, these results suggest that the two nonhomologous chromosomes disjoined in most asci. Therefore, authentic yeast chromosomes are capable of distributive disjunction.

Formation of strains that contain a monosomic bisected copy of chromosome *I*: Chromosome *I* was bisected into two functional chromosome fragments as described in MATERIALS AND METHODS and Figure 4. Haploid strain VG37-8B was transformed with linear plasmid YLpVG47 to bisect chromosome *I* at *CEN1*. Several stable Ura$^+$ transformants were examined by TAFE. Two transformants that lacked the full length copy of chromosome *I* and contained two smaller chromosomes, *IL* and *IR*, were identified (Figure 5). As predicted for bisection at *CEN1*, these chromosome fragments were 100 kb (*IR*) and 160 kb (*IL*) in size.

To bisect chromosome *I* at *MAK16*, haploid strain VG37-8B was transformed with linear plasmid YLpVG59. Again, stable Ura$^+$ transformants were examined by TAFE. One transformant was missing the full length copy of chromosome *I* and contained the predicted 125 kb (*IA*) and 140 kb (*IB*) chromosome *I* fragments (Figure 5).

The TAFE gels were blotted and hybridized to *CEN1* and *URA3* probes. As predicted, the *CEN1* probe hybridized to both chromosomal fragments in both bisections while the *URA3* probe hybridized to only fragment *IL* in the YLpVG47 transformants and fragment *IA* in the YLpVG59 transformant (data not shown). In addition, genomic blot hybridization experiments confirmed that the linear plasmids integrated at the expected sites (data not shown). Therefore, YLpVG47 and YLpVG59 successfully bisected chromosome *I* into two functional linear chromosome fragments. Each pair of chromosome fragments was nonhomologous with the exception of *CEN1* and the target sequences used for the bisections (Figure 4).

One transformant for each bisection (strains VG37-8B:YLpVG47 and VG37-8B:YLpVG59) was crossed



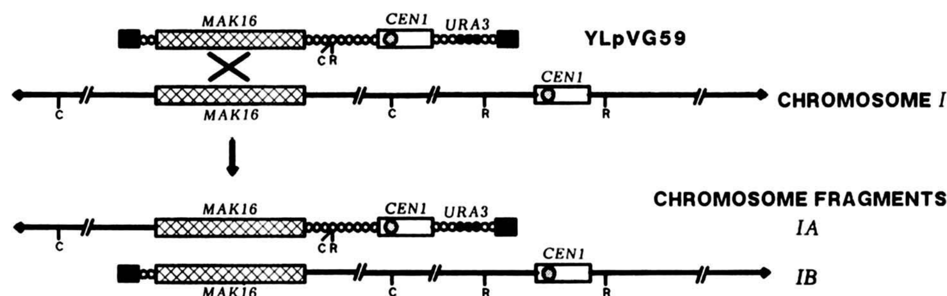


FIGURE 4.—Bisection of chromosome *I*. A, Bisection at *CEN1*. Recombination between linear plasmid YLpVG47 and the full length copy of chromosome *I* generates two telocentric chromosome fragments, *IL* and *IR*. B, Bisection I at *MAK16*. Recombination between linear plasmid YLpVG59 and the full length copy of chromosome *I* generates telocentric and metacentric chromosome fragments, IA and IB, respectively. ooo YIp5 DNA; ●●● *URA3* gene; ■ tetrahymena rDNA ends; [hatched box] DNA fragment carrying *MAK16*; [box] DNA fragment carrying *CEN1* with the stipled circle denoting the position of *CEN1*; ▬ the remainder of chromosome I DNA molecule; R *Eco*RI; C *Cla*I.

to strain VGGALCEN yielding diploids VG79 and VG80 (Table 1). These diploids, which contained a conditional centromere on the full length copy of chromosome *I* and a bisected copy of chromosome *I*, were grown on galactose to induce the loss of the full length copy of chromosome *I* (Figure 6). A His$^-$ colony from each strain was obtained and examined by TAFE. The electrophoretic karyotypes indicated these colonies lacked the full length copy of chromosome *I* and contained only a single bisected copy of chromosome *I* (Figure 7). These monosomic strains were called VG79-BM (BM for *b*isected *m*onosome) and VG80-BM for the bisections at *CEN1* and *MAK16*, respectively.

Distributive disjunction of chromosome *I* fragments: To further investigate whether yeast chromosomes were capable of distributive disjunction, we examined the meiotic segregation of the two mostly nonhomologous chromosome *I* fragments in strains VG79-BM and VG80-BM as described in MATERIALS AND METHODS. Similar to the double monosome experiment, disjunction of the chromosome fragments yields asci containing four inviable spores (the 0:4 [viable:inviable] class) while nondisjunction yields asci with either two viable and two inviable spores (the 2:2 class) or one viable and three inviable spores (the 1:3 class). If the two chromosome halves always undergo distributive disjunction, all asci will be in the 0:4 class while random segregation will result in the 0:4 class equalling the total in the 2:2 and 1:3 classes (Figure 8).

The results showed that 89% of the asci from both VG79-BM and VG80-BM were in the 0:4 class while only 9% were in the 2:2 class and 2% were in the 1:3

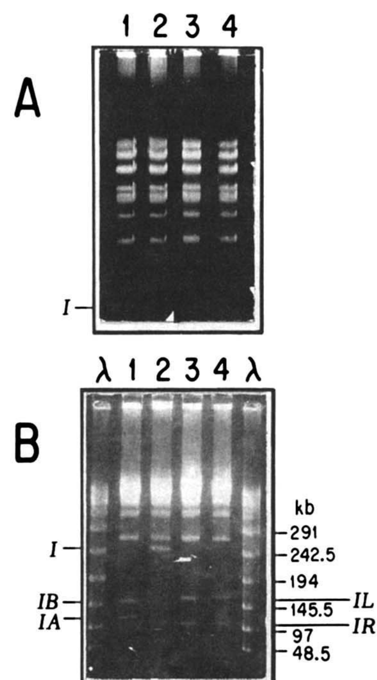


FIGURE 5.—Physical detection of chromosome bisection using TAFE. Electrophoresis was carried out for 20 hr using 55-sec pulses (panel A) and for 16 hr using 25-sec pulses (panel B) as described in MATERIALS AND METHODS. Lane 1, strain VG37-8B:YLpVG59 containing bisection at *MAK16*; lane 2, parent haploid VG37-8B containing full length copy of chromosome *I*; lanes 3 and 4, strain VG37-8B:YLpVG47 (transformants 1 and 2) containing bisection at *CEN1*. λ designates concatemeric bacteriophage lambda DNA size markers.

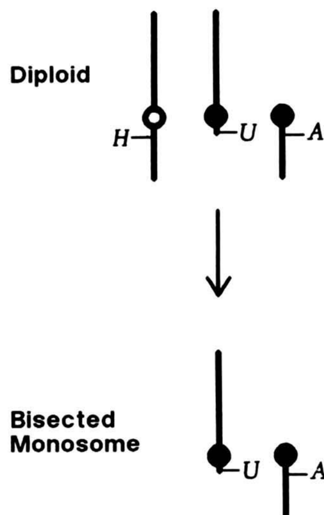


FIGURE 6.—Formation of strains monosomic for a bisected copy of chromosome *I*. Diploid strains containing a full-length copy of chromosome *I* carrying a conditional centromere and a bisected copy of chromosome *I* were grown on galactose to induce loss of the full length copy of chromosome *I*. (○) Conditional centromere; (●) wild-type centromeres; U, *URA3*; A, *ADE1*; H, *ade1::HIS3*.

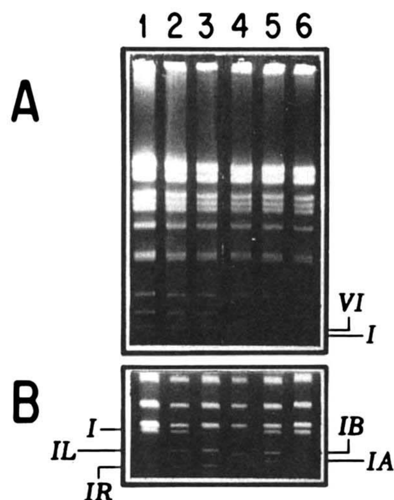


FIGURE 7.—Physical detection of chromosome *I* loss and chromosome *I* reconstitution using TAFE. Electrophoresis was carried out for 20 hr using 55-sec pulses (panel A) and 16 hr using 40-sec pulses to better visualize the small chromosomes (panel B) as described in MATERIALS AND METHODS. Lane 1, reconstituted chromosome *I* monosomic strain VG79-MR; lane 2, diploid strain VG79; lane 3, bisected monosomic strain VG79-BM; lane 4, bisected monosomic strain VG80-BM; lane 5, diploid strain VG80; lane 6, reconstituted chromosome *I* monosomic strain VG80-MR.

class (Table 3). Chi-squared analysis indicated that these results were significantly different ($P < 10^{-6}$) from that predicted by random segregation.

Viable spores in all the 2:2 class asci and all but two of the 1:3 class asci were Ura^+ indicating the chromosomes remained bisected. Both VG79-BM and VG80-BM gave rise to one ascus containing a single viable Ura^- spore. These asci were not included in the results because they probably were due to reconstitution of chromosome I (see below). In addition, viable spores in the 2:2 class asci were always sisters indicating the two half chromosomes cosegregated at the first meiotic division.

To determine whether nuclear division was occurring normally, sporulated cultures of both bisected monosomic strains were treated with DAPI and examined by fluorescence microscopy. All four-spored asci contained four normal appearing nuclei that fluoresced with approximately equal intensity (data not shown). Thus, these strains undergo two apparently normal meiotic divisions. Again, spore inviability was not caused by a failure to complete meiosis.

To show that the high percentage of asci containing four inviable spores was not due to genetic background, we examined the spore viability of strain VG90. This isogenic variant of both VG79-BM and VG80-BM contains two full-length copies of chromosome *I* and yielded 95% 4:0 (viable:inviable) asci (Table 3). In addition, the parent diploids VG79 and VG80 also exhibited very good spore viability giving mostly 4:0 and a few 3:1 asci (data not shown).

Congenic diploid strains VG86 and VG87 containing homozygous diploid copies of the bisected chromosome also gave rise to mostly 4:0 (viable:inviable) asci (Table 3) indicating that each chromosome fragment was able to segregate efficiently from its homolog during meiosis. Thus, spore inviability in strains VG79-BM and VG80-BM was not due to either some aberrant behavior of the chromosome fragments or the bisection of a gene essential for spore germination or viability.

To prove that spore inviability in strains VG79-BM and VG80-BM was not due to loss of additional chromosomes or some other unexpected cause, we obtained isogenic variants of these strains that had undergone mitotic recombination between the homologous regions of the bisected chromosome fragments and now contained a reconstituted intact copy of chromosome *I*. Strains VG79-MR (MR for *mono*somic *r*econstituted chromosome) and VG80-MR were selected directly from the bisected monosomic strains as Ura^- colonies on FOA medium as described in MATERIALS AND METHODS. TAFE (Figure 7) and blot hybridization (data not shown) indicated that VG79-MR and VG80-MR lacked the chromosome fragments and contained a single 250-kb full length copy of chromosome *I*. Meiotic analysis of these strains (Table 3) indicated that 83–87% of the asci contained two viable spores and 5–15% contained a single viable spore. Only 2–8% were in the 0:4 class. This level of spore viability is comparable to that seen in other strains monosomic for a single chromosome (BRUENN and MORTIMER 1970; KABACK 1989).

Thus, we observed a high level of asci in the 0:4 class only when two monosomic chromosome fragments were present. The most likely explanation for

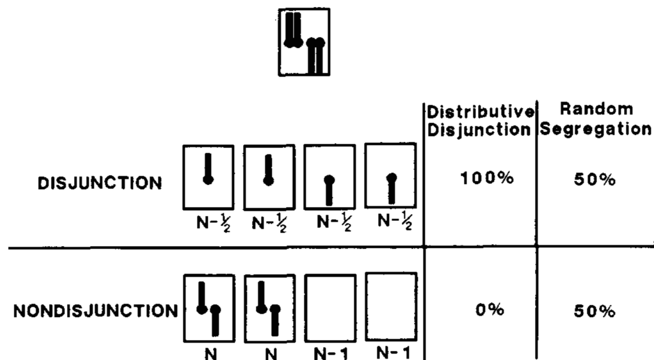


FIGURE 8.—Predicted consequences of segregation of chromosome *I* fragments in bisected monosomic strains. Disjunction results in 0:4 (viable:inviable) asci; Nondisjunction results in 2:2 (viable:inviable) asci. In some cases nondisjunction can also yield 1:3 (viable:inviable) asci due to spore inviability of unknown origin. Spore viability predicted for 100% distributive disjunction and random segregation is shown.

these results is that these fragments disjoined during meiosis. Since these chromosomes are mostly nonhomologous and rarely if ever underwent recombination, we conclude they segregated by distributive disjunction.

Effect of a centromere plasmid on spore viability in the double monosomic strain: When three chromosomes distributively disjoin, two chromosomes cosegregate to one pole and the third segregates to the opposite pole (GRELL 1976; DAWSON, MURRAY and SZOSTAK 1986). Since a *CEN* plasmid distributively disjoins from one full length chromosome (KABACK 1989), it may be able to participate with two monosomic chromosomes in distributive disjunction. When the two full length monosomic chromosomes cosegregate to one pole and the *CEN* plasmid segregates to the opposite pole, two viable spores and two inviable *CEN* plasmid containing spores, respectively, are produced. Therefore, participation of a *CEN* plasmid in distributive disjunction could actually restore some spore viability to the double monosomic strain. However, if the spore inviability observed in the double monosomic strain was not caused by distributive disjunction or the *CEN* plasmid cannot participate when two monosomic chromosomes are present, the plasmid would not affect spore viability.

Plasmid pVG96, the *CEN* plasmid used for this experiment, contains *CEN4, ARS1*, the *ARG4* gene and a 12-kb insert from chromosome *XII*. This plasmid was constructed as described in MATERIALS AND METHODS from plasmid pWB2, which was previously shown to exhibit relatively efficient meiotic segregational behavior (KABACK 1989).

First, we examined the efficiency that plasmid pVG96 was transmitted during meiosis in diploid strain VG72. The plasmid segregated 2:2 (Arg^+:Arg^-) at the first meiotic division in 80%, 1:3 in 12%, 0:4 in 3% and 4:0 in 4% of the 76 asci analyzed. These results indicate that plasmid pVG96 is maintained as a single copy in most cells and is transmitted efficiently during meiosis in diploids. Plasmid segregation is reported for only asci containing four viable spores. However, the few asci containing three viable spores gave data that was consistent with results from asci with four viable spores. Second, we introduced this plasmid into strain VG89-M3 and examined whether plasmid pVG96 is capable of distributive disjunction from a single monosomic copy of chromosome *III*. 93% of the asci examined contained two viable Arg^- (plasmid negative) spores suggesting that the plasmid segregated from the monosomic chromosome into the inviable spores at approximately the same frequency observed for other *CEN* plasmids (KABACK 1989).

To determine if plasmid pVG96 increases spore viability in the double monosomic strain, plasmid containing VG72-DM was isolated in two ways as described in MATERIALS AND METHODS. TAFE generated karyotypes showed that these strains were double monosomes and genomic blot hybridization confirmed they contained the full length plasmid (data not shown).

Four types of asci can be formed due to segregation of plasmid pVG96 and the two monosomic chromosomes (MATERIALS AND METHODS; Figure 9). In type 1, both monosomic chromosomes cosegregate to one pole and the plasmid segregates to the opposite pole. This segregation leads to mostly 2:2 (viable:inviable) and in some cases 1:3 asci (due to spore inviability of unknown origin) where all viable spores lack plasmid. In type 2, the plasmid and chromosome *I* cosegregate while chromosome *III* segregates to the opposite pole. In type 3, plasmid and chromosome *III* cosegregate

TABLE 3

Meiotic analysis of monosomic strains containing a bisected copy of chromosome *I*

Strain	Chromosome *I* form	No. of asci analyzed	Percentage of asci with ascus type (viable:inviable) 4:0	3:1	2:2	1:3	0:4
VG79-BM	O/IL,IR[a]	79	0	0	9	2	89
VG80-BM	O/IA,IB[a]	79	0	0	9	2	89
VG90	I/I[b]	20	95	0	5	0	0
VG86	IL,IR/IL,IR[c]	40	75	15	7	0	2
VG87	IA,IB/IA,IB[c]	40	88	12	0	0	0
VG79-MR	O/I[d]	40	0	0	83	15	2
VG80-MR	O/I[d]	40	0	0	87	5	8

Meiotic segregation of functional chromosome *I* fragments in diploid and monosomic strains was analyzed as described in MATERIALS AND METHODS.

[a] Chromosome *I* monosome containing two chromosome *I* fragments.

[b] Diploid containing two full length copies of chromosome *I*.

[c] Diploid for both chromosome *I* fragments. No full length copies of chromosome *I* are present.

[d] Chromosome *I* monosome containing a reconstituted full length chromosome *I*.

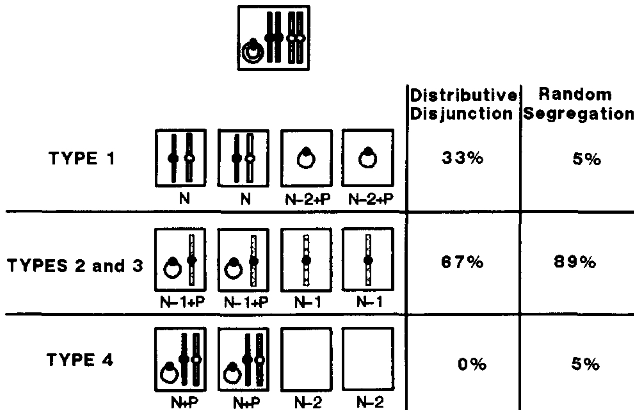


FIGURE 9.—Predicted consequences of segregation of a *CEN* plasmid and two monosomic chromosomes. Strains that are monosomic for both chromosomes *I* and *III* and contain plasmid pVG96 were analyzed as described in MATERIALS AND METHODS. Segregation of the *CEN* plasmid and two monosomic chromosomes generates the ascus types shown. Type 1 asci are either 2:2 or 1:3 (viable:inviable) where all viable spores lack plasmid. Type 2 and type 3 asci are 0:4 (viable:inviable). Since the specific chromosome which cosegregated with the *CEN* plasmid cannot be determined, a single generic chromosome (hatched) is depicted. Type 4 asci are either 2:2 or 1:3 (viable:inviable) where all viable spores contain plasmid. Predicted percentages of type 1, types 2 and 3 or type 4 asci are shown for distributive disjunction when the plasmid and monosomic chromosomes are equivalent and for random segregation of the plasmid.

while chromosome *I* segregates to the opposite pole. Types 2 and 3 are indistinguishable since they both give rise to 0:4 (viable:inviable) asci. In type 4, the plasmid and both monosomic chromosomes all cosegregate to the same pole. This cosegregation leads to mostly 2:2 (viable:inviable) and in some cases 1:3 asci (due to spore inviability of unknown origin) where all viable spores contain plasmid.

If distributive disjunction occurs and the plasmid participates, we expect most asci to be of types 1, 2 and 3 and few if any to be of type 4. The number of type 1 asci will be dependent on the efficiency at which the plasmid participates. If the *CEN* plasmid and the monosomic chromosomes are equivalent, one third of the asci should be type 1 and two thirds should be types 2 and 3. If the plasmid participates in distributive disjunction but not as an equal partner, the two monosomic chromosomes would preferentially disjoin leading to fewer type 1 and more types 2 and 3 asci. Alternatively, if spore inviability in the double monosomic strain is not due to distributive disjunction or the plasmid does not participate, the plasmid should not cause an increase in spore viability. Approximately 90% of the asci would still contain four inviable spores and the number of type 1 and type 4 asci would be equal due to random segregation of the plasmid.

Analysis of the plasmid pVG96 containing double monosome, VG72-DM indicated that a total of 27% of the asci were type 1 (Table 4). 67% of the asci

TABLE 4

Effect of a *CEN* plasmid on spore viability in the double monosomic strain

		Percentage of asci with ascus type (viable:inviable)				
		Type 1		Types 2 + 3	Type 4	
Strain[a]	No. of asci analyzed	2:2	1:3	0:4	2:2	1:3
+Plasmid	319	21	6	67	5	1
		Ascus type (viable:inviable)				
		2:2	1:3	0:4		
−Plasmid	220	10	2	88		
Untransformed	80	9	2	89		

[a] Strain VG72-DM containing *CEN* plasmid pVG96 (+plasmid) was isolated and analyzed as described in MATERIALS AND METHODS. Isolates that spontaneously lost plasmid (−plasmid) and untransformed strain VG72-DM were analyzed as controls.

contained four inviable spores and were classified as types 2 and 3 and only 6% of the asci were type 4. As a control, colonies that spontaneously lost plasmid were examined. Only 12% of these asci contained viable spores, in close agreement with the 11% obtained with the original untransformed double monosomic strain. Chi-squared analysis indicated that the observed increase in asci with viable spores to 27% (type 1 asci) in plasmid containing VG72-DM was significant and could not be caused by random chance ($P < 4 \times 10^{-3}$). In addition, in the 2:2 (viable:inviable) asci (types 1 and 4), 81 out of 82 contained sister spores indicating that the monosomic chromosomes cosegregated at the first meiotic division.

We also transformed the bisected monosomic strains, VG79-BM and VG80-BM, with plasmid pVG96 and found that spore viability was similarly increased due to the presence of plasmid (data not shown).

These results show that this *CEN* plasmid increased spore viability in the double monosomic strains. The most likely explanation is that the *CEN* plasmid participated in distributive disjunction causing both full length chromosomes to segregate away from the plasmid in a significant fraction of asci (type 1 asci). The observation that type 1 asci were fivefold more predominant than type 4 asci is also consistent with distributive disjunction since random segregation of the plasmid predicts that these classes would be equal. The fact that nearly one third of the asci were type 1 indicates that the plasmid participated with the two chromosomes almost as an equal partner in distributive disjunction. These results further demonstrate that authentic chromosomes are capable of undergoing distributive disjunction in *S. cerevisiae* and eliminate the possibility that spore inviability is caused by factors other than chromosome segregation.

DISCUSSION

We examined meiotic segregation of unpaired copies of chromosome *I* and chromosome *III* in *S. cerevisiae* strains monosomic for both chromosomes. Disjunction of the monosomic chromosomes yields asci with four inviable spores while nondisjunction yields asci with two or one viable spores. Eighty-nine percent of the asci dissected contained four inviable spores suggesting that the two monosomic chromosomes disjoined in most meiotic cells. Since these chromosomes are nonhomologous, authentic yeast chromosomes appear to be capable of distributive disjunction.

In these experiments most asci had four inviable spores making it necessary to show that chromosome segregation was the only cause of spore inviability. Several lines of evidence eliminated other possible causes of spore inviability. First, the parent diploid exhibited 96% spore viability demonstrating that the genetic background did not contribute significantly to spore inviability. Second, isogenic single monosomic strains isolated from the parent diploid yielded mostly asci with two viable and two inviable spores demonstrating that the method of inducing chromosome loss did not cause any unexpected spore inviability. Third, electrophoretic karyotyping showed that spore inviability in the double monosomic strain was not due to the loss of additional chromosomes. The double monosomic strain was clearly monosomic for chromosomes *I* and *III* and as best could be judged, diploid for all other chromosomes. The karyotype contained two chromosomal doublets (*VII-XV* and *XIII-XVI*) making it possible that a doublet contained three copies of one chromosome and a single copy of the other. However, formation of such a complex aneuploid (monosomic for three chromosomes and trisomic for another) would require two additional mitotic nondisjunctional events concommittant with or following growth of the parent diploid on galactose. Presumably these complex aneuploids could arise because strains missing additional chromosomes could have some selective advantage over the double monosomic strain. However, such complex events would be expected extremely infrequently making it difficult to generate the double monosomes. Alternatively, we could expect slow growing primary isolates from which faster growing variants arise. Contrary to these predictions, the double monosomic strains arose at a high frequency and grew rapidly from the time they were first isolated. Thus, we can conclude that our strains were indeed monosomic for chromosomes *I* and *III* and diploid for all other chromosomes. Finally, the presence of a *CEN* plasmid in the double monosomic strain increased spore viability significantly. This result cannot be explained by the loss of additional chromosomes but is the expected finding for distributive disjunction of two monosomic chromosomes and a nonessential *CEN* plasmid. Therefore, the high percentage of asci with four inviable spores obtained with the double monosomic strain is most likely the result of distributive disjunction of the two monosomic chromosomes.

It is possible that failure to undergo distributive disjunction in the double monosomes can affect the formation of four-spored asci. If this were true, dissection of only four-spored asci might have selected those that underwent disjunction. However, all euploid, single monosomic and double monosomic strains produced similar high percentages of four-spored asci making this possibility unlikely.

To provide additional evidence for distributive disjunction, we bisected chromosome *I* into two functional chromosome fragments and examined their meiotic segregation in a yeast strain monosomic for both fragments. The chromosome was bisected near *CEN1* and near *MAK16* and the results from both bisections were identical. Disjunction of the monosomic chromosome fragments produces an ascus containing four inviable spores while nondisjunction produces an ascus with two viable and two inviable spores. 89% of the asci analyzed contained four inviable spores suggesting that the chromosome fragments disjoined in most meiotic cells. Coincidentally, this was the same percentage obtained using the double monosomic strain. Thus, the two almost completely nonhomologous chromosome *I* fragments also appear to undergo distributive disjunction.

It was necessary to exclude all other potential causes of spore inviability to show that disjunction of the chromosome fragments caused the high percentage of 0:4 asci in the bisected monosomic strains. First, an isogenic strain containing two full length copies of chromosome *I* had 98% spore viability demonstrating that the genetic background did not contribute significantly to spore inviability. Second, most of the asci from congenic diploids homozygous for both chromosome fragments had four viable spores demonstrating that spore inviability is not due to either the inability of the chromosome fragments to segregate as normal chromosomes or the bisection of an essential gene. Finally, we reconstituted chromosome *I* in each bisected monosomic strain and found that most asci from these strains had two viable and two inviable spores as expected for a strain monosomic for a single chromosome (BREUNN and MORTIMER 1970). This result directly demonstrates that neither loss of additional chromosomes nor anything else in the genetic background contributed to spore inviability. Thus, we observed a high percentage of asci with four inviable spores only when strains contained a bisected monosomic copy of chromosome *I*. The most probable explanation for these results is that the two chromo-

some *I* fragments distributively disjoined during meiosis.

Homologous recombination between the two chromosome *I* fragments in the bisected monosomic strains or between the two monosomic chromosomes in the double monosomic strain could not have been a factor in these experiments. Each pair of chromosome fragments contained only 2 or 7 kb of homologous DNA. Based on the average cM/kb observed for yeast, recombination between these regions of homology is expected to occur in less than 5% of meiotic cells (KABACK, STEENSMA and DEJONGE 1989). A single crossover between the chromosome fragments reconstitutes a full length chromosome and gives rise to an ascus with a single viable spore containing the intact chromosome. Only 2 out of the 160 tetrads examined were of this type. Since the chromosomes segregated from each other in 89% of the asci and recombination was a rare event, recombination could not have been involved in the segregation of the chromosome fragments. The precise amount of sequence homology shared by chromosomes *I* and *III* is not known. However, it probably is no more than 10 kb. Each chromosome has a related but not identical 120 bp *CEN* sequence (HIETER *et al.* 1985b), approximately 200 bp of related telomeric sequences (SZOSTAK and BLACKBURN 1982) and both contain 5.6-kb Tyl retroposons (STEENSMA, CROWLEY and KABACK 1987; KLEIN and PETES 1984). Chromosome *I* also has a few repeated tRNA genes but there is no evidence that these genes are repeated on chromosome *III* (LIEBMAN *et al.* 1984; STEENSMA, CROWLEY and KABACK 1987; COLEMAN *et al.* 1986; A. BARTON and D. B. KABACK, unpublished results). Based on the limited homology, recombination is expected to be rare and is not likely to be involved in the segregation of the two intact monosomic chromosomes. We cannot eliminate the possibility that the telomeres recombined since these events cannot be detected. However, based on the observations that circular chromosomes undergo distributive disjunction (MANN and DAVIS 1986; KABACK 1989), it is unlikely that telomeric recombination plays a role.

We also wanted to investigate distributive disjunction of authentic chromosomes where all the products of meiosis would be viable. It was previously demonstrated that a synthetic circular minichromosome segregated from a trisomic copy of chromosome *I* (KABACK 1989). Therefore, we examined a strain trisomic for both chromosome *I* and chromosome *VI* (double trisomic strain) to determine if the two extra chromosomes underwent distributive disjunction. The extra copies of chromosomes *I* and *VI* did not distributively disjoin, but instead segregated randomly with respect to each other (GUACCI 1990). It is known that three homologous chromosomes undergo trivalent pairing during meiosis (SHAFFER *et al.* 1971; CULBERTSON and HENRY 1973). Therefore, we suggest that trivalent pairing among each set of trisomic chromosomes prevents the two extra chromosomes from distributively disjoining. Accordingly, the use of double trisomic strains cannot be used to examine distributive disjunction in *S. cerevisiae*.

Our results demonstrate that authentic nonhomologous yeast chromosomes undergo distributive disjunction about 90% of the time. This frequency is no better than has been observed between two nonhomologous circular *CEN* plasmids, an unpaired chromosome and a *CEN* plasmid or a nonhomologous synthetic linear chromosome and a chromosome *III* fragment (MANN and DAVIS 1986; KABACK 1989; DAWSON, MURRAY and SZOSTAK 1986). In contrast, authentic Drosophila chromosomes undergo distributive disjunction 99.9% of the time. Therefore, distributive disjunction in yeast is much less efficient than in Drosophila, and the previously observed inefficiency of distributive disjunction in yeast was not related to the use of synthetic chromosomes.

The relative efficiencies observed in yeast and Drosophila are consistent with the fact that distributive disjunction is rarely if ever needed in yeast but is always needed in Drosophila. Virtually all yeast chromosomes undergo meiotic recombination (KABACK, STEENSMA and DE JONGE 1989), whereas the smallest Drosophila chromosome rarely, if ever, undergoes recombination. Thus, it makes sense that Drosophila has an efficient mechanism for segregating nonrecombinant chromosomes. In contrast, the role of distributive disjunction in *S. cerevisiae* is enigmatic because there is no obvious need for it. It was suggested that distributive disjunction is a backup system for segregating the occasional nonrecombinant yeast chromosome (DAWSON, MURRAY and SZOSTAK 1986). However, it also is possible that distributive disjunction is simply a result of the normal processes and interactions involved in segregating homologous chromosomes (NOVITSKI 1964).

The presence of a *CEN* plasmid in the double monosomic strain significantly increased the number of asci containing two viable spores. The *CEN* plasmid appeared to distributively disjoin from the two monosomic chromosomes in 27% of the asci. Chi-squared analysis showed that the percentage of asci with viable spores was not significantly different from the 33% predicted if the plasmid and chromosomes were equivalent in their ability to undergo distributive disjunction. However, the appearance of a small number of asci with viable spores in the untransformed double monosomic strains combined with the infrequent mitotic loss of plasmid or increase in plasmid copy number makes it impossible to determine the real efficiency at which the plasmid participates in distributive

disjunction. Nevertheless, the level of asci containing viable spores was not significantly different from 33%. Therefore, it is likely that the *CEN* plasmid and the monosomic chromosomes are almost equivalent in their ability to undergo distributive disjunction.

Our results may be in contrast with the findings in Drosophila, where the two nonhomologous chromosomes closest in size preferentially disjoined while the third usually cosegregated with one of the other two chromosomes. Since the *CEN* plasmid was less than 1/15 the size of the two chromosomes, preferential segregation based on size does not appear to occur in *S. cerevisiae*. However, it must be noted that the lack of size preference could be related to the use of a circular chromosome in yeast since only linear chromosomes were examined in Drosophila.

In summary, we have found that authentic nonhomologous chromosomes undergo distributive disjunction in *S. cerevisiae*. Disjunction occurs about 90% of the time and does not appear to be dependent on the size of the chromosomes involved. At present the role of this type of segregation in meiosis is not known. Perhaps the isolation and analysis of mutants that cannot undergo distributive disjunction will enable the mechanism and function of distributive disjunction in yeast to be understood.

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