DIS1: A Yeast Gene Required for Proper Meiotic Chromosome Disjunction

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ABSTRACT

Mutants at a newly identified locus, *DIS1* (*dis*junction), were detected by screening for mutants that generate aneuploid spores (chromosome *VIII* disomes) at an increased frequency. Strains carrying the partially dominant alleles, *DIS1-1* or *DIS1-2*, generate disomes at rates up to 100 times the background level. Mitotic nondisjunction is also increased 10- to 50-fold over background. Half-tetrad analysis of disomes for a marked interval on chromosome *VIII* yields wild-type map distances, indicating that a general recombination deficiency is not the cause of nondisjuction. Meiotic nondisjunction in *DIS1* mutants is not chromosome specific; 5% of the spores disomic for chromosome *VIII* are also disomic for chromosome *III*. Although only one disomic spore is found per exceptional ascus most of the disomes appear to be generated in the first meiotic division because recovered chromosome *VIII* disomes contain mostly nonsister chromosomes. We propose that disome generation in the *DIS1* mutants results from precocious separation of sister centromeres.

THE proper segregation of genetic material during meiosis requires accurate chromosome replication, the synapsis of homologous chromosomes, genetic recombination and two rounds of chromosome segregation. In synaptic organisms, homologous chromosomes disjoin from each other during the first round of chromosome segregation (the reductional division). Proper segregation at meiosis I requires synapsis and recombination, presumably because these processes establish connections (chiasmata) between bivalents and allow them to be properly oriented on the metaphase plate. Mutants that perturb meiotic recombination often produce aneuploid meiotic products (reviewed in BAKER *et al.* 1976, BAKER and HALL 1976, and ESPOSITO and KLAPHOLZ 1981). There are two mutants in yeast that are not defective in meiotic recombination, yet appear to affect the reductional division (KLAPHOLZ and ESPOSITO 1980). These mutants, known as *spo12* and *spo13*, apparently bypass the reductional division and proceed directly to the second (equational) division. In wild-type cells, the equational division of meiosis appears similar to the mitotic divisions since sister chromatids disjoin. At least two genes in yeast required for normal mitotic chromosome segregation, *NDC1* (THOMAS and BOTSTEIN 1986) and *CDC31* (BYERS 1981), are also essential for the equational division of meiosis.

In an effort to identify other genes involved in meiotic chromosome segregation in yeast, we developed a screen to detect meiotic mutants that generate aneuploid spores at a high rate. We describe two alleles, one dominant and one semi-dominant, of a previously unidentified gene *DIS1* (for *dis*junction).

MATERIALS AND METHODS

Media: Synthetic complete medium (SC), rich media (YEPD and GNA), and sporulation medium (KAc) were prepared according to CAMPBELL, FOGEL and LUSNAK (1975). Synthetic complete medium lacking a nutrient is denoted by SC followed by the nutrient it lacks (*e.g.*, SC-thr is SC without threonine). Cycloheximide medium (SC + cyh) is SC medium supplemented with 0.7 mg/liter cycloheximide. Copper medium (SC + cup) is SC medium made with 1.5% phytagar (Grand Island Biochemicals) and supplemented to a final concentration of 0.02 mM copper sulfate unless otherwise stated (*e.g.*, SC + cup (0.04 mM) is SC supplemented with 0.04 mM $CuSO_4$). YEPAD is YEPD supplemented with 38 mg/liter adenine-HCl.

Strain construction: Most yeast genetic markers used in this study were obtained from stocks constructed in this laboratory. The two complementing alleles, *his4-280* and *his4-290*, were provided by G. FINK and the complementing *arg4-8* and *arg4-9* alleles were from R. K. MORTIMER. Table 1 lists yeast strains and genotypes.

Tetrad dissection was performed by the method of MORTIMER and HAWTHORNE (1969) as adapted to plates (FOGEL *et al.* 1979). When large ascosporal colonies were required, 12 asci were dissected on a single plate in a 8 mm by 8 mm grid. Complementation tests for *MAT*, *HIS4* and *ARG4* alleles were performed by spraying replicas with tester strains on YEPD and selecting diploids by replica-plating to the appropriate omission media. Allelism tests for *THR1* alles were accomplished by replica-plating diploids (obtained from crosses with tester strains as described above) to KAc media to induce meiotic recombination and then to SC-thr to select prototrophic recombinants. Tester strains for all of the allele tests were BR1493-4Ba, BR1493-1Dα, BR1454-13Dα, and BR1454-19Ba. Matings between homothallic strains were accomplished by mixing spores and selecting for complementation at *HIS4*.

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TABLE 1

Yeast strains

BR700	*HO arg4-8 cyh2 ade2-1* *HO arg4-9 CYH2 ade2-1*
BR1027-27C	*MAT***a** *ade1 tyr1 trp1-1 ura3-1 leu1 thr1 arg4-9 his6 ura4 lys7 lys9 met2 arg1 tyr7*
BR1197	*HO DIS1-1 his4-280 arg4-8 thr1-1 met13 trp1-1 ade2-1* *HO DIS1-1 his4-290 arg4-9 THR1 MET13 trp1-1 ade2-1*
BR1389	*DIS1-1 his4-280 arg4-9 thr1-4 trp1-1 ura3-1 ade2-1* *DIS1-1 his4-290 arg4-8 thr1-1 trp1-1 URA3 ade2-1*
BR1454-19B	*MAT***a** *his4-280 arg4-8 thr1-1 ade8*
BR1454-13D	*MAT*α *his4-280 arg4-8 thr1-1 ade8*
BR1479	*leu2-27 his4-280 arg4-8 thr1-1 trp1-1 ura3-1 ade2-1* *leu2-27 his4-290 arg4-9 THR1 trp1-1 URA3 ade2-1*
BR1490	*DIS1-2 his4-280 arg4-8 thr1-1 cyh10-100 met13 trp1-1 ura3-1 ade2-1* *DIS1-2 his4-290 arg4-9 THR1 CYH10 met13 TRP1 URA3 ade2-1*
BR1493-4B	*MAT***a** *ade1 his4-290 arg4-9 thr1-4*
BR1493-1D	*MAT*α *his4-290 arg4-9 thr1-4 ade1*
BR1499	*his4-280 arg4-8 cyh2 ade2-1* *his4-290 arg4-8 CYH2 ade2-1*
BR1500	*DIS1-2 his4-280 arg4-8 thr1-1 trp1-1 ura3-1 ade2-1* *DIS1-2 his4-290 arg4-9 THR1 trp1-1 ura3-1 ade2-1*
BR1502	*DIS1-2 his4-280 arg4-8 thr1-1 met13 ade2-1* *DIS1-2 his4-290 arg4-9 THR1 met13 ade2-1*
BR1504	*DIS1-1 leu2-27 his4-280 arg4-8 thr1-1 ura3-1 ade2-1* *DIS1-1 LEU2 his4-290 arg4-9 THR1 URA3 ade2-1*
BR1551	*HO DIS1-1 leu2-27 his4-280 arg4-8 ade2-1* *HO dis1+ LEU2 his4-290 arg4-8 ade2-1*
BR1553	*HO DIS1-1 his4-280 ura3-1 arg4-8 ade2-1* *HO DIS1-2 his4-290 URA3 arg4-8 ade2-1*
BR1559	*DIS1-1 his4-280 arg4-9 thr1-4 trp1-1 ura3-1 ade2-1* *DIS1-1 his4-290 arg4-8 thr1-1 trp1-1 URA3 ade2-1*
BR1570	*DIS1-2 leu2-27 HIS4 arg4-8 thr1-1 trp1-1 ura3-1 ade2-1* *DIS1-2 LEU2 his4-280 arg4-9 thr1-4 TRP1 URA3 ade2-1*
BR1643	*HO DIS1-2 leu2-27 his4-280 arg4-8 thr1-4 trp1-1 ade2-1* *HO dis1$^+$ LEU2 his4-290 arg4-8 THR1 TRP1 ade2-1*
BR1646	*HO DIS1-2 leu2-27 HIS4 arg4-8 thr1-4 ade2-1* *HO dis1$^+$ LEU2 his4-280 arg4-8 THR1 ade2-1*
BR1658	*DIS1-1 arg4-8 met13 trp1-1 ura3-1 ade2-1* *dis1$^+$ arg4-8 MET13 TRP1 URA3 ade2-1*
BR1660	*DIS1-1 his4-280 arg4-8 thr1-1 cyh3 trp1-1 uura3-1 ade6 ade2-1* *dis1 his4-290 arg4-8 THR1 CYH3 TRP1 URA3 ADE6 ade2-1*
BR1669	*his4-280 arg4-8 thr1-1 cyh10-100 met13 ura3-1 ade2-1* *his4-290 arg4-8 THR1 CYH10 MET13 URA3 ade2-1*
BR1757	*his4-280 arg4-8 trp1-1 ade6 ade2-1* *his4-290 arg4-8 TRP1 ADE6 ade2-1* *arg4-8*
BR1794	*DIS1-1 his4-280 arg4-9 thr1-4 trp1-1 ura3-1 ade2-1* *DIS1-1 his4-290 arg4-8 thr1-1 trp1-1 URA3 ade2-1*

All strains are heterothallic (*ho/ho*), *MAT***a**/*MAT*α, *dis1*$^+$/*dis1*$^+$ and *CUP1/CUP1* unless otherwise indicated.

Resistance to copper is dependent upon the gene copy number at the *CUP1* locus (FOGEL and WELCH 1982) which is located on chromosome *VIII*, 28 cM distal to *THR1*. Strains used in the present study carry only a single copy of the *CUP1* gene (J. WELCH, unpublished results) and have been derived from BR622. BR622 is an extremely copper-sensitive strain which was identified by screening laboratory strains. The copper sensitivity of BR622 reflects at least two other genes, in addition to *CUP1*, that moderate copper sensitivity compared to the wild-type, copper-resistant strain, X2180. Vegetative cells of BR622 and its derivatives grow confluently on SC + cup (0.015 mM) medium and fail to grow on SC + cup (0.02 mM) medium. At higher copper ion concentration and after several days of incubation, copper-resistant outgrowths appear on a background of inhibited cells. These stable outgrowths tolerate 0.02 mM copper but are sensitive to concentrations of 0.04 mM. Since the *CUP1* locus contains only a single copy of the *CUP1* gene, the enhanced copper resistance is not due to gene amplification or unequal sister exchange. The copper-resistant outgrowths from these strains contain extra copies of chromosome *VIII* and are generated by mitotic nondisjunction events.

Identification of chromosome *VIII* disomes: To test both mitotically and meiotically derived copper-resistant outgrowths for disomy, the segregation of chromosome *VIII* markers was followed. Presumptive chromosome *VIII* disomes were crossed to copper-sensitive tester strains marked on chromosome *VIII* with *arg4* and *thr1*. If the strain tested is disomic for chromosome *VIII*, then the auxotrophic markers exhibit typical trisomic segregation ratios ($4^+:0^-$, $3^+:1^-$ and $2^+:2^-$) while only $2^+:2^-$ segregations for copper resistance:copper sensitivity are observed. However, if the strain is not a disome, then all chromosome *VIII* markers segregate $2^+:2^-$.

To select for hyperploidy among meiotic cells, patches of diploids were replica-plated to sporulation media (KAc); 3 days later the KAc plates were replica plated to SC + cup (0.02 mM). More than 100 copper-resistant outgrowths obtained by this procedure were tested for disomy for chromosome *VIII* by segregation (as above) and found to be disomic. Thus, the ability to select disomic spores permits a search for mutants that generate increased numbers of meiotic aneuploids.

Isolation of mutants: BR700 was grown in liquid YEPAD to early stationary phase, centrifuged, resuspended in 1% KAc, and shaken vigorously for three days to induce sporulation. Unless otherwise stated, all incubations were at 30°. The sporulated culture was treated with 2.5% ethylmethane sulfonate (EMS) (GOLIN and ESPOSITO 1977) for 70 min. Viability was reduced to 30%. The mutagenized culture was plated on SC + cyh medium to minimize the contribution of unsporulated diploid cells. The plated spores diploidized via *HO*-mediated mating type switching and subsequent mating. Diploid colonies were patched to GNA plates and then replica-plated to KAc medium. Six of 2597 colonies tested were threonine auxotrophs, indicating effective mutagenesis. After four days, the KAc plates were replica-plated to SC + cup (0.02 mM) and these replicas were screened, 3 days later, for copper-resistant outgrowths.

Quantitation of mitotic and meiotic aneuploidy: Chromosome *VIII* disomic cells plated at low densities on SC + cup (0.02 mM) fail to form large colonies so are indistinguishable from sensitive cells. However, disomic cells in a background of copper-sensitive cells can be distinguished by replica-plating to copper media, indicating that a relatively high density of cells permits the detection of single copper-resistant cells. Thus, to measure rates of chromosome gain, two methods involving the scoring of copper-resistant papillae were used.

Method 1. This method was used for strains that generate disomes at a relatively high frequency ($>10^{-4}$). Strains were patched onto GNA medium in 7.5 mm × 10 mm rectangles. The GNA plates were incubated overnight and replica-plated to one SC + cup plate (to assay any mitotic contribution to nondisjunction) and to two KAc plates. After three days, sporulation was scored and the KAc plates were replica-plated to SC + cup (0.02 mM) media. To estimate the number of asci transferred from the patches to the SC + cup (0.02 mM) plates, the agar of one rectangle from each plate was transferred to a test tube containing 5 ml of sterile 1 M sorbitol and then vortexed to suspend the cells. Dilutions were plated on YEPD to determine the number of colony-forming units. The second set of SC + cup plates was incubated at 30° for 3 days to allow the development of copper-resistant papillae. The number of copper-resistant papillae per patch divided by the estimated number of cells transferred per patch and multiplied by the percent sporulation provided an estimate of the rate of meiotic nondisjunction.

Method 2. To estimate the nondisjunction rates for strains that produce relatively few outgrowths ($<10^{-4}$), an adaptation of the method of LURIA and DELBRUCK (1943) (as adapted to yeast by MALONEY and FOGEL 1980) was used. Single colonies 3 mm in diameter were suspended in YEPAD. Once the cultures reached stationary phase, each was diluted and 100 to 200 cells were plated on each of six or more GNA plates. After 4 days of incubation, the number of colonies was recorded for each plate, and each plate was replica-plated to a KAc plate to induce sporulation. Three days later, the KAc plates were replica-plated to SC + cup (0.02 mM). The cells from one SC + cup (0.02 mM) plate (per strain) were washed off as before and the percent sporulation and cell number were estimated visually. The remaining five SC + cup (0.02 mM) replicas were incubated for four days, then the number of colonies without copper-resistance papillae was counted. The fraction of colonies with papillae [the P_0 term of the Poisson distribution (RYAN 1963)] and the number of sporulated cells per colony can be used to calculate the meiotic nondisjunction rate. Method 2 was also used to determine the mitotic nondisjunction rates except that the colonies were not replicated to sporulation medium.

RESULTS

Identification of mutants that exhibit high rates of meiotic nondisjunction. A screen was developed to identify mutants that generate aneuploid spores at high frequencies. The starting strain was homothallic (*HO*) to permit the recovery of recessive mutations. Homothallic haploids (spores) switch mating type within the first few cell divisions (STRATHERN and HERSKOWITZ 1979) enabling the cells to mate and form a diploid colony homozygous for any induced mutation. A sporulated culture of a homothallic diploid strain, BR700 (*HO/HO arg4-8/arg4-8 cyh2^R/CYH2 ade2-1/ade2-1*) was treated with EMS (see MATERIALS AND METHODS) and plated on cycloheximide-containing medium (SC + cyh). Unsporulated diploids are sensitive to cycloheximide because cycloheximide resistance is recessive. Thus, the selection

for resistance recovers mostly meiotic products carrying the *cyh2*^{R} allele. These diploid colonies were sporulated and screened for the production of chromosome *VIII* disomic spores.

Chromosome *VIII* disomes were recognized by replica-plating the sporulated colonies to copper-containing media, SC + cup (0.02 mM), and scoring copper-resistant outgrowths. The strains used in this study all have a single copy of the *CUP1* gene at the *CUP1* locus on chromosome *VIII* (J. Welsh, unpublished results). *CUP1* displays a gene dosage effect for the level of copper resistance; thus, strains disomic for chromosome *VIII* are resistant to copper (see Materials and Methods). Mutants that produce aneuploid spores at an increased rate should also produce copper-resistant outgrowths at a high frequency after sporulation.

Of 5625 respiratory-competent isolates that survived EMS mutagenesis, 159 colonies exhibiting elevated frequencies of copper-resistant outgrowths were isolated and retested. Nearly half of these colonies either failed to sporulate (35 of 149) or were copper-resistant prior to sporulation (42 of 149). Of the remaining 72 isolates, ten colonies displayed a reproducible phenotype when colony-purified isolates were retested. Two of these were chosen for further study because they exhibited Mendelian segregation. The mutants were designated *DIS1-1* and *DIS1-2*.

Segregation and dominance: The two original mutant isolates of *DIS1-1* and *DIS1-2* were sporulated and tetrads were dissected and analyzed. The ascosporal colonies were scored directly for their ability to generate aneuploid spores, since they gave rise to homothallic diploid colonies capable of sporulation. As expected, the *DIS1-2* isolate displayed high frequencies of copper-resistant outgrowths in all spore colonies examined. *DIS1-1* segregated 2:2 for the meiotic phenotype indicating that it was heterozygous for the induced mutation; the *DIS1-1* mutation must therefore be dominant. A spore colony from the original *DIS1-1* isolate that expressed the mutant phenotype was used for further analyses.

Segregation tests were performed on *DIS1* heterozygotes constructed by crossing the mutants to wild-type strains. Heterozygous diploids, both *DIS1-1*/+ and *DIS1-2*/+, exhibited elevated levels of copper-resistant outgrowths on patch tests from sporulated cells, indicating that both mutants are at least partially dominant. More than 50 unselected tetrads from *HO*/*HO* strains heterozygous for *DIS1-1* (BR1551) or *DIS1-2* (BR1646) exhibited $2^{+}:2^{-}$ segregation for elevated meiotic frequencies of copper-resistant papillae. Cross BR1551 also exhibited three $3^{+}:1^{-}$ tetrads, either due to gene conversions or difficulty in scoring. Figure 1 shows a typical segregation of the *DIS1* phenotype from a heterozygous diploid.

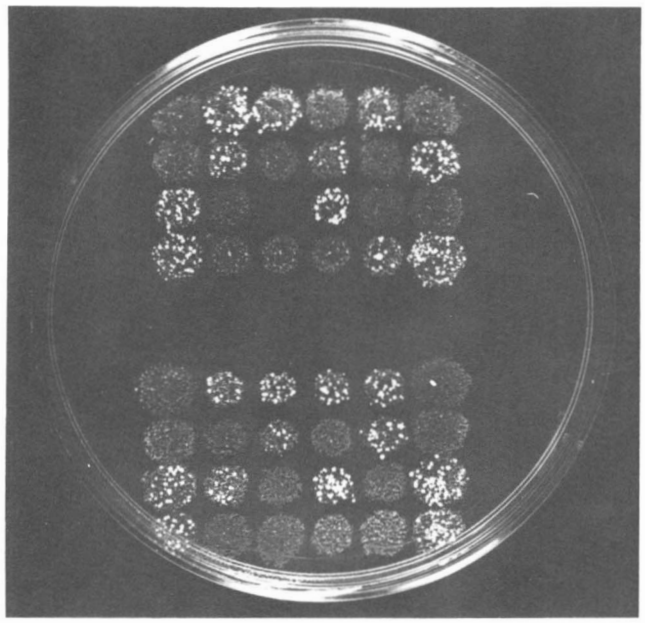

Figure 1.—Segregation of *DIS1-2* in tetrads. Dissected tetrads from BR1646, *DIS1-2*/*dis1*$^{+}$ *HO*/*HO*, were scored for the meiotic production of chromosome *VIII* disomes. *HO* spores were directly replica plated to sporulation medium and then to SC + cup (0.02 mM) after 3 days. The high frequency of copper-resistant papillae was used to follow the segregation of the *DIS1-2* mutant.

Both *DIS1-1* and *DIS1-2* mutants have have extensively back-crossed to wild-type copper-sensitive strains, and all crosses described below display wild-type spore viability (>90%).

***DIS1-1* and *DIS1-2* are allelic:** To determine whether *DIS1-1* and *DIS1-2* represent mutations at a single locus or two distinct loci, a hybrid between them (BR1553) was constructed and analyzed. Since *DIS1-1* and *DIS1-2* display dominant phenotypes, a segregational test for allelism was performed. All spores from 81 tetrads with four surviving spores displayed a mutant phenotype. These results suggest that *DIS1-1* and *DIS1-2* are either alleles of the same locus or represent two linked genes of similar function located less than 2 cM apart.

It has been difficult to accumulate genetic map information for *DIS1* because both *DIS1* alleles are dominant and the phenotype must be scored in diploids. In addition, scoring depends on the proper copper-sensitive background, not found in most available mapping strains. The *DIS1* gene is not located on chromosome *VIII* and it is not centromere-linked.

Copper-resistant papillae from *DIS1* strains are disomic: Sporulated cultures of the two *DIS1* mutants exhibit a higher frequency of outgrowths on SC + cup (0.02 mM) than does the parental strain. To demonstrate that the copper-resistant meiotic products reflect the accumulation of an additional chromosome *VIII*, copper-resistant meiotic products were crossed to the tester strain BR1027-27C**a** (*leu1*, *ura3*) and the resulting diploids were sporulated, dissected

TABLE 2

Segregation of copper resistance among tetrads from copper-resistant outgrowths mated to a copper-sensitive tester

Strain	Parental genotype	Cu^R tested[a]	Ditype tetrads[b]	First division tetrads[c]	Second division tetrads[d]	% FDS[e]
BR1499	$\frac{dis1^+}{dis1^+}$	24	165	157	8	95.1
BR1504	$\frac{DIS1\text{-}1}{DIS1\text{-}1}$	41	308	296	12	96.1
BR1502	$\frac{DIS1\text{-}2}{DIS1\text{-}2}$	47	351	342	9	97.4

[a] Independent copper-resistant outgrowths were isolated from sporulated cultures.

[b] Each ascosporal outgrowth was crossed to a haploid copper-sensitive strain bearing the centromere markers, *leu1* and *ura3* (BR1027-27Ca). Ten tetrads were dissected from each hybrid. Four-spored viable tetrads which exhibited a ditype segregation for both *leu1* and *ura3* and segregated 2:2 for copper resistance:copper sensitivity are listed.

[c] First division tetrads are the number of tetrads where copper resistance segregated PD or NPD relative to the centromere markers.

[d] Second division tetrads are tetrads where copper-resistance failed to segregate PD (parental) or NPD (nonparental) relative to the centromere markers.

[e] % FDS is the percentage of first division tetrads.

and analyzed for the segregation of copper resistance. If the copper-resistant outgrowths are disomic, then the tetrads from the trisomic diploids should segregate 2 copper-resistant: 2 copper-sensitive spores, and this segregation should occur at the first meiotic division. The centromere-linked markers *LEU1* (3.1 cM from *CENIII*) and *URA3* (8 cM from *CENV*) were heterozygous in all diploids and their segregations were used to identify the first division. In these crosses, sister spores can be distinguished from greater than 99% confidence

$$\left[\frac{0.5(0.06 \times 0.16)}{(0.94 \times 0.84) + [0.5(0.06 \times 0.16)]}\right].$$

Colonies from each of three strains BR1499 (*dis1*$^+$/*dis1*$^+$), BR1502 (*DIS1-1/DIS1-1*) and BR1504 (*DIS1-2/DIS1-2*) were sporulated, and subsequently replica-plated to SC + cup (0.02 mM). To insure that the copper-resistant outgrowths arose independently, a single copper-resistant outgrowth was picked from each sporulated colony and colony purified. A total of 113 copper-resistant colonies (24 from wild type, 41 from *DIS1-1* and 48 from *DIS1-2* meioses) were crossed to the tester strain. The resulting diploids were sporulated and ten tetrads dissected from each (Table 2). Of 113 crosses, 112 segregated predominantly $2^+:2^-$ for copper-tolerance on SC + cup (0.02 mM); these presumably represent chromosome *VIII* disomes. The one exceptional cross was from the *DIS1-2* strain and segregated as follows: six $1^+:3^-$ tetrads, three $0^+:4^-$ tetrads and one $2^+:2^-$ tetrad. A second gene affecting the expression of copper resistance was probably segregating in this diploid. The spores from all crosses were sensitive on SC + cup (0.04), *i.e.*, the copper-resistance in the resistant spore clones is equivalent to the resistance level of a strain disomic for chromosome *VIII*.

In the tetrads where copper-resistance segregated $2^+:2^-$, the segregation pattern was compared to markers segregating at the first meiotic division. The copper-resistant phenotype segregated predominantly (about 95%) at the first meiotic division. Since the tetrad data was homogeneous (with the removal of the one aberrant *DIS1-2* cross), the values in Table 2 represent pooled sums for each original diploid. The frequency of first division segregation was 95.1%, 96.1% and 97.4% for *dis1*$^+$/*dis1*$^+$, *DIS1-1/DIS1-1* and *DIS1-2/DIS1-2*, respectively. Thus, the copper-resistant phenotype displayed close centromere linkage. The apparent centromere linkage of copper-resistance can be explained by the meiotic segregation of three homologous chromosomes, each carrying a copy of the *CUP1* gene. Reductional segregation of one pair and an independent segregation of the extra homolog at anaphase I followed by equational division at anaphase II results in disomic sister spores. The occasional tetratypes (second division segregants) may reflect a precocious sister centromere separation at the first meiotic anaphase or a misdiagnosis of the first division.

To provide additional evidence that the copper-resistant spores were disomic for chromosome *VIII*, 15 representative copper-resistant spores colonies from *DIS1-1* and *DIS1-2* were crossed to a haploid strain carrying *thr1* on chromosome *VIII*. Five tetrads were dissected from each diploid and all exhibited trisomic segregations for threonine auxotrophy. Taken together with the apparent centromere linkage of copper resistance, and the uniform level of resistance from all copper-resistant isolates, this result strongly suggests that the elevated levels of copper-resistant outgrowths generated by *DIS1* mutants are due to disomy for chromosome *VIII*.

Rates of both meiotic and mitotic nondisjunction are elevated in *DIS1* mutants: The meiotic nondisjunction rates in mutant and wild-type strains were estimated by determining the frequencies of copper-resistant papillae produced among asci (described in MATERIALS AND METHODS) (Table 3). The meiotic nondisjunction rates calculated for wild type (BR1479) is approximately 5 disomes per 10^6 asci. The homozygous mutant strains, BR1389 (*DIS1-1/DIS1-1*) and BR1490 (*DIS1-2/DIS1-2*), displayed rates of 4–6 disomes per 10^4 asci. Thus, the mutant rates are increased about 100-fold above wild type.

To quantitate the dominance of the mutant alleles, the phenotypes of heterozygous and homozygous mutants were compared. The estimated nondisjunc-

TABLE 3

Rates of chromosome *VIII* nondisjunction

Strains	Genotype	Mitotic rate[a] $Cu^R/10^{-9}$ cells/division	Meiotic rate $Cu^R/10^{-6}$ meioses
BR1479	$\frac{dis1^+}{dis1^+}$	7[b]	5
BR1660	$\frac{DIS1\text{-}1}{dis1^+}$	ND	180
BR1389	$\frac{DIS1\text{-}1}{DIS1\text{-}1}$	65	430
BR1643	$\frac{DIS1\text{-}2}{dis1^+}$	ND	140
BR1490	$\frac{DIS1\text{-}2}{DIS1\text{-}2}$	380	580

[a] Meiotic rates for BR1479 and all mitotic rates were determined by method II (described in MATERIALS AND METHODS). Other meiotic rates were determined by method I.

[b] Standard deviations were large and the numbers shown are meant to represent relative estimates (the relative rates remained fairly constant within repeated experiments although the actual numbers varied).

tion rate of the *DIS1-1* heterozygote, BR1658, is similar to the homozygote, *DIS1-1/DIS1-1*, BR1389. The *DIS1-2* heterozygote, BR1643, displays an intermediate nondisjunction rate (about sevenfold down), when compared to the *DIS1-2* homozygote, BR1502.

Since meiotic mutants often express a detectable phenotype during vegetative growth (SIMCHEN 1974; BAKER, CARPENTER and RIPOLL 1978), mitotic rates of chromosome *VIII* nondisjunction were determined. The rates estimated for homozygous *DIS1-1* and *DIS1-2* strains are increased 10-fold and 50-fold, respectively, relative to the wild-type control. This result indicates that the disjunction component perturbed by the *DIS1* mutation is involved in both mitotic and meiotic chromosome segregation.

Disomic spores have undergone normal levels of recombination: A possible explanation for the nondisjunction observed in *DIS1* mutants is that these mutants are defective in meiotic recombination. We have not seen any gross alterations in meiotic intragenic recombination or intergenic recombination in five intervals examined in the *DIS1* mutants (data not shown) (ROCKMILL 1983). Since the nondisjunction phenotype in *DIS1* mutants appears to affect only a small fraction of meioses, a recombination defect causing nondisjunction might be evident only in the products of those particular meiotic events. We have, therefore, estimated recombination frequencies from those meioses in which *DIS1*-mediated nondisjunction is evident. This was accomplished by selecting copper-resistant (disomic) meiotic products and scoring crossovers between two markers, *THR1* and *ARG4*, on the disomic chromosome. *DIS1-1* and *DIS1-2* homozygous diploids that were heteroallelic

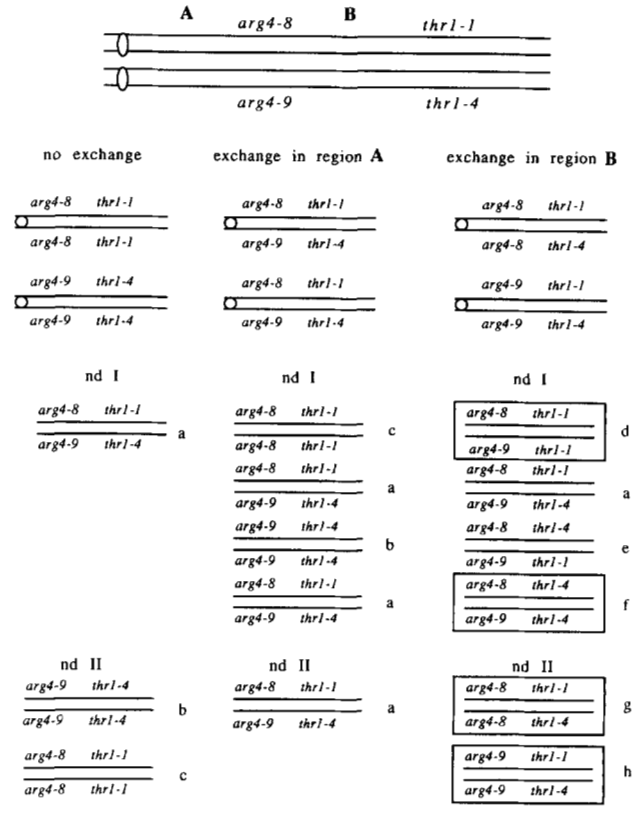

FIGURE 2.—Genotypes of disomic meiotic products. Predicted genotypes of disomic spores from meiotic cells experiencing no genetic exchanges, or one exchange in either interval A or B, are diagrammed. The disomes arise by nondisjunction during meiosis I (nd I) or meiosis II (nd II). Genotypes d, f, g and h are distinguishible genotypes derived from meioses in which an exchange occurred in interval B. Map distance is calculated as

$$\frac{2(d + f) + g + h}{\text{total}} \times 50\%.$$

This formula, A,B double exchanges, although it overestimates the map contribution of these E_2's from for meiosis I nondisjunctants by one fourth. Half the B,B double exchanges will go undetected, but double exchanges in this interval should occur infrequently (less than $0.26^2 = 0.068$).

at both *ARG4* and *THR1* were constructed; disomic spores of all possible genotypes resulting from these crosses can be distinguished. The *ARG4* phenotypes can be distinguished by the temperatures at which the various disomic strains can grow on SC-arg. Homoallelic *arg4-9* disomes are auxotrophic on SC-arg at all temperatures. The *arg4-8* allele is temperature sensitive; homoallelic *arg4-8* disomes are capable of growth on SC-arg at temperatures up to 33°, but not at higher temperatures. (Since a haploid *arg4-8* strain fails to grow on SC-arg above 30°, the *arg4-8/arg4-8* disome exhibits a gene dosage effect.) The heteroallelic disome displays allelic complementation, and grows on SC-arg at temperatures up to 35°. The *THR1* heteroalleles were distinguished by allelism tests with two sets of testers (described in MATERIALS AND METHODS).

TABLE 4

Allelic constitution at *ARG4* and *THR1* among disomic spores,[a] as percent of total

Strains	Parental genotype	a+e[b] 8 1 / 9 4	b 9 4 / 9 4	c 8 1 / 8 1	d 8 1 / 9 1	f 8 4 / 9 4	g 8 1 / 8 4	h 9 1 / 9 4	i[c] 9 1 / 9 1
BR1794 / BR1559	*DIS1-1* / *DIS1-1*	75.2	0	1.8	4.0	8.4	7.0	2.6	0.4
BR1570	*DIS1-2* / *DIS1-2*	60.2	10.1	9.6	4.5	2.6	6.1	5.3	1.1

[a] Copper-resistant spores derived from mutant diploids were scored for *ARG4* alleles as described in MATERIALS AND METHODS. *arg4-8* and *arg4-9* alleles are represented by "8" and "9," respectively; *thr1-1* and *thr1-4* alleles are represented by "1" and "4," respectively.

[b] The major class, a+e, represents both nonrecombinants and double crossovers. Classes a–h are labeled as in Figure 3.

[c] Classes i and j represent double crossovers in the *ARG4-THR1* interval with or without a third crossover between *CENVIII* and *ARG4*.

The consequences of exchange in the *CENVIII-ARG4* (A) and *ARG4-THR1* (B) intervals are diagrammed in Figure 2. Among the meioses that have a single exchange in region B, those that undergo nondisjunction at the first division will form four types of products; half of these products (*d* and *f*) have diagnostic phenotypes. Exchange in region B followed by nondisjunction at the second division generates phenotypes *g* and *h*. We can therefore estimate the map distance between *ARG4* and *THR1* by using the formula $50\% \times [2(d + f) + g + h)]/$ total disomes.

Single copper-resistant outgrowths were taken from individual sporulated isolates. Disomy was confirmed by restreaking each isolate on medium containing 0.02 mM copper. The 113 analyzed disomes for *DIS1-1* and 375 from *DIS1-2* were grouped into the nine possible phenotypes with respect to *arg4* and *thr1* alleles (Table 4). The calculated map distance for the *ARG4-THR1* intervals is 17 cM and 13 cM in the *DIS1-1* and *DIS1-2* mutants respectively, as compared to 13 cM for wild type (MORTIMER and SCHILD 1980). This result suggests that the production of disomes by *DIS1* mutants is not the consequence of a lack of recombination.

***DIS1* mutants generate chromosome *VIII* disomes predominantly at the first meiotic division:** Aneuploid spores may result from nondisjunction at the first or second meiotic division. To determine whether the effect of the *DIS1* mutants is specific for meiosis I or meiosis II, the fraction of aneuploids generated at each of the two meiotic divisions was determined among a population of meiotically-generated disomes. The diploids constructed for this study were heteroallelic at the centromere-linked chromosome *VIII* gene, *ARG4*. Disomic spores selected from these strains may be homoallelic (*arg4-8/arg4-8* or *arg4-9/arg4-9*) or heteroallelic (*arg4-8/arg4-9*) and these are distinguishable (see above).

The possible chromatid segregations from heteroallelic diploids experiencing an aberrant first or second meiotic division are displayed in the first two columns of Figure 2. The proportion of heteroallelic disomes is affected by the proportion of nondisjunction at the second division and by the proportion of exchange between *ARG4* and *CENVIII*. Let x equal the frequency of exchange (then $1 - x$ is the frequency of E_0 tetrads) and y equal the relative frequency of nondisjunction at the second division (then $1 - y$ is the relative frequency of nondisjunction at the first division). If the probabilities of exchange and division of nondisjunction are independent, then $(b + c)$, the proportion of homoallelic disomes observed (see Figure 2), is $y(1 - x) + 0.5x(1 - y)$, which simplifies to $(b + c) = y + 0.5x(1 - 3y)$.

Since there is but one independent observation but two unknowns, this equation does not have a unique solution; however, it does permit calculations of limits and of solutions for one unknown if a value for the other is assumed. For example, it is reasonable to assume that the frequency of exchange in the *CENVIII-ARG4* interval is not affected by these *DIS* mutants, since it has just been shown that the frequency of exchange in a adjacent interval is not affected. In these and related strains, *ARG4* is 15.5 cM from its centromere (*i.e.*, 31% exchange). Substituting 0.31 for x yields

$$y = \frac{[(b + c) - 0.155]}{0.535},$$

which can then by used to solve for $1 - y$, the proportion of disomes that are due to nondisjunction at the first meiotic division. It should be noted that an aberrant first meiotic division can occur by both bivalents segregating to the same pole or by one bivalent plus a single chromatid segregating to one pole. Both possibilities predict the same proportion of heteroallelic to homoallelic disomes.

Copper-resistant disomes derived from individual sporulated diploid patches were scored for their arginine phenotype. The results are presented in Table 5. Most of the disomes recovered from all *DIS1* mutants were heteroallelic for the *ARG4* alleles. Calculations using the relationship given above indicate

TABLE 5

Fraction of disomes derived from the first meiotic division

Strain	Genotype	Disomes tested[a]	Heteroallelic (*arg4-8/arg4-9*) disomes observed	Calculated frequency derived from first division[b]
BR1794[c]	$\frac{DIS1\text{-}1}{DIS1\text{-}1}$	232	200 (86%)	1.0
BR1550	$\frac{DIS1\text{-}1}{dis1^+}$	234	157 (67%)	0.7
BR1570[c]	$\frac{DIS1\text{-}2}{DIS1\text{-}2}$	375	253 (67%)	0.7
BR1549	$\frac{DIS1\text{-}2}{dis1^+}$	58	39 (67%)	0.7

[a] Meiotically derived copper-resistant outgrowths were examined from sporulated mutant diploids containing heteroalleles *arg4-8* and *arg4-9*. Each disomic outgrowth was scored for its arginine phenotype and mating ability (to ensure haploidy).

[b] The fraction of disomes formed at the first meiotic division was calculated from the Equation 1, see text.

[c] Data included from Table 3.

that all of the disomes from the *DIS1-1/DIS1-1* crosses and 70% of the disomes from the *DIS1-1/dis1*$^+$, *DIS1-2/DIS1-2* and *DIS1-2/dis1*$^+$ crosses arose from nondisjunction at the first meiotic division.

Since all the nondisjunction appears to occur at the first meiotic division in *DIS1-1*, the recombination data in Table 4 can be interpreted further. Most of the disomes homoallelic for *ARG4* and therefore presumably recombinant between *CENVIII* and *ARG4* (classes b, c, f, g, i and j), are also recombinant in the *ARG4-THR1* interval (classes f, g, i and j). This may be the result of high negative interference in which exchange in one interval increases the chance of exchange in another interval or that multiple-exchange tetrads are more sensitive to disruptions in proper disjunction (MERRIAM and FROST 1964). Some of these disomes may have been the result of meiosis II nondisjunction suggesting that *DIS1-1* does nondisjoin at meiosis II to some extent. Alternatively, post-meiotic nondisjunction may account for some of these aberrant events although most of the nondisjunction occuring in the *DIS1-1* mutant is meiotic, since recovered disomes have predominantly heteroallelic markers.

A single chromosome *VIII* disome is generated per aberrant meiosis in *DIS1-1* heterozygotes: Disomic spores derived from an aberrant first meiotic division may be generated in two ways. If a bivalent fails to disjoin and both homologs move to the same pole at the first division, the resulting ascus will consist of two disomic sister spores and two dead spores. If a single homolog segregates equationally at anaphase I, the resultant tetrad will contain one disomic spore, one dead spore and two haploid spores. These two types of nondisjunction events are distinguishable since the resulting tetrads contain either one or two disomic spores. The type of nondisjunction event may be inferred by determining the number of viable disomic spores that arise in a given ascus. Each copper-resistant outgrowth, originating from asci plated onto SC + cup (0.02 mM) medium, represents a single aberrant meiosis. Tetrads containing two spores disomic for chromosome *VIII* should form a single copper-resistant outgrowth on SC + cup (0.02 mM) due to the proximity of the spores, and these outgrowths should consist of cells from two genetically distinct sister spores.

To readily identify asci containing two disomic spores, a red/white indicator system was used to signal heterogeneous colonies. BR1660 is *ade2/ade2, ade6/ADE6* generating ascosporal colonies which are 50% white (*ade2 ade6*) and 50% red (*ade2 ADE6*). The *ade2 ADE6* copper-resistant outgrowths do develop red pigment on the SC + cup (0.02 mM) medium, so red/white sectored colonies can be recognized when they occur. Since *ade6* is 29 cM from *CENVII*, 58% of the asci containing two disomic sister spores should form red/white sectored colonies. This scheme was tested by constructing a diploid trisomic for chromosome *VIII* (BR1757), heterozygous for *ade6* and the centromere marker *trp1* and homozygous for *ade2*. All the tetrads from this diploid should produce two disomic sister spores. To simulate a *DIS1* diploid, BR1757 was mixed with a wild-type diploid, BR1373 (since copper-resistant cells require a high cell density to form papillae, see MATERIALS AND METHODS), and the mixture was sporulated on solid medium and replica-plated to SC + cup (0.02 mM). Approximately 30% of the copper-resistant outgrowths were sectored. Since only half of the expected sectored colonies were observed, this suggests that the ability of a disomic spore to form a copper-resistant outgrowth is about 0.7. Both sides of 25 sectored copper-resistant outgrowths were colony purified and tested for their Trp phenotype. Of these sectored colonies, 22 carried the same *TRP1* allele in both sectors, indicating that they were probably sister spores. Thus, this test allows the detection of sectored colonies arising from a single tetrad.

BR1660 (*DIS1-1/+*) is heterozygous for two centromere markers, *CYH3* (10 cM from *CENVII*) and *TRP1* (0.5 cM from *CENIV*), heterozygous for *ade6* and homozygous for *ade2*. This diploid was sporulated and replica-plated as before. Of approximately 3400 papillae, only nine sectored colonies were seen and these were isolated and purified. The colonies were scored for tryptophan auxotrophy and cycloheximide resistance. Only one contained two cell types that may have arisen from sister spores (*i.e.*, both half sectors carried the same centromere marker configuration for *CYH3* and *TRP1*). On the unlikely assumption that these spores arose from the same

ascus, the maximum frequency of asci with two sister disomic spores is 6 per 10^4 disome-containing asci. Thus, the production of two disomic spores per ascus is at most a rare outcome of *DIS1*-mediated nondisjunction. Apparently, the disomes generated in *DIS1* mutants result from events generating a single hyperploid spore per ascus.

***DIS1*-generated disomes are often disomic for more than one chromosome:** Nondisjunction of chromosome *VIII* in the *DIS1* mutants was detected by the presence of copper-resistant spores; disomy of other chromosomes was not assayed. Nondisjunction of multiple chromosomes in *DIS1* mutants would imply that the *DIS1* gene product is required for chromosome segregation in general and not for chromosome *VIII* disjunction specifically. If multiple nondisjunction events occur among a rare subpopulation of meiotic cells, a significant fraction of chromosome *VIII* disomes should simultaneously carry extra copies of other chromosomes.

To seach for such multiply disomic spores, spore colonies hyperploid for chromosome *VIII* were screened for the presence of additional copies of chromosome *III*. Chromosome *III* disomes are relatively stable (SHAFFER *et al.* 1971). In diploids heterozygous for *his4-280* and *his4-290*, chromosome *III* disomes may be identified by histidine prototrophy due to intragenic complementation. A wild-type strain (BR1499) and a *DIS1-1* homozygote (BR1504) were sporulated and 117 and 402 copper-resistant outgrowths, from wild type and *DIS1-1* respectively, were selected. These were tested for histidine prototrophy and then scored for mating type expression and sporulation capability (Table 6).

No histidine prototrophs were recovered from wild type and 19 histidine prototrophs were found among the disomes generated from the *DIS1-1* diploid. Of the 19, 7 were histidine prototrophs, nonmaters and sporulation-deficient and these were classified as chromosome *III* disomes, since they represent the expected phenotype of a spore colony disomic for chromosome *III*. Another 7 of the 19 were histidine-prototrophic, mating-competent strains. These were crossed to a haploid strain and tetrads were analyzed for the segregation of chromosome *III* markers. All seven sporulated hybrids exhibited trisomic segregations ($2^+:1^-$, $1^+:3^-$ and $0^+:4^-$ for His^+ and **2a**:1α:1**a**α or 1**a**:2α:1**a**α for *MAT*) and thus were classified as originating from trisomic parents. The remaining five histidine prototrophs were capable of sporulation. Four of these yielded trisomic segregation ratios for *HIS4* and *MAT* but failed to display the expected $2^+:2^-$ segregations of *URA3* and *CYH10*. These isolates may have arisen from doubly trisomic diploid spores or from a mating of a doubly disomic spore with another spore. One sporulation-proficient, His^+ strain segregated 2:2 for mating type and was therefore not disomic for chromosome *III*. Thus, 18 of 402 or 4.5% of selected chromosome *VIII* hyperploids were additionally hyperploid for chromosome *III*. This is a slight underestimate because half the disomes from meioses in which there was an exchange between *HIS4* and *CENIII* will be homozygous for a *HIS4* allele, and therefore not His^+, the phenotype diagnostic for disomy.

TABLE 6

Frequency of chromosome *III* disomes among copper-resistant outgrowths[a]

Strains disomes	Diploid genotype	Cu^R outgrowths % disomes	His prototrophs				
			His^+	Spo^- Mat^+	Spo^+ Mat^-	Spo^- Mat^-	III
BR1499	$\frac{dis1^+}{dis1^+}$	117	0	0	0	0	0
BR15044.5	$\frac{DIS1\text{-}1}{DIS1\text{-}1}$	402	19	7	5[b]	7	18

[a] Diploids (*his4-280/his4-290*) were sporulated and replicated to SC+cup (0.02). Independent copper-resistant outgrowths were isolated and scored for histidine prototrophy (intragenic complementation), sporulation ability, and mating competence.

[b] One isolate in this category was not disomic for chromosome *III*, see text.

DISCUSSION

***DIS1* mutants are disjunction-defective:** *DIS1* mutants were isolated by screening for mutants that produce increased frequencies of ascospores containing an extra copy of chromosome *VIII*. Chromosome *VIII* disomes were distinguished by their increased resistance to ionic copper in the medium, reflecting a gene dosage effect mediated by the copper-resistance locus, *CUP1*, on chromosome *VIII*. Although the mechanism for generating copper resistance in many strains involves tandem gene amplification at *CUP1* (FOGEL and WELCH 1982), tandem gene duplication events are not found among the copper-resistant isolates identified in this study. This is because the starting strain used for the mutagenesis carries a single copy *CUP1* gene, a sequence which does not undergo amplification (WELCH *et al.* 1983; J. WELCH, personal communication).

DIS1 mutant strains demonstrate enhanced production of aneuploids both meiotically and mitotically. The meiotic rates of nondisjunction of chromosome *VIII* for the *DIS1-1* and *DIS1-2* mutants are increased approximately 100-fold over wild type. Nondisjunction rates in heterozygotes show that the *DIS1-1* and *DIS1-2* mutations are semidominant for the generation of meiotically derived aneuploids. Relative to wild type, the mitotic nondisjunction rates for the *DIS1-1* and *DIS1-2* mutants are increased 10-fold and 50-fold, respectively. Increased mitotic non-

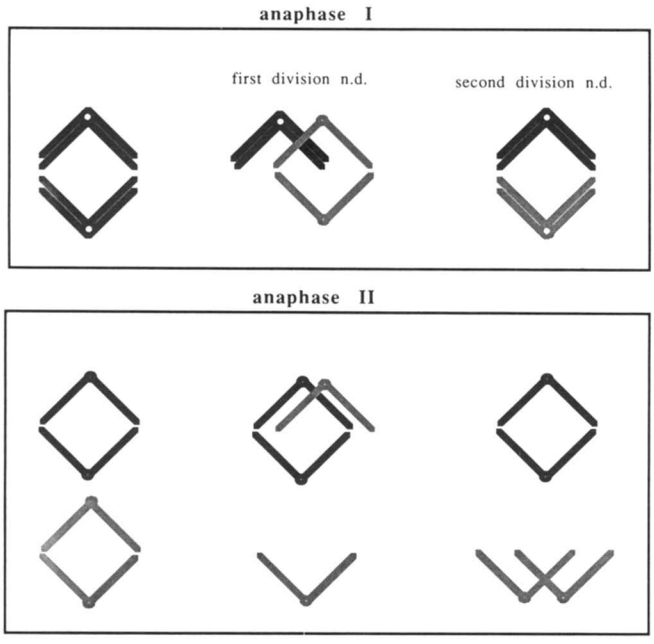

FIGURE 3.—Model for the meiotic chromosome segregation in *DIS1* mutants. The upper panel outlines the possible segregations of bivalents and separated chromatids during anaphase I of meiosis. The lower panel shows meiosis II chromosome segregation. The chromosomes on the left side of the figure represents normal disjunction of both meiotic divisions. The middle set of chromosomes depict nondisjunction at meiosis I where one pair of sister chromosomes undergoes an equational disjunction. The segregation of these chromosomes at meiosis II results in the generation of one disomic spore containing nonsister chromatids. The right set of chromosomes disjoin properly at meiosis I, but nondisjoin at meiosis II, resulting in one disomic spore containing sister chromatids per ascus.

disjunction in *DIS1* strains implies that the *dis1*$^+$ gene product functions during both meiotic and mitotic divisions.

A general defect in meiotic recombination does not cause the elevated nondisjunction levels observed in the *DIS1* mutants. Map distance was calculated from *DIS1* meioses in which nondisjunction of chromosome *VIII* took place. The map distance for the *ARG4-THR1* interval on chromosome *VIII* from the two *DIS1* mutants approximates the map distance found in tetrads from wild-type strains.

All disomic spore colonies recovered from a *DIS1-1* diploid were the products of nondisjunction events occurring at the first meiotic division. In contrast, only 70% of the disomic spores recovered from a *DIS1-2* diploid originated through aberrant reductional divisions. Both heterozygotes, *DIS1-1*/*dis1*$^+$ and *DIS1-2*/*dis1*$^+$, produced 70% of the disomic spores from nondisjunction at the first meiotic division.

The *DIS1* mutants probably affect the segregation of all chromosomes. In *DIS1-1* strains, 5% of the spores disomic for chromosome VIII are simultaneously disomic for chromosome *III*. The recovery of a particular disome may be dependent on disomy of one or more different chromosomes (HILGAR and MORTIMER 1980; PARRY and COX 1970). This type of interaction is probably not responsible for the observations of chromosome *VIII* and *III* disomy because each disome is stable independently. The coupling of the individually rare events involving chromosome *III* and *VIII* suggests that a fraction of the *DIS1* meioses undergo high levels of nondisjunction. [It is difficult to measure spontaneous rates of chromosome *III* nondisjunction in wild-type strains, but no disomes were observed among 700 spores tested, so the rate is less than 4.7×10^{-3} (our unpublished results).] If we assume that all chromosomes respond equally to the *DIS1-1* allelle, we can estimate that an average of three chromosomes nondisjoin in an affected meiosis and that the frequency of affected meioses is 2×10^{-3}.

Assuming that all nondisjunction occurs by premature centromere separation, then the 5% doubly disomic spore colonies arose from one-quarter of the meioses in which both chromsomes *III* and *VII* nondisjoined, since three-quarters of the time the disomic chromosome *III* would segregate to a spore not disomic for chromosome *VIII*. This suggests that the probability of chromosome *III* misbehavior given an affected meiosis is 20%. If all chromosomes are equally affected, then since there are 16 chromosomes on average 0.2×16 or 3.2 chromosomes will be undergoing nondisjunction in an affected meiosis. Moreover, since 4×10^{-4} of total meioses are observed to give rise to a disomic *VIII* spore, the total frequency of affected meioses is estimated to be $4 \times 10^{-4}/0.2 = 2 \times 12^{-3}$.

Model for disjunction in *DIS1* mutants: Isolated copper-resistant papillae represent only one spore product from a single ascus, rather than the mixture of two disomic spores expected from reductional nondisjunction, where both homologs segregate to the same pole at anaphase I. Since the nondisjunction event occurs most often at the first meiotic division, this can be explained by the precocious separation of a sister chromatid pair.

A model of chromosome segregation consistent with the data observed is diagrammed in Figure 3. For nondisjunction occurring during meiosis I, a pair of sister chromatids separates prematurely and one of these chromatids disjoins both from the half-bivalent and from its sister. A meiosis II, the pair of sister chromatids segregates equationally, while the unpaired chromatids segregate randomly, generating one disome containing nonsister chromatids, two haploids and one nullosomic, dead spore. Nondisjunction at meiosis II is a consequence of separation of sister chromatids sometime after anaphase I; the separated chromatids segregate randomly with respect to each other, generating one disomic spore

containing sister chromatids, two haploids and one nullosomic spore.

The model postulates an equational chromosome segregation at meiosis I rather than a random segregation of separated chromatids because a model with random segregation of chromosomes makes three predictions, only one of which is observed. A random segregation model predicts that one seventh of the recovered disomes should be equational, one-seventh should be trisomes, and one-seventh should occur as two reductional disomes per ascus. Although one-third equational disomic spores were recovered in some genotypes, no trisomic spore colonies were detected among the 901 outgrowths tested. (Trisomic papillae can be detected on SC + cup (0.04 mM) media since they are viable and resistant to this level of copper.) The proportion of asci with two disomic spores is effectively zero. We therefore postulate that an equational division at anaphase I is the mechanism of formation of disomic spores containing nonsister chromatids.

DIS1 mutants mirror many features of the Drosophila mutants *mei-S332* (DAVIS 1971), *ord* (MASON 1976; LIN and CHURCH 1982) and *G67* (GETHMANN 1984). These mutants cause nondisjunction to occur at both meiotic divisions and altered recombination levels are not responsible. As in the *DIS1* mutants, the Drosphila mutants display semi-dominant phenotypes, affect all chromosomes (except *G67*), and have elevated mitotic nondisjunction rates. Cytological observations of the *ord* mutant support the conclusions of the genetic analyses that the aneuploid gametes are the products of precocious separation of sister chromatids (GOLDSTEIN 1980, LIN and CHURCH 1982).

Nature of *DIS1-1* and *DIS1-2* alleles: *DIS1* mutants cause nondisjunction in meiosis and mitosis. This is a reasonable phenotype for mutants defective in sister chromatid cohesion, *i.e.*, a defect in the equational segregation of chromosomes. *DIS1-1* strains appear to undergo nondisjunction more often at meiosis I than do *DIS1-2* strains. The phenotypic differences between the two *DIS1* alleles may be explained by either change of function mutations (heteromorphic mutants) or by a change in gene expression. To account for both partial and full dominance as well as altered timing, it is easier to envision changes in the regulation of gene expression. If the *DIS1* gene product is required for chromosome separation, then the time of its expression could relate to when it works. Thus, mutants that express the gene product too early in meiosis might be expected to allow chromosomes to separate earlier, and not segregate properly.

Possible roles for the *DIS1* gene product: Chromatids may be held together by kinetochores through meiosis I; at the appropriate time in meiosis these connections are resolved. GOLDSTEIN (1981) describes the differentiation of kinetochores during meiosis I anaphase in Drosophila and postulates that bivalents segregate reductionally because each pair of sister chromatids has only one functionally differentiated kinetochore at meiosis I metaphase. Mutants that differentiate kinetochores early might cause altered segregation patterns at both meiotic divisions. If a kinetochore becomes prematurely differentiated (*e.g.*, prior to metaphase I), the kinetochores from sister chromatids might become attached to opposite spindle poles and segregate at anaphase I. Functional kinetochore differentiation after metaphase I may result in separated chromatids, allowing the kinetochores to attach to the spindle randomly at metaphase II. The segregation phenotypes of *DIS1* mutants can be explained by such as defect.

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LITERATURE CITED

BAKER, B. S., and J. C. HALL, 1979 Meiotic mutants: genic control of meiotic recombination and chromosome segregation. pp. 352–434. In: *The Genetics and Biology of Drosophila*, Vol. 1a, Edited by E. NOVITSKI and M. ASHBURNER. Academic Press, New York.

BAKER, B. S., A. T. C. CARPENTER and P. RIPOLL, 1978 The utilization during mitotic cell division of loci controlling meiotic recombination and disjunction in *Drosophila melanogaster*. Genetics **90:** 531–578.

BAKER, B. S., A. T. CARPENTER, M. S. ESPOSITO, R. E. ESPOSITO and L. SANDLER, 1976 The genetic control of meiosis. Annu. Rev. Genet. **10:** 53–134.

BYERS, B., 1981 Multiple roles of the spindle pole bodies in the life cycle of *Saccaromyces cerevisiae*. pp. 119–133. In: *Molecular Genetics in Yeast. Alfred Benzon Symposium 16*, Edited by D. VON WETTSTEIN, J. FRIIS, M. KIELLAND-BRANDT and A. STENDERUP. Munksgaard, Copenhagen.

CAMPBELL, D., S. FOGEL and K. LUSNAK, 1975 Mitotic chromosome loss in a disomic haploid of *Saccharomyces cerevisiae*. Genetics **79:** 383–396.

DAVIS, B. K., 1971 Genetic analysis of a meiotic mutant resulting in precocious sister-centromere separation in *Drosophila melanogaster*. Mol. Gen. Genet. **113:** 251–272.

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

FOGEL, S., R. MORTIMER, K. LUSNAK and F. TRAVARES, 1979 Meiotic gene conversion: a signal of the basic recombination event in yeast. Cold Spring Harbor Symp. Quant. Biol. **43:** 1325–1341.

FOGEL, S., and J. WELCH, 1982 Tandem gene amplification mediates copper resistance in yeast. Proc. Natl. Acad. Sci. USA **79:** 5342–5346.

GETHMANN, R. C., 1984 The genetic analysis of a chromosome-specific meiotic mutant that permits a premature separation of sister chromatids in *Drosophila melanogaster*. Genetics **107:** 65–77.

GOLDSTEIN, L. S. B., 1980 Mechanisms of chromosome orienta-

tion revealed by two meiotic mutants in *Drosophila melanogaster*. Chromosoma **78:** 79–111.

GOLDSTEIN, L. S. B., 1981 Kinetochore structure and its role in chromosome orientation during the first meiotic division in male *Drosophila melanogaster*. Cell **25:** 591–602.

GOLIN, J. E., and M. S. ESPOSITO, 1976 Evidence for joint genic control of spontaneous mutation and genetic recombination during mitosis in *Saccharomyces*. Mol. Gen. Genet. **150:** 127–135.

HILGAR, F., and R. K. MORTIMER, 1980 Genetic mapping or *arg1* and *arg8* in *Saccharomyces cerevisiae* by trisomic analysis combined with interallelic complementation. J. Bacteriol. **141:** 270–274.

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

LIN, H-P. P., and K. CHURCH, 1982 Meiosis in *Drosophila melanogaster*. III. The effect of orientation disrupter (*ord*) on gonial mitotic and the meiotic divisions in males. Genetics **102:** 751–770.

LURIA, S. E., and M. DELBRUCK, 1943 Mutations of bacteria from virus sensitivity to virus resistance. Genetics **28:** 491–511.

MALONEY, D., and S. FOGEL, 1980 Mitotic recombination in yeast: isolation and characterization of mutants with enhanced spontaneous mitotic gene conversion rates. Genetics **94:** 825–839.

MASON, J. M., 1976 Orientation disrupter (*ord*): a recombination defective and disjunction-defective meiotic mutant in *Drosophila melanogaster*. Genetics **84:** 545–572.

MERRIAM, J. R., and J. M. FROST, 1964 Exchange and nondisjunction of the *X*-chromosomes in female *Drosophila melanogaster*. Genetics **49:** 109–122.

MORTIMER, R. K., and D. C. HAWTHORNE, 1969 Yeast genetics. pp. 385–460. In: *The Yeasts*, Vol. 1, Edited by A. H. ROSE and J. S. HARRISON. Academic Press, New York.

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

PARRY, E. M., and B. S. COX, 1970 The tolerance of aneuploidy in yeast. Genet. Res. **16:** 333–340.

ROCKMILL, B., 1983 Meiotic nondisjunction mutants in yeast, Ph.D. thesis, University of California, Berkeley.

RYAN, J., 1963 Mutation and population genetics. pp. 39–82. In: *Methodology in Basic Genetics*, Edited by W. L. BURDETTE. Holden-Day, New York.

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

SIMCHEN, G., 1974 Are mitotic functions required in meiosis? Genetics **76:** 745–753.

STRATHERN, J. N., and I. HERSKOWITZ, 1979 Assymetry and directionality in production of new cell types during clonal growth: the switching pattern of homothallic yeast. Cell **17:** 371–387.

THOMAS, J. H., and D. BOTSTEIN, 1986 A gene required for the separation of chromosomes in the spindle apparatus in yeast. Cell **44:** 65–76.

WELCH, J., S. FOGEL, G. CATHALA and M. KARIN, 1983 Industrial yeasts display tandem gene iteration at the *CUP1* region. Mol. Cell. Biol **3:** 1353–1361.

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