Segregation of Recombinant Chromatids Following Mitotic Crossing Over in Yeast

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ABSTRACT

It has long been assumed that chromatid segregation following mitotic crossing over in yeast is random, with the recombinant chromatids segregating to opposite poles of the cell (x-segregation) or to the same pole of the cell (z-segregation) with equal frequency. X-segregation events can be readily identified because heterozygous markers distal to the point of the exchange are reduced to homozygosity. Z-segregation events yield daughter cells which are identical phenotypically to nonrecombinant cells and thus can only be identified by the altered linkage relationships of genetic markers on opposite sides of the exchange. We have systematically examined the segregation patterns of chromatids with a spontaneous mitotic exchange in the *CEN5-CAN1* interval on chromosome *V*. We find that the number of x-segregation events is equal to the number of z-segregations, thus demonstrating that chromatid segregation is indeed random. In addition, we have found that at least 5% of the cells selected for a recombination event on chromosome *V* are trisomic for this chromosome, indicating a strong association between mitotic recombination and chromosome nondisjunction.

MITOTIC recombination was originally proposed by STERN (1936) to explain the phenomenon of twin spots in Drosophila. Twin spots are adjacent patches of homozygous recessive, phenotypically mutant tissue arising in flies heterozygous for linked markers. Such spots result from mitotic exchange between the markers and their centromere, followed by segregation of the recombinant chromatids to opposite poles of the cell. Since the early work of STERN, mitotic recombination has been studied extensively in the genetically amenable yeast *Saccharomyces cerevisiae* (for reviews see ESPOSITO and WAGSTAFF 1981; ROEDER and STEWART 1988; ORR-WEAVER and SZOSTAK 1985). Spontaneous levels of mitotic recombination in yeast occur at frequencies several orders of magnitude below the corresponding meiotic levels, but can be induced to high levels by DNA-damaging agents. This induction suggests a role for mitotic recombination in DNA repair and this has been confirmed genetically by the isolation of recombination-defective yeast strains exhibiting increased sensitivity to physical and chemical DNA-damaging agents (for a review see FRIEDBERG 1988). As noted above, an important consequence of mitotic recombination is the reduction to homozygosity (homozygosis) of heterozygous markers, resulting in the appearance of twin spots in Drosophila or sectored colonies in yeast. It should be noted that the "unmasking" of recessive genes by recombination-associated homozygosis has been implicated in carcinogenesis in higher eukaryotes (STANBRIDGE 1990). Finally, mitotic recombination events involving like sequences at nonhomologous chromosomal locations can result in genome rearrangements and are important in the concerted evolution of multigene families (PETES and HILL 1988).

Recombination in yeast can be either reciprocal or nonreciprocal in nature. There is a strong association between the nonreciprocal (gene conversion) events and crossing over in both mitosis and meiosis and this association is a central feature in models of genetic recombination (for a review see ORR-WEAVER and SZOSTAK 1985). While there are similarities between mitotic and meiotic recombination in yeast, there are mechanistic differences as well. In contrast to meiotic recombination, which is thought to occur exclusively in the G2 stage of the cell cycle after chromosomes have replicated, the majority of mitotic recombination appears to initiate in G1, before DNA replication (WILDENBERG 1970; ESPOSITO 1978; FABRE 1978; GOLIN and ESPOSITO 1981). As illustrated in Figure 1, resolution of a G1-initiated interaction by cleavage of the uncrossed strands prior to DNA replication yields exclusively crossover chromatids, and both daughter cells remain heterozygous for markers distal to the point of the exchange. Cleavage of the crossed strands in G1 does not alter genetic linkages. To explain the recombination-associated homozygosis of heterozygous markers, it has been suggested that the interacting molecules formed by initiation in G1 are resolved by DNA replication to yield the same recombinant-nonrecombinant sister chromatid pairs result-

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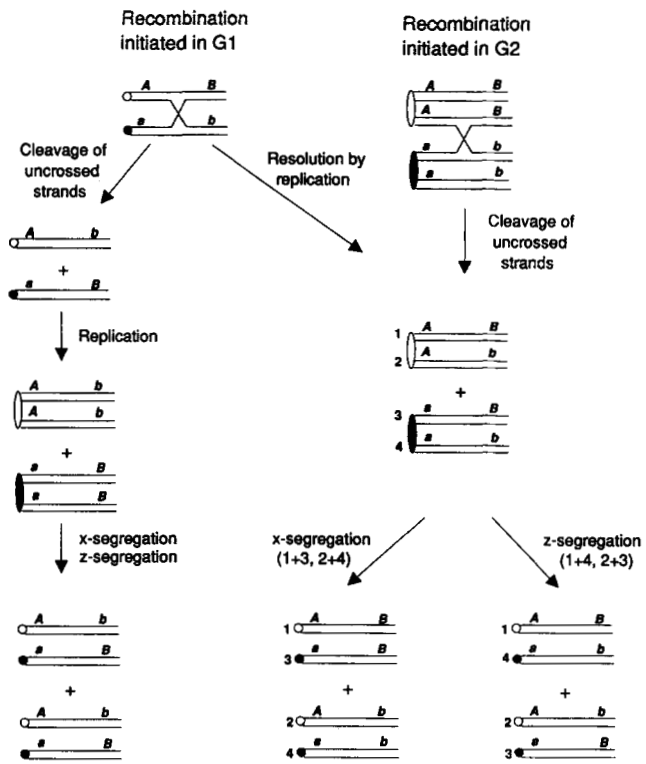

FIGURE 1.—G1 *vs.* G2 initiation of mitotic recombination. Each line represents a single strand of DNA; open and closed circles represent centromeres. Note that sister chromatids always segregate away from each other so that daughter cells get one of each type of centromere. G1 resolution of the crossed strands by cleavage of the uncrossed strands, followed by DNA synthesis and chromatid segregation, yields *B/b* daughter cells and is thought not to occur (left side of figure). Resolution of the strands crossed in G1 by DNA replication yields products identical to those resulting from cleavage of the uncrossed strands in G2-initiated recombination (right side of figure). X-segregation gives *B/B* or *b/b* homozygous daughter cells whereas z-segregation yields *B/b* daughters. Resolution in G1 by cleavage of the crossed strands followed by an associated G2 exchange would yield a similar pattern, although the crossover chromatids would not necessarily be derived from the G1-interacting strands.

ing from mitotic exchange in G2 (ESPOSITO 1978; see Figure 1). An alternative explanation for the observed homozygosis of heterozygous markers is that events initiated in G1 are resolved by cleavage of the crossed strands, but are often accompanied by an exchange in G2 (ROMAN and RUZINSKI 1990). According to this model, the recombination-associated alignment of chromosomes in G1 is maintained after chromosome replication, thus facilitating additional G2 interactions. Although most mitotic recombination is thought to initiate in G1, there is evidence that events can be initiated in G2 as well (ROMAN and FABRE 1983; FABRE, BOULET and ROMAN 1984). Another difference between meiotic and mitotic recombination is that the formation of heteroduplex intermediates is almost exclusively asymmetric in the former (FOGEL, MORTIMER and LUSNAK 1981) but is often symmetric in the latter (ESPOSITO 1978; GOLIN and ESPOSITO 1981). Finally, mutations have been characterized which are specific either to meiotic or mitotic recombination (ORR-WEAVER and SZOSTAK 1985).

Mitotic gene conversion events in yeast are detected experimentally by the production of wild-type recombinants in an auxotrophic diploid strain heterozygous for different mutant alleles (heteroalleles) at the locus being monitored. Mitotic crossing over is detected by monitoring the homozygosis of heterozygous markers. As illustrated in Figure 1, segregation of exchange chromatids away from one another (defined as x-segregation by STERN 1936) yields daughter cells homozygous for markers centromere-distal to the point of exchange. In contrast, segregation of the recombinant chromatids to one pole and the nonrecombinant chromatids to the opposite pole (defined as z-segregation by STERN 1936), results in daughter cells heterozygous for distal markers and hence phenotypically indistinguishable from the parental cell. While meiotic crossing over is always followed by x-segregation of homologous chromosomes, chromatid segregation following mitotic crossing over is generally assumed to be random so that the frequency of x-segregation equals that of z-segregation. Thus the total frequency of mitotic exchange is calculated routinely by doubling an experimentally determined frequency of homozygosis, which corresponds to the frequency of x-segregation. It has, however, been reported by PIMPINELLI and RIPOLL (1986) that mitotic x-segregation exceeds z-segregation by at least a factor of two in Drosophila. This conclusion was reached by examining the segregation of cytologically marked chromosomes in X-irradiated larval brain cells and suggests that frequencies of mitotic exchange may have been overestimated by simply doubling homozygosis frequencies. In this paper we report the results of experiments designed to examine the randomness of chromatid segregation following spontaneous mitotic crossing over in yeast.

MATERIALS AND METHODS

Strain, media and growth conditions: Strain SJR58 (JINKS-ROBERTSON and PETES 1986) was used for the mitotic analysis of recombinant chromatid segregation and has the following genotype: *MATα/MAT***a** *his3/HIS3*$^+$ *his4/his4 ura3-3ΔBgl/ura3-50 leu2-3,112/leu2-3,112 trp1/TRP1*$^+$ *ade2/ADE2*$^+$ *met8-1/met8-1 CAN1*$^+$*/can1-101 CEN5/CEN5-LEU2*$^+$. Standard yeast media and genetic techniques were used in all experiments (SHERMAN 1991). Yeast strains were grown vegetatively at 30° and sporulated at room temperature. YPD (1% yeast extract, 2% Bacto-peptone, 2% dextrose; 2.5% agar for plates) was used for nonselective growth. For the selection of recombinants and the scoring of nutritional markers, SC drop-out plates were made by supplementing SD minimal medium (0.17% yeast nitrogen base without amino acids and ammonium, 0.5% NH_4SO_4, 2% dextrose, 2.5% agar) with all but the one relevant amino acid or base. SC-ura, for example, contained all amino acids and adenine, but no uracil. For the analysis of haploid strains

derived from SJR58, SC omission media were supplemented with five times (0.4 g/liter) the normal amount of leucine to ensure proper growth of Leu$^-$ isolates. Canavanine resistance was scored on SC minus arginine medium supplemented with 60 μg/ml L-canavanine sulfate (SC-arg+can). Sporulation medium contained 1% K-acetate, 0.1% yeast extract, 0.05% dextrose, 2.5% agar and required amino acids and bases. For dissection, asci were pretreated for 10 min with a reducing agent (0.1 M β-mercaptoethanol in 0.02 M EDTA, 0.2 M Tris-HCl, pH 9; DAVIDOW and BYERS 1984) and then incubated with 10% glusolase to digest the ascal wall. Tetrads were dissected by micromanipulation on YPD plates and ascosporal clones were allowed to grow for four days nonselectively before scoring nutritional markers. The meiotic segregations of relevant heterozygous markers were scored by directly replica-plating from the dissection plates to appropriate omission media.

Selection and characterization of Ura$^+$ recombinants: Single colonies of strain SJR58 were picked from YPD plates and patched onto SC-ura plates. Ura$^+$ recombinants appeared as papillae (paps) against a background of no growth after approximately 3 days. To ensure the independence of Ura$^+$ recombinants, we picked only a single pap from each patch. A single colony derived from the nonselective purification of each pap was saved for genetic analyses.

The genotype of Ura$^+$ recombinants at the *CAN1* locus was ascertained by replica-plating patches of cells from YPD plates to SC-arg+can plates. The canavanine-containing plates were irradiated with UV and wrapped with aluminum foil to prevent the repair of DNA damage by visible light. Isolates homozygous for the canavanine-resistant allele (*can1-101/can1-101*) were identified as confluently growing patches after one day. Ura$^+$ recombinants heterozygous at the *CAN1* locus (*CAN1$^+$/can1-101*) could be distinguished from those homozygous for the canavanine-sensitive allele (*CAN1$^+$/CAN1$^+$*) after two days of growth. Homozygous sensitive patches failed to show any growth while the heterozygous patches had numerous canavanine-resistant paps resulting from UV-induced recombination. Homozygosity at the *CAN1* locus was confirmed by tetrad dissection.

Induction of chromosome loss in recombinants heterozygous at *CAN1*: Linkage of the *can1* allele to the *LEU2$^+$*-marked (*CEN5-LEU2$^+$*) *vs.* unmarked (*CEN5*) chromosome *V* centromere was determined by inducing loss of the chromosome carrying the sensitive *CAN1$^+$* allele using the microtubule depolymerization drug methyl 2-benzimidazole carbamate (MBC) (WOOD 1982). Single colonies containing approximately 3×10^6 cells were excised from YPD plates and resuspended in 1 ml liquid YPD. Ten microliters of a 20 mg/ml stock solution of MBC (obtained from the Yeast Genetic Stock Center) in dimethyl sulfoxide were added and cells were incubated for 8 hr on a roller drum at 30°. After 8 hr in MBC-containing medium, there was an approximately 100-fold increase in the frequency of canavanine-resistant cells (from 10^{-4} to 10^{-2}; data not shown). Twenty-five microliters of the MBC-treated culture were plated on SC-arg+can to select for canavanine-resistant cells that had presumably lost the copy of chromosome *V* carrying the wild-type, sensitive allele. To eliminate "jackpot" colonies having high frequencies of canavanine resistance due to an early mitotic event, the same volume of cells was also plated on canavanine medium prior to the addition of MBC. After 4–5 days of growth, canavanine-resistant colonies were picked and purified nonselectively on YPD medium. Purified colonies were then scored for the Leu, Ura and Can phenotypes. In early experiments, three colonies of each heterozygous recombinant were treated with MBC; in later experiments only a single colony was treated with MBC, but at least three canavanine-resistant isolates were examined.

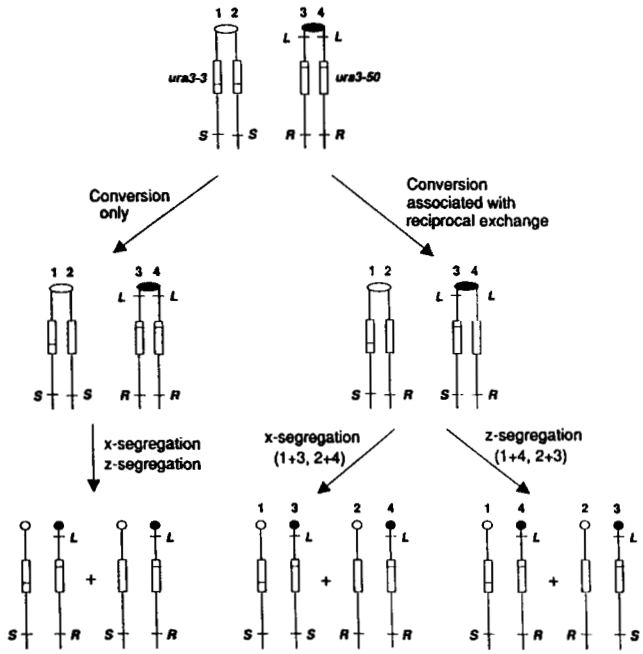

FIGURE 2.—Chromosome *V* recombination in strain SJR58. Each line represents a chromatid and, where relevant, chromatids are numbered. Circles represent centromeres and the open boxes the *URA3* locus. The relative positions of the mutations in the *ura3-3* and *ura3-50* alleles are indicated by the lines within the open boxes. "*L*" represents the *LEU2$^+$*-marked *CEN5*, *S* is the *CAN1$^+$* allele and *R* is the *can1–101* allele. The *URA3* and *CAN1* loci are approximately 11 and 50 cM, respectively, from *CEN5* (MORTIMER *et al.* 1989). The inserted *LEU2$^+$* gene is within 1 kb of *CEN5* (see JINKS-ROBERTSON and PETES 1986). In the examples shown, *ura3-3* is converted to *URA3$^+$*, but either heteroallele may be converted. It should be noted that although this figure assumes that the wild-type allele is on one of the crossover chromatids, this is not necessarily true for events initiated in G1 (see Figure 4).

RESULTS

Experimental system: Chromatid segregation following mitotic crossing over between the centromere of chromosome *V* (*CEN5*) and the *CAN1* locus was analyzed in the diploid strain SJR58. The construction of SJR58 was described in detail by JINKS-ROBERTSON and PETES (1986) and the relevant features of this strain are illustrated in Figure 2. Heteroalleles (*ura3-3* and *ura3-50*) are present at the *URA3* locus on chromosome *V* and recombination between them can be detected by selecting for Ura$^+$ segregants. While the majority of intragenic Ura$^+$ recombinants would be expected to result from gene conversion rather than exchange between the *ura3* heteroalleles, 10–50% of the conversion events at *URA3* should be associated with crossing over elsewhere on chromosome *V* (ESPOSITO and WAGSTAFF 1981). The *CAN1*

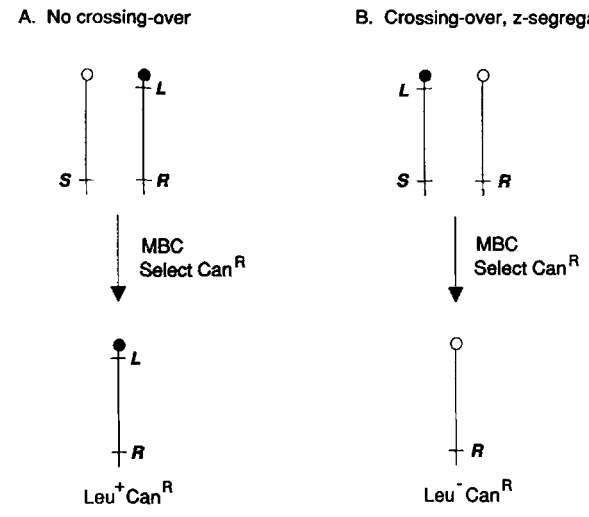

FIGURE 3.—Determination of *CEN5-CAN1* linkage by MBC-induced chromosome loss. Symbols are as described in Figure 2.

locus is distal to *URA3* and is heterozygous for a wild-type, canavanine-sensitive allele (*CAN1*$^+$) and a mutant, canavanine-resistant allele (*can1-101*). The *CAN1* locus encodes arginine permease, which is responsible for the uptake of the toxic arginine analog canavanine. Heterozygous cells produce functional permease and are thus phenotypically sensitive to the drug. The centromere of the copy of chromosome *V* carrying the canavanine-resistant allele is marked by insertion of the yeast *LEU2*$^+$ gene; SJR58 is homozygous for a mutant allele at the normal *LEU2* locus on chromosome *III*.

As illustrated in Figure 2, gene conversion in G2 at the *URA3* locus with no associated exchange followed by either x-segregation or z-segregation yields daughter cells which remain heterozygous at *CAN1*. An exchange between the *ura3* heteroalleles (or a gene conversion at *URA3* associated with crossing over) followed by z-segregation similarly results in daughter cells heterozygous at *CAN1*. In these cells, however, the linkage between *CEN5* and *CAN1* is reversed relative to the parental strain so that the *LEU2*$^+$ allele is linked to the canavanine-sensitive (Can^s) allele and the unmarked *CEN5* is linked to the canavanine-resistant (Can^r) allele. The reversed linkage can be detected using the chromosome loss assay described in detail below. Crossing over followed by x-segregation of the recombinant chromatids results in homozygosis of the alleles at *CAN1*. While recombinants homozygous for the resistant allele are phenotypically Can^r, those homozygous for the sensitive allele remain Can^s like the parental strain. The homozygous sensitive recombinants, however, can be distinguished from the heterozygous recombinants following irradiation with UV. Heterozygotes frequently give rise to Can^r segregants as a result of UV-induced recombination, chromosome loss or mutational inactivation of the sensitive allele. Homozygous sensitive strains do not segregate resistant colonies at a detectable frequency since this would require inactivation of both wild-type alleles.

TABLE 1

Classification of Ura$^+$ recombinants

Phenotypic classification	MBC/tetrad classification	Number observed
"S/R"	S/R, no crossover	798
	S/R, crossover and z-segregation	73
	S/R/R trisome	33
	S/S/R trisome	2
		906
"S/S"	S/S, crossover and x-segregation	14
	S/S/R trisome	19
	Other	4
		37
"R/R"	R/R, crossover and x-segregation	89
	Other	3
		92
Total analyzed		1035

The *CAN1*$^+$ allele is designated as "S" for sensitive; the *can1-101* allele is designated "R" for resistant.

Reversed linkage of *CAN1* and *CEN5* in the Ura$^+$ recombinants heterozygous at *CAN1* can be detected as illustrated in Figure 3. Recombinants are grown in the presence of the microtubule depolymerization drug MBC to induce chromosome loss (WOOD 1982) and cells which have lost the copy of chromosome *V* carrying the Can^s allele are selected by plating in the presence of canavanine. Can^r colonies are then scored for their leucine phenotype. Ura$^+$ recombinants with the parental linkage of the centromere and the *CAN1* locus segregate Can^{r}Leu$^+$ colonies whereas recombinants with reversed linkage (*i.e.*, a crossover between the centromere and *CAN1* followed by z-segregation) segregate Can^{r}Leu$^-$ colonies.

Isolation and characterization of Ura$^+$ recombinants: A total of 1035 independent Ura$^+$ recombinants were isolated from strain SJR58 as described in MATERIALS AND METHODS and the data obtained from genetic analyses of these recombinants are summarized in Table 1. As expected, the majority (906) of the Ura$^+$ recombinants were classified initially as heterozygous at the *CAN1* locus, indicating either no crossing over between *CAN1* and *CEN5* or crossing over followed by z-segregation. Of the Ura$^+$ recombinants, 37 were phenotypically Can^s and 92 were Can^r; this bias is addressed below. To confirm the genotypes of the Can^r and Can^s recombinants, each was sporulated and subjected to tetrad analysis. Recombinants homozygous at *CAN1* would be expected to yield exclusively Can^s or Can^r spores whereas recombinants heterozygous at *CAN1* should exhibit 2:2 segregation

of the canavanine phenotype. Eighty-nine of the 92 Can^r isolates were confirmed to be homozygous for the *can1-101* allele. Unexpectedly, only 14 of the 37 Can^s isolates were confirmed to be *CAN1/CAN1*. Nineteen of the remaining 23 Can^s recombinants appeared to be trisomic for chromosome V (*CAN1$^+$/CAN1$^+$/can1-101*); the genetic analysis of these presumptive trisomes is described in detail below. The remaining four Can^s recombinants were either petite or had the very poor spore viability typical of triploid strains (PARRY and COX 1970).

Each of the 906 Ura$^+$ recombinants classified as heterozygous at *CAN1* was treated with MBC and the linkage of the *can1-101* allele to the *LEU2*$^+$-marked *vs.* unmarked centromere was determined. While the majority of the heterozygous recombinants exhibited clear linkage relationships in the MBC analysis, approximately 5% yielded mixed results in that the Can^r segregants analyzed differed in either the Ura or Leu phenotype. These ambiguous recombinants were, therefore, further characterized by tetrad dissection. Recombinants heterozygous at *CAN1* produce predominantly tetratype asci, but those with the parental linkage of the *CEN5* and *CAN1* loci can be distinguished from those with the recombinant configuration by the production of parental ditype (PD) and nonparental ditype (NPD) asci, respectively. That the production of PD *vs.* NPD asci is a valid means of determining *CEN5-CAN1* linkage was confirmed by dissecting tetrads derived from the parental strain SJR58; of 389 tetrads analyzed, 161 were PD and only 5 were NPD. From MBC analysis and tetrad analysis, 798 of the heterozygous Ura$^+$ recombinants maintained the parental linkage of the *can1-101* allele to the *LEU2*$^+$-marked centromere and hence had no crossing over. Seventy-three of the Ura$^+$ recombinants had the reversed linkage of *CEN5* and *CAN1* which is diagnostic of mitotic crossing over followed by z-segregation. The remaining 35 Ura$^+$ recombinants were tentatively classified as trisomic for chromosome V based on a non-2:2 segregation pattern of the alleles at the *CAN1* locus (see below). In summary, of the 1035 Ura$^+$ recombinants analyzed 103 (10.7%) were homozygous at *CAN1* and thus represented mitotic crossing over followed by segregation of the recombinant chromatids to opposite poles of the cell. A combination of mitotic MBC treatment and meiotic tetrad dissection detected 73 cases (7.0%) of reversed linkage of *CEN5* and *CAN1* corresponding to exchange followed by segregation of the recombinant chromatids together. The observed x-segregation bias is significant ($\chi^2 = 4.78$; $0.02 < P < 0.05$) and thus x-segregation appears to exceed z-segregation by approximately 50%.

A potential source of error which could contribute to the observed x-segregation bias is the formation of a *URA3*$^+$ allele on each of the exchange chromatids via a symmetric heteroduplex intermediate. Subsequent x-segregation would yield two Ura$^+$ daughter cells, each of which would have been detected in the initial screen for Ura$^+$ recombinants. In contrast, z-segregation would yield only a single Ura$^+$ daughter in which both exchange chromatids are Ura$^+$. To investigate this possibility, nineteen of the z-segregation recombinants were sporulated and tetrads were dissected. In all cases, the Ura phenotype segregated 2+:2− in meiotic tetrads (data not shown), suggesting that the formation of a *URA3*$^+$ allele on both exchange chromatids is a rare event. This observation is consistent with the formation of a predominantly asymmetric heteroduplex recombination intermediate.

A second possible source of the observed x-segregation bias is the phenomenon of chromosome loss associated with *RAD52*-independent mitotic recombination (HABER and HEARN 1985). In *rad52* mutants, the z-segregation class is greatly reduced relative to the x-segregation class, but this reduction is accompanied by a comparable increase in chromosome loss events. It was suggested by HABER and HEARN (1985) that in *rad52* strains, one of the chromatids participating in the recombination event remains broken and is subsequently lost from the cell. Since the detection of z-segregation depends on the cosegregation of both exchange chromatids, z-segregation would decrease at the expense of increased aneuploidy. Although we have detected a substantial amount of aneuploidy associated with recombination, we observed chromosome gain rather than chromosome loss in our system (see below). In any case, the level of *RAD52*-independent recombination would be expected to be a small fraction of the wild-type level of recombination (MALONE and ESPOSITO 1980; MALONE *et al.* 1988) and thus would not be expected to contribute significantly to the bias reported here.

A third potential source of the observed x-segregation bias is the underlying assumption that the Ura$^+$ gene is always on one of the crossover chromatids (see Figure 2). While this assumption is valid for recombination events initiated in G2, it is not necessarily valid for G1-initiated events. Figure 4 illustrates potential Ura$^+$ products produced if recombination initiates in G1 and an asymmetric heteroduplex intermediate is formed. If the mismatches are repaired such that each strand involved in the heteroduplex is converted to *URA3*$^+$, then resolution by DNA replication will yield Ura$^+$ sister chromatids, only one of which has undergone an exchange. Subsequent x-segregation would yield two daughter cells, each of which is Ura$^+$ and thus would be detected by the initial screen. Since each daughter produced by x-segregation contains an exchange chromatid, each would be classified as a crossover event. While z-segregation

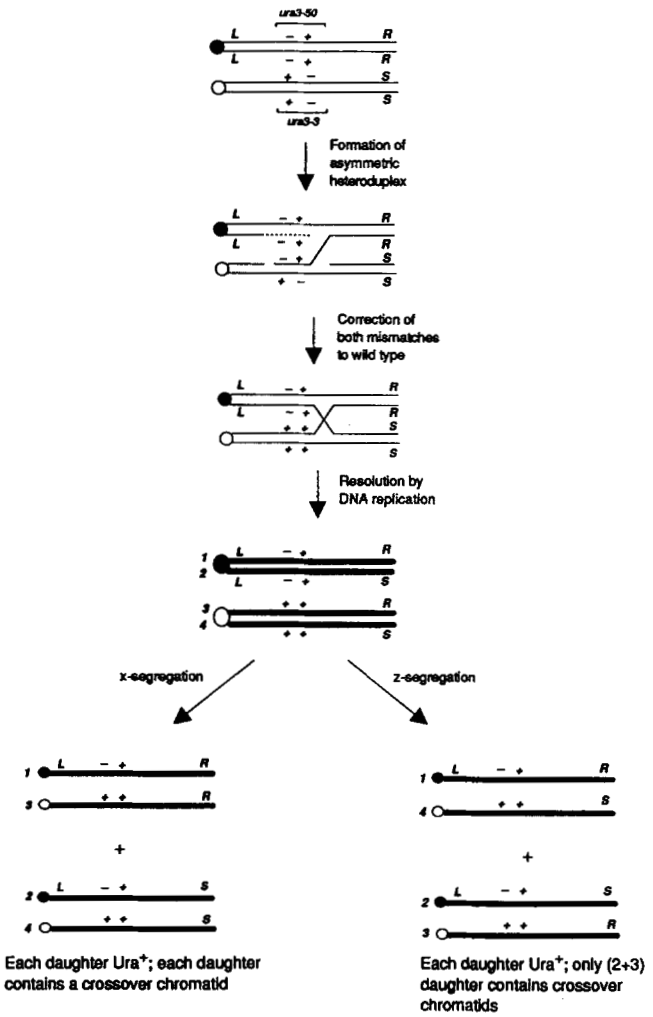

FIGURE 4.—Formation of *URA3*$^+$ alleles in G1-initiated recombination. Thin lines correspond to single strands of DNA and thick lines correspond to double-stranded chromatids. The dotted line corresponds to gap-filling by DNA polymerase. The positions of wild-type and mutant information in the mutant *ura3* alleles are indicated by "+" or "−," respectively. Other symbols are as in Figure 2. Asymmetric heteroduplex is formed covering both mutant sites in the *URA3* locus and the resulting mismatches are repaired to wild type. Note that many different repair patterns are possible; heteroduplex may be asymmetric or symmetric and may cover only one or both sites. Resolution by cleavage of the crossed strands in G1 followed by exchange in G2 is also possible.

would also yield two Ura$^+$ daughters, only the daughter containing both exchange chromatids would be classified as a crossover event. Thus, the scenario depicted in Figure 4 would result in an apparent excess of x-segregation relative to z-segregation events. While a specific mechanism is depicted in Figure 4, it should be noted that the actual mechanism of generating a crossover associated with G1 gene conversion is irrelevant to the results being considered here. The relevant point is that, in the screen used to enrich for cells with an exchange on chromosome *V*, z-segregation events are not detected unless the *URA3*$^+$ allele is on one of the crossover chromatids. The frequency with which the *URA3*$^+$ allele is on the noncrossover chromatid in the x-segregation recombinants can be determined by tetrad analysis. In the case of the *CAN1*$^+$/*CAN1*$^+$ recombinants, the nonexchange chromatid will be Leu$^-$ and the exchange chromatid will be Leu$^+$; the reverse is true of the *can1/can1* homozygotes. The linkage of the *URA3*$^+$ allele to *LEU2*$^+$-marked *vs.* unmarked centromere was determined in 54 of the homozygotes and, in nine cases (17%), the *URA3*$^+$ allele was on the nonexchange chromatid. The x-segregation bias introduced by the presence of the *URA3*$^+$ allele on the nonexchange chromatid can be corrected for by subtracting these events from the total number of *CAN1* homozygotes. Assuming that 83% of the homozygotes have the *URA3*$^+$ gene on the exchange chromatid, the corrected number of homozygotes produced by x-segregation is 86. A statistical comparison of the corrected number of x-segregation events (86) with the observed number of z-segregations (73) indicates that segregation of recombinant chromatids is random ($\chi^2 = 0.9$; $0.3 < P < 0.5$).

Parity *vs.* disparity in conversion of the *ura3* heteroalleles: An excess of Can^r to Can^s homozygous recombinants (89 *vs.* 14; see Table 1) resulting from x-segregation of the recombinant chromatids was noted above. This is the bias expected given the relative orientation of the *ura3* heteroalleles; the *ura3-3* allele is centromere-distal relative to *ura3-50* and is linked to the *CAN1*$^+$ allele. A simple crossover between the *ura3* heteroalleles will yield a wild-type gene linked to the *can1-101* allele and hence an excess of Can^r recombinants over Can^s recombinants (see Figure 2). The same holds true for crossovers linked mechanistically to G1 or G2 gene conversion events. MBC analysis of the 73 z-segregation heterozygotes revealed a bias similar to that observed in the x-segregation homozygotes; the *URA3*$^+$ allele was linked to *can1-101* in 59 recombinants and to *CAN1*$^+$ in only 14 recombinants. The biases seen in the x-segregation *vs.* z-segregation classes are not statistically different ($\chi^2 = 1.01$; $0.30 < P < 0.50$) and hence do not affect our conclusions concerning the randomness of chromatid segregation.

Consistent with our interpretation that the conversion biases associated with crossing over reflect a constraint imposed by the relative orientations of the *ura3* heteroalleles, there was parity in conversion of the heteroalleles in the nonexchange *CAN1*$^+$/*can1-101* recombinants. In the MBC analysis, 385 and 412 of the nonexchange recombinants had the *URA3*$^+$ allele linked to *can1-101* and *CAN1*$^+$, respectively ($\chi^2 = 0.92$; $0.30 < P < 0.50$).

Analysis of trisomic recombinants: Ura$^+$ recombinants tentatively classified as trisomic for chromosome *V* were identified by non-2:2 or non-4:0 segregation of the Can phenotype. Figure 5 presents typical

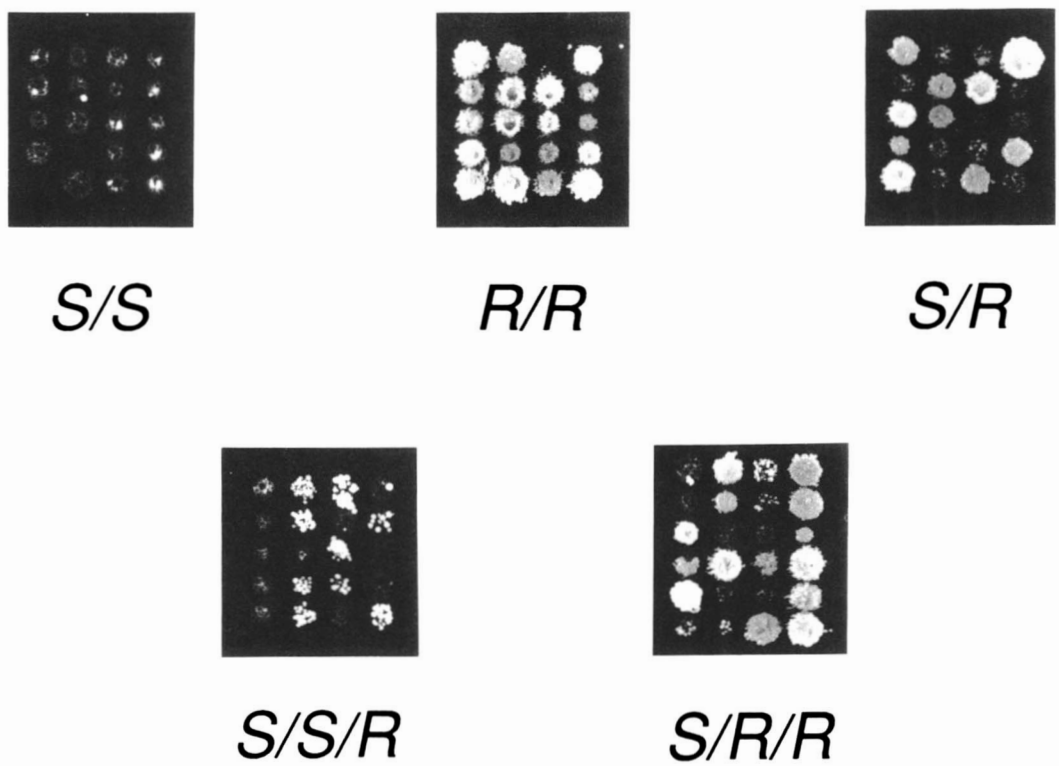

FIGURE 5.—Segregation of the Can phenotype in meiotic tetrads. The meiotic segregation of the Can phenotype in representative tetrads from the indicated diploids or chromosome *V* trisomes is shown. Each row corresponds to a complete tetrad; *R* and *S* correspond to the Can^r (*can1-101*) and Can^s (*CAN1*$^+$) alleles, respectively. Note that many of the sensitive spores derived from the trisomic strains segregate Can^r papillae while those derived from the diploid strains do not.

meiotic segregation patterns of the canavanine phenotype produced by *CAN1*$^+$/*can1* heterozygotes; *CAN1*$^+$/*CAN1*$^+$ and *can1*/*can1* homozygotes; and the putative *CAN1*$^+$/*CAN1*$^+$/*can1* and *CAN1*$^+$/*can1*/*can1* trisomes. Figure 6 schematically illustrates possible meiotic segregation patterns of the Can phenotypes in a trisomic strain carrying two *CAN1*$^+$ alleles and a single *can1* allele. The diagnostic feature of such chromosome *V* trisomes is an excess (>50%) of Can^s spores with the remaining spores being predominantly canavanine-sensitive but segregating resistant cells at a high frequency (the Can^p or papping phenotype). The Can^p spores are disomes having two copies of chromosome *V*, one carrying the *CAN1*$^+$ allele and the other carrying the *can1* allele. Such disomes are unstable and lose one copy of chromosome *V* at a high frequency, thus resulting in the appearance of the Can^r papillae. Depending on the meiotic exchange and subsequent chromatid segregation patterns, *CAN1*$^+$/*CAN1*$^+$/*can1* trisomes will occasionally produce Can^r spores. The *CAN1*$^+$/*can1*/*can1* trisomes yield a meiotic segregation pattern which is essentially the reverse of the pattern seen with the *CAN1*$^+$/*CAN1*$^+$/*can1* trisomes; most spores are Can^r or Can^p and only an occasional Can^s spore is seen.

Ura$^+$ recombinants suspected of being trisomic for chromosome *V* were initially detected among the Can^s class of recombinants; one-half (19/37) of the Can^s isolates appeared to be *CAN1*$^+$/*CAN1*$^+$/*can1* in meiotic analyses. An additional 35 trisomes (33 *CAN1*$^+$/*can1*/*can1* and 2 *CAN1*$^+$/*CAN1*$^+$/*can1*) were detected among the Ura$^+$ recombinants originally classified as heterozygous at *CAN1*. These were picked up on the basis of mixed MBC results (31/35) or by dissection of random SJR58 Ura$^+$ isolates (4/35). Thus a total of 54 presumptive trisomes were identified, which corresponds to 5.0% of the total Ura$^+$ recombinants analyzed.

The recombinants exhibiting the aberrant segregation of the canavanine phenotype were assumed to be trisomic for all of chromosome *V*, but it was possible that these strains were trisomic for only the left arm of chromosome *V* carrying the *CAN1* locus, or that they contained an unidentified chromosomal rearrangement. To address this issue, we crossed ten Can^p spores derived from independent presumptive trisomes to an appropriate haploid strain with a recessive marker (*hom3*) on the right arm of chromosome *V*. If all of chromosome *V* is duplicated, the marker on the right arm of chromosome *V* should, like the *CAN1* locus on the left arm, exhibit non-2:2 meiotic segregation. The alleles at the *HOM3* locus segregated 4+:0−, 3+:1− and 2+:2− in all ten Can^p spores analyzed, thus confirming that these spores were trisomic

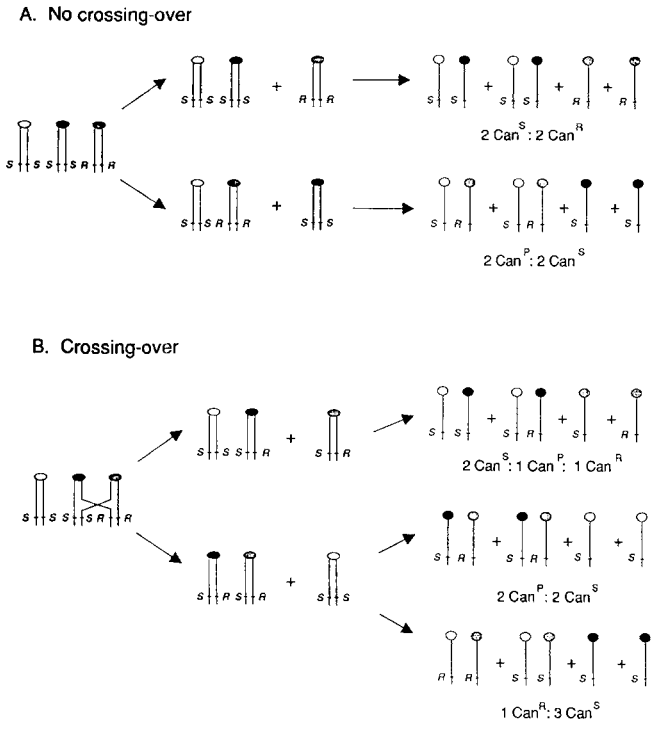

FIGURE 6.—Meiotic segregation patterns of the Can phenotype in tetrads derived from a *CAN1*$^+$/*CAN1*$^+$/*can1-101* trisome. Symbols are as described in Figure 2. Disomic spores heterozygous at *CAN1* are canavanine-sensitive but segregate resistant papillae at a high frequency (the papping or Can^p phenotype).

for both arms of chromosome *V*. While this does not rule out the occurrence of partial trisomes or chromosomal rearrangements in some recombinants, the data suggest that these events are rare relative to trisomy for the entire chromosome.

Approximately 100 of the SJR58 Ura$^+$ recombinants were subjected to tetrad dissection in order to confirm the results inferred from the canavanine phenotype and the MBC analysis of heterozygotes. As noted above, four additional trisomes were picked up by this random tetrad dissection. This indicates that we probably failed to identify a substantial number of trisomic recombinants and that the actual frequency of chromosome *V* nondisjunction among recombinants is likely to be greater than 5%. In addition, the reciprocal class of monosomic recombinants was not detected. Although the initial recombinants isolated may have been biased in favor of those growing rapidly, later recombinants were isolated after a longer incubation and without regard to size. Reduplication of a monosomic chromosome would have resulted in a recombinant homozygous at the three loci monitored on chromosome *V*; none was detected.

Chromosome stability is not affected by the *LEU2*$^+$ insertion: The association of nondisjunction with mitotic recombination is not unprecedented (CAMPBELL, FOGEL and LUSNAK 1975; CAMPBELL and FOGEL 1977), but the relatively high frequency we observed (at least 5%) caused us to consider the possibility that the insertion of *LEU2*$^+$ adjacent to *CEN5* in our strain might contribute to the observed chromosome instability. This possibility was examined in two ways. First, we dissected meiotic tetrads derived from approximately 100 random colonies of the parental strain SJR58 and detected no phenotypic evidence of trisomy for chromosome *V*. It should be noted that spore viability was greater than 95% and that, as expected, the *LEU2*$^+$ gene showed first division segregation relative to the centromere-linked *TRP1* locus. The second experiment involved estimating the mitotic frequency of chromosome *V* loss by measuring the frequency of Can^r segregants in strains heterozygous at *CAN1*. That this approach accurately reflects the frequency of chromosome *V* loss was shown in experiments reported by HARTWELL and SMITH (1985), in which approximately 40% of spontaneous Can^r segregants from a *CAN1*$^+$/*can1* diploid were the result of chromosome loss. Two sets of diploid strains heterozygous at *CAN1* were constructed by mating appropriate haploids derived from the sporulation of strain SJR58. In the control set of diploids, neither copy of *CEN5* contained the *LEU2*$^+$ insert; in the experimental set, the chromosome with the *CAN1*$^+$ allele also had the *LEU2*$^+$ insertion adjacent to *CEN5*. If chromosome *V* is destabilized by the *LEU2*$^+$ insertion, then one would expect the experimental diploids to produce many more Can^r segregants than the control strains. The median frequency of Can^r colonies was 9.2×10^{-5} for control diploids and 8.2×10^{-5} for the experimental strains. Chromosome *V* monosomes grow much slower than diploids (HARTWELL and SMITH 1985; WOOD 1982), so measuring the fraction of small colonies appearing between 3 and 7 days after plating on selective medium should provide a crude estimate of the percentage of Can^r colonies derived from nondisjunction. 22% and 28% of the Can^r colonies were slow growing from the control and experimental strains, respectively. Based on these data, we conclude that insertion of the *LEU2*$^+$ gene adjacent to *CEN5* does not appreciably affect centromere function.

DISCUSSION

We have systematically examined the mitotic segregation of exchange chromatids in a diploid yeast strain. Mitotic crossing over in the *CEN5-CAN1* interval on chromosome *V* was monitored using a strain heterozygous for a *LEU2*$^+$ marker inserted adjacent to *CEN5*, heteroallelic at the *URA3* locus between *CEN5* and *CAN1*, and heterozygous at the distal *CAN1* locus. Independent Ura$^+$ recombinants were initially selected and approximately 20% of these were the result of or had an associated crossover event. Of the mitotic crossovers, 59% (103/176) were classified as x-segregation events while only 41% (73/176) were

classified as z-segregations. A critical assumption in the experimental design is that the *URA3*$^+$ gene resulting from recombination between the *ura3* heteroalleles will always be on the crossover chromatid(s) and never on one of the noncrossover chromatids. If the *URA3*$^+$ allele is on a nonexchange chromatid, then z-segregation (but not x-segregation) will give rise to a Ura$^-$ daughter that will go undetected in the initial screen. A detailed characterization of the x-segregation class revealed that the *URA3*$^+$ gene was in fact present on the noncrossover rather than the crossover chromatid in approximately 17% of the recombinants. As illustrated in Figure 4, this anomaly can be explained by gene conversion events occurring in G1. Correcting for this 17% bias, the number of x-segregations (86) is not statistically different from the number of z-segregations (73). Thus, in contrast to the x-segregation bias observed in Drosophila (PIMPINELLI and RIPOLL 1986), chromatid segregation following mitotic crossing over on chromosome *V* in yeast is random. It is likely that chromatid segregation is similarly random for the other yeast chromosomes.

The reason for the discrepancy between the Drosophila and yeast results is not obvious. The experimental protocol in the Drosophila experiments involved X-irradiation and subsequent microscopic examination of cytologically marked chromosomes (PIMPINELLI and RIPOLL 1986). In our experiments, spontaneous rather than mutagen-induced recombinants were isolated and these were characterized based on genetic criteria. The bias observed in Drosophila could be due to the use of a mutagen to induce crossing over or could be related to somatic chromosome pairing, a phenomenon that is thought to be unique to Dipteran species (SEMIONOV and SMIRNOV 1984). In addition, it should be noted that the detection of z-segregation depends on the integrity of both chromatids participating in the crossover. If one chromatid failed to be repaired in the Drosophila experiments, it likely would be lost and this could lead to an underestimate of z-segregations (see below for the description of such a phenomenon in a *rad52* yeast strain).

Previous studies have indicated that 30–40% of the heteroduplex formed during mitotic recombination in yeast is symmetric (ESPOSITO 1978; GOLIN and ESPOSITO 1981). In the current study, this would be manifested as *URA3*$^+$ alleles on both recombinant chromatids in cells derived from z-segregation. Of the z-segregation recombinants analyzed, none (0/19) contained two *URA3*$^+$ alleles. While this does not exclude the formation of symmetric heteroduplex intermediates, our data are more consistent with the formation of an asymmetric heteroduplex intermediate.

The occurrence of a *URA3*$^+$ allele on the noncrossover rather than on the exchange chromatid in x-segregation recombinants is consistent with initiation of a substantial fraction (at least 17%) of mitotic recombination in the G1 stage of the cell cycle (see ESPOSITO 1978; GOLIN and ESPOSITO 1981). We assume that G1 interactions between nonsister chromatids either are resolved by cleavage of the crossed strands and then followed by an associated G2 crossover (ROMAN and RUZINSKI 1990), or are simply resolved by DNA replication (ESPOSITO 1978). If, however, cleavage of the uncrossed strands occurred at an appreciable frequency in G1, all four chromatids would be recombinant after DNA replication and heterozygosity at *CAN1* would result regardless of the pattern of chromatid segregation. Such events would be scored as z-segregations in our assay. There is not a z-segregation bias in our data, although one was reported by ROMAN (1980) in an examination of X-ray induced recombination in yeast. While it is possible that a G1-generated z-segregation bias is balanced by an x-segregation bias in G2 to give the approximate equality of segregation patterns observed in the current study, we think that this is unlikely.

The occurrence of co-conversion tracts extending from *URA3* through *CEN5* would not affect the results reported here. While long mitotic conversion tracts have been reported in yeast (GOLIN, FALCO and MARGOLSKEE 1986; VOELKEL-MEIMAN and ROEDER 1990), tracts encompassing the *URA3* locus have been estimated to be 4–10 kb in length (JUDD and PETES 1988), thus making it unlikely that tracts would extend from *URA3* to *CEN5*. If such events did occur, they would most likely result in homozygosity at *CEN5* and heterozygosity at *CAN1*, a pattern not detected in our analyses (data not shown). It should be noted that an identical pattern would result from a meiotic-like reductional rather than the normal mitotic equational segregation of chromatids, a pattern termed y-segregation by STERN (1936). In agreement with Drosophila results (PIMPINELLI and RIPOLL 1986), there is no evidence of mitotic y-segregation in yeast.

A striking phenomenon observed in this study is the association of nondisjunction with mitotic recombination. At least 5% of the Ura$^+$ recombinants isolated were trisomic strains containing an extra copy of chromosome *V*. Chromosome loss associated with recombination in chromosome *III* disomes has been previously reported by FOGEL and co-workers (CAMPBELL, FOGEL and LUSNAK 1975; CAMPBELL and FOGEL 1977). In their early experiments, chromosome *III* loss events in **a**/α disomic strains were detected by selecting for mating-competent segregants. Approximately 10% of the haploid segregants were recombinant with respect to marker genes on chromosome *III* (CAMPBELL, FOGEL and LUSNAK 1975).

In a subsequent study, recombination events on chromosome *III* in disomic strains were selected ini-

tially and then were subsequently examined for associated chromosome loss (CAMPBELL and FOGEL 1977). Results indicated that chromosome loss was enhanced when recombination events occurred close to the centromere and it was suggested that precocious sister centromere segregation might be associated with events occurring close to the centromere. Since chromosome gain events could not be detected in these studies, it was not clear whether the chromosome loss events were actually the result of aberrant chromatid segregation or of some anomaly in chromosome replication. HABER and HEARN (1985) suggested that the chromosome loss observed by FOGEL and co-workers might be due to *RAD52*-independent recombination in which one of the involved chromatids fails to be repaired and is lost. *RAD52*-independent recombination, however, cannot account for the occurrence of recombination-associated chromosome gain in the present study. Chromosome gain associated with recombination is not unique to our strain since it has been noted in at least two other studies (MCGILL *et al.* 1990; THOMAS and ROTHSTEIN 1989). It is likely that recombination-associated nondisjunction reflects either a general problem in DNA metabolism in the affected cells (see HARTWELL and SMITH 1985) or that recombination in some way interferes with chromosome alignment and/or spindle attachment. If there was a problem in DNA metabolism, one might expect to see coincident nondisjunction for several chromosomes. Although the strain used in the present work was not designed to systematically examine this, we did look for coincident disomy of chromosome *III* or *XV* with chromosome *V* and none was found (data not shown). It should be noted that we did not detect the reciprocal of the trisomic class of recombinants: strains monosomic for chromosome *V*. Ura^+ monosomes, however, would be expected to be less frequent that Ura^+ trisomes. If only one of the four chromatids in a recombinant carries a $URA3^+$ allele, the wild-type allele is more likely to end up in the trisomic daughter than in the monosomic daughter. In addition, we may have inadvertently selected against monosomes in our initial screen for recombinants since such strains are slow growing (HARTWELL and SMITH 1985; WOOD 1982).

While it is not possible to ascribe a cause and effect relationship to mitotic recombination and nondisjunction, we favor the view that recombination interferes with chromosome alignment on the metaphase plate and/or spindle attachment. Consistent with this idea is the general observation that the spore viability of *spo13* strains is increased and aneuploidy decreased by mutations which reduce or eliminate meiotic recombination (HOLLINGSWORTH and BYERS 1989; KLAPHOLZ, WADDELL and ESPOSITO 1985; ENGEBRECHT and ROEDER 1989; MENEES and ROEDER 1989; MALONE 1983). Thus, meiotic recombination seems to interfere with the single mitotic-like division that occurs in *spo13* mutants. We imagine that chromatid segregation is delayed until the centromeres of sister chromatids are attached to opposite poles of the cell and the chromatids are thus experiencing forces in opposite directions. A crossover chromatid possibly might encounter such tension as a result of its interaction with a nonsister chromatid, rather than its attachment to the mitotic spindle. If anaphase then ensues, nondisjunction would occur at a high frequency.

In conclusion, the mitotic segregation of exchange chromatids is random in yeast, with recombinant chromatids segregating to the same pole and to opposite poles with equal frequency. The general method of calculating mitotic crossing over frequencies by simply doubling the homozygosis frequency of heterozygous markers is thus valid. In addition, there is a strong association of mitotic recombination with nondisjunction which is manifested as chromosome gain events in the present system.

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