ASSOCIATION OF CHROMOSOME LOSS WITH CENTROMERE-ADJACENT MITOTIC RECOMBINATION IN A YEAST DISOMIC HAPLOID

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ABSTRACT

Experiments designed to characterize the association between disomic chromosome loss and centromere-adjacent mitotic recombination were performed. Mitotic gene convertants were selected at two heteroallelic sites on the left arm of disomic chromosome III and tested for coincident chromosome loss. The principal results are: (1) Disomic chromosome loss is markedly enhanced (nearly 40-fold) over basal levels among mitotic gene convertants selected to arise close to the centromere; no such enhancement is observed among convertants selected to arise relatively far from the centromere. (2) Chromosome loss is primarily associated with proximal allele conversion at the centromere-adjacent site, and many of these convertants are reciprocally recombined in the adjacent proximal interval. (3) Partial aneuploid exceptions provisionally identified as carrying left arm telocentrics have been found. A testable model is proposed suggesting that centromere involvement in genetic recombination may precipitate segregational disfunction leading to mitotic chromosome loss.

WE have shown that cells of *Saccharomyces cerevisiae* disomic ($n + 1$) for chromosome III spontaneously lose at low frequency one of the two chromosomes present in duplicate (CAMPBELL, FOGEL and LUSNAK 1975). Most of the euploid chromosomes recovered after mitotic events leading to chromosome loss retained the parental (disomic) marker configuration expected from chromosome segregation alone; a significant minority (about 10 percent), however, were recombinant for marker genes. The observed frequency of recombinant exceptions exceeded by at least 100-fold that expected if chromosome loss and mitotic recombination in the disome were independent events. Nearly all the recombinant exceptions could be accounted for by mitotic exchange, or especially mitotic gene conversion, near the centromere.

The centromere proximity of mitotic exchanges associated with chromosome loss suggests a possible causal relationship between these two events. Recombination in or near the centromeric region could, for example, interfere with normal chromosome segregation and promote, directly or indirectly, chromo-

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some loss. These considerations imply that the association between chromosome loss and mitotic recombination should be preserved, independent of which mitotic event is initially selected. Two specific predictions can then be made: (1) chromosome loss in the disome should be enhanced over the spontaneous background among mitotic recombinants selected to arise near the centromere; and (2) there should be little or no enhancement of chromosome loss among recombinants selected to arise far from the centromere. This report describes experiments which test and confirm these predictions.

MATERIALS AND METHODS

Media

Ingredients are given in amounts per liter of distilled water. Media were solidified with 15 g/l agar.

YEPD: D-glucose 20 g, Difco Bacto-peptone 20 g, Difco yeast extract 10 g.

YPMAL: maltose 40 g, Difco Bacto-peptone 20 g, Difco yeast extract 10 g; bromthymol blue was added as color (pH) indicator to the medium after autoclaving as a filter-sterlized aqueous solution.

Synthetic complete medium: D-glucose 20 g, Difco yeast nitrogen base without amino acids 6.7 g, nutrient supplement as follows (μg/ml final concentrations): adenine-SO_4 20, L-arginine-HCl 50, L-histidine-HCl 20, L-isoleucine 50, L-leucine 50, L-lysine-HCl 50, L-methionine 50, L-phenylalanine 50, DL-threonine 600, L-tryptophan 50, L-tyrosine 50, uracil 20, L-valine 50.

Dropout media: Synthetic complete medium lacking single nutrilite supplements.

Sporulation medium: Potassium acetate 20 g, Difco yeast extract 2.2 g, D-glucose 0.5 g, complete nutrient supplement as listed for synthetic complete medium.

GNA: D-glucose 50 g, Difco Bacto-peptone 5 g, Difco yeast extract 10 g, Difco beef extract 3 g, complete nutrient supplement as listed for synthetic complete medium.

Yeast strains

Genotypes of the haploid mutant strains of *Saccharomyces cerevisiae* employed in this work are:

5003–21A–1A $\dfrac{+ \quad his4\text{–}4 \quad leu2\text{–}1 \quad +}{his4\text{–}290 \quad + \quad + \quad leu2\text{–}27} \bigcirc \dfrac{\mathbf{a} \quad + \quad mal2}{\alpha \quad thr4 \quad +}$; *ade2–1*; *trp1*; *met2*.

5037–5A *his4–290 leu2–27* · *α mal2*; *ade8–18*; *ura3*; *lys2*.

5037–5B *his4–290 leu2–27* · **a** *mal2*; *ade8–18*; *ura3*; *lys2*.

S181 **a**; *ade6*.

S185 *α*; *ade6*.

Origins of the several alleles, properties of the disomic haploid strain 5003–21A–1A, and the genetic map of markers on linkage group III are given in CAMPBELL, FOGEL and LUSNAK (1975). Allele map order at *his4* is that reported by FINK and STYLES (1974). The allele order at *leu2* is not known (FOGEL and ROTH 1974), and the order shown in Figure 1 is arbitrary. Data presented in this work, however, are consistent with the map order *leu2–1*, *leu2–27*, centromere. Strains S181 and S185 (from DR. T. R. MANNEY) were employed in mating-type tests of spore clones based on complementation between the *ade6* marker in the tester(s) and the *ade2* and *ade8* markers segregating in meiotic tetrads. Even in those cases where a spore clone was *ade*+, the vigorous diploid growth indicative of mating was clearly distinguishable from the nonmating (**a**/*α*) *ade*+ phenotype.

Recovery of prototrophic gene convertants

Cells of the disomic strain 5003–21A–1A, heteroallelic at *his4* and *leu2*, were streaked for single colonies on *GNA*. After 2 days incubation at 30°, a single colony was picked, resuspended

in water, diluted and plated by the soft agar (0.7 percent) overlay method on several *GNA* plates to give approximately 100 colonies per plate. After 2 days incubation, single disomic clones were transferred to *GNA* master plates; all well-separated colonies from a given plate were transferred before going on to the next plate. The master plates were incubated one day and then replica-plated to leucineless and histidineless plates. The replicas were incubated 3–4 days, at which time prototrophic papillae, representing *leu*+ and *his*+ mitotic gene convertants, were readily apparent.

The prototrophic papillae were subjected to two distinct isolation regimes. In the "low resolution" procedure (experiments 1, 2, 3, 4) single *leu*+ and *his*+ papillae from each original disomic clone were transferred by toothpick directly to leucineless and histidineless master plates, respectively. Care was taken to transfer only well-separated single papillae. The master plates were incubated for 2–5 days and then replica-plated to appropriate dropout media, and to *YEPD* for mating-type tests against haploid tester strains 5037-5A (α) and 5037-5B (**a**). In the "high resolution" procedure (experiments 5 and 6) single *leu*+ and *his*+ papillae from each original disomic clone were streaked for single colonies on leucineless and histidineless plates, respectively. The resultant clones (5 from each streak in experiment 5, and 2 from each streak in experiment 6) were transferred to *GNA* master plates, incubated one day, and then replica-plated and mating-type tested as above.

In the six independent experiments, 3877 independent *leu*+ papillae and 3901 independent *his*+ papillae were tested. The isolated papillae were, for the most part, pure clones. However, despite precautions taken to isolate only well-separated papillae, 43 *leu*+ papillae (1.1 percent) and 30 *his*+ papillae (0.8 percent) were, upon testing, sectored for two phenotypes, and are judged to represent inadvertant mixing of adjacent prototrophic papillae, or secondary mitotic segregations after initial isolation. Mixed clones were detected somewhat more frequently in the "high resolution" experiments (5, 6) than in the "low resolution" experiments (1, 2, 3, 4): among *leu2*+, 1.8 percent *vs.* 0.7 percent; among *his4*+, 1.2 percent *vs.* 0.5 percent. The 73 mixed clones were included in the count totals.

Alleles *leu2-1* and *his4-4* are ochre suppressible, and prototrophs at the two heteroallelic loci can arise by extragenic suppression. Simultaneous reversion to prototrophy at both heteroallelic loci *and* at the ochre-suppressible *ade2-1* site was taken to signify the presence of a suppressor. Among 3920 total *leu*+ prototrophs, 328 (8.4 percent) were also *his*+ and *ade*+; among 3931 total *his*+ prototrophs, 74 (1.9 percent) were also *leu*+ and *ade*+. These were judged to have arisen by extragenic suppression and were excluded from the count totals.

Genetic characterization of mating-competent prototrophic gene convertants

Diploids from matings between *leu*+ and *his*+ gene convertants of disomic strain 5003-21A-1A and haploid strains 5037-5A (α) or 5037-5B (**a**) were purified by streaking on adenineless medium. Clones were transferred to *GNA* master plates, and the masters were replica-plated to appropriate selective media and to sporulation medium. The sporulation plates were incubated for 3 days and maintained thereafter in the cold; they provided material for ascus dissection with little loss in spore viability over a period of several weeks. After brief digestion with Glusulase (Endo Laboratories) (JOHNSTON and MORTIMER 1959) asci were dissected by micromanipulation directly on the surface of freshly poured (2–24 hr) *GNA* plates (22.5 g/l agar), which were held in inverted position in a Plexiglas device attached to the microscope stage. (We are indebted to MR. N. INOUYE for assistance in constructing these devices.) The dissection plates, each accommodating 20–22 tetrads, served directly as the master plates for subsequent replica platings.

Decisions on the genetic structure of recovered diploids were based on tetrad analyses of dissected asci. For the right arm of linkage group III, tetrads from trisomic ($2n + 1$) and normal ($2n$) diploids are distinguishable on the basis of mating-type phenotype. Trisomic diploids produce, after meiosis, two haploid spores and two disomic spores; the presence of spores unable to mate with either **a** or α haploid testers (hence of disomic genotype **a**/α) is an unambiguous determinant of trisomy. Segregations other than 2+:2*m* at *thr4* and *mal2* also contributed to the determination in some instances. For the left arm of linkage group III, the presence of

spores which yield prototrophic intragenic recombinants at *leu2* or *his4* after exposure to sublethal doses (>95 percent survival) of ultraviolet (254 nm) light (hence of disomic genotype *leu2-1/leu2-27* or *his4-4/his4-290*) is an unambiguous determinant of trisomy.

RESULTS

In earlier experiments (CAMPBELL, FOGEL and LUSNAK 1975) we detected chromosome loss by challenging a nonmating yeast strain, disomic ($n + 1$) for linkage group III and heterozygous at the mating-type locus (**a**/α), to mate with standard haploid testers. Mating-competent haploid segregants (genotype **a** or α) were recovered in diploids and characterized by ascus dissection and tetrad analysis. Many of the chromosomes thus isolated were recombinant in the vicinity of the centromere with respect to the original disomic configuration, specifically in the *leu2*-centromere interval by reciprocal exchange, or at the *leu2* locus by mitotic gene conversion.

These findings have led us to postulate a causal relationship between mitotic chromosome loss and mitotic recombination near the centromere (CAMPBELL and FOGEL 1975). One prediction of this proposal is that the relationship should still obtain in the reverse experiment: the chromosome loss frequency among mitotic gene convertants selected to occur near the centromere (at *leu2*) should be elevated over the spontaneous background; no enhancement should be seen among mitotic gene convertants selected to occur relatively far from the centromere (at *his4*).

The experimental design is outlined in Figure 1. Prototrophic gene convertants arising at heteroallelic loci *leu2* and *his4* were selected in the nonmating (**a**/α) disomic haploid. Only a single prototrophic clone (*leu*$^+$ and *his*$^+$) was taken from each original disomic clone, thus ensuring the complete independence of recovered mitotic events. The purified gene convertants were then tested for mating ability against standard haploid testers. The experiment thus detects primary instances of concomitant chromosome loss among the mitotic gene convertants.

Mating ability can arise by two means: (1) chromosome loss ($n + 1 \rightarrow n$), resulting in mating-competent (**a** or α) haploid segregants; and (2) reciprocal mitotic exchange between the centromere and the mating-type locus (or, rarely, mitotic gene conversion at the mating-type locus), resulting in mating-competent (**a**/**a** or α/α) disomic segregants. These two configurations are clearly distinguished by tetrad analyses of recovered diploids. Genotypic expectations from both chromosome loss and mitotic recombination are listed in Figure 1.

In six independent experiments, 7449 independent prototrophic (*leu*$^+$ or *his*$^+$) clones were characterized phenotypically and tested for mating ability. These clones are almost exclusively spontaneous mitotic gene convertants at one of the two heteroallelic loci; prototrophs formed by extragenic suppression of alleles *leu2-1* or *his4-4* were eliminated by tests for simultaneous reversion of other suppressible markers (chiefly *ade2-1*) carried in the disome (see MATERIALS AND METHODS).

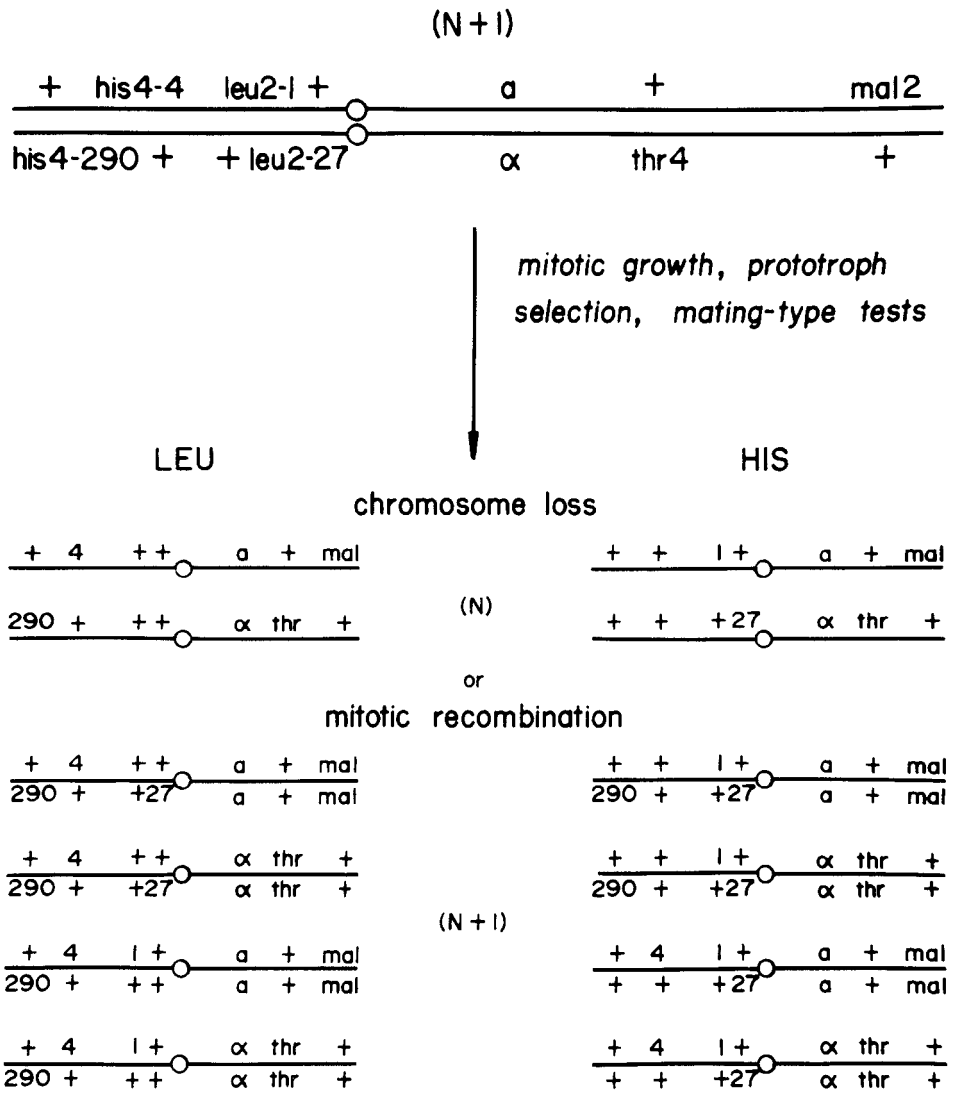

FIGURE 1.—Experimental design outline. The parental disomic haploid ($n+1$) is shown at the top. Genetic map distances are: *his4* (14.2) *leu2* (5.8) *centromere* (26.0) *mating-type locus* (26.2) *thr4* (37.8) *mal2* (CAMPBELL, FOGEL and LUSNAK 1975). *His*+ and *leu*+ prototrophic gene convertants were selected in the disome and subjected to mating-type tests. The original disome is nonmating (**a**/α); mating ability can arise by chromosome loss or by reciprocal mitotic recombination between the centromere and the mating-type locus. Genotypic expectations from both these cases are listed below. The resultant diploid products are thus either $2n$ or $2n+1$ and can be distinguished by tetrad analysis.

TABLE 1

Recovery of mating-competent cells among mitotic gene convertants

Expt	Among *LEU*+ Chr. loss (n)	Mitotic recomb. ($n + 1$)	Exceptions ($n + 1$. n)	Total clones	Among *HIS*+ Chr. loss (n)	Mitotic recomb. ($n + 1$)	Exceptions ($n + 1$. n)	Total clones
1	[3]	[3]	[0]	180	0	0	0	196
2	10	4	1	671	0	0	0	633
3	3	4	0	762	0	2	0	857
4	7	1	2	760	0	2	0	862
5	4	1	1	248	0	1	0	268
6	9	6	1	971	1	1	1	1041
Total	36	19	5	3592	1	6	1	3857

Recovered mating-competent gene convertants were categorized by tetrad analysis as representing chromosome loss ($2n$ diploids), mitotic recombination between the centromere and the mating-type locus ($2n + 1$ diploids), or partial aneuploid exceptions (see text). For experiments 1, 2, 3, and 4, total clones represent all clones tested, corrected for mixed clones and for extragenic suppressors; for experiments 5 and 6, total clones represent the number of *primary* clones tested (actual clones tested: 2922 *leu*+ and 3129 *his*+), corrected for mixed clones and suppressors (see MATERIALS AND METHODS). The genetic structure decisions were based on 2281 dissected asci from 62 recovered diploids. Numbers in brackets [ ] in experiment 1 signify that these diploids were not tetrad analyzed; structure assignments in these cases were based on haploid and diploid phenotypes only.

Mating-competent prototrophic clones are shown in Table 1. The principal results are listed in the columns headed "chr. loss." Among 3592 *leu*+ convertants, 60 mating-competent individuals were found, of which 36 (frequency $1.00 \pm 0.17 \times 10^{-2}$) are instances of chromosome loss. Among 3857 *his*+ convertants, 8 mating-competent individuals were found, of which only one (frequency $2.59 \pm 2.60 \times 10^{-4}$) represents a clear instance of chromosome loss. Chromosome loss is nearly 40 times as frequent among *leu*+ gene convertants as among *his*+ gene convertants. The difference is highly significant ($P \ll 0.001$). Moreover, the chromosome loss frequency among *his*+ convertants, which serves here as an internal control, does not differ significantly ($P > 0.45$) from the previously observed unselected chromosome loss frequency in the disome ($1.29 \pm 0.41 \times 10^{-4}$) (CAMPBELL, FOGEL and LUSNAK 1975). These data confirm our prediction that mitotic recombinants arising near the centromere should be significantly enriched for chromosome loss events, whereas recombinants arising far from the centromere should not. The correlation is thus preserved independent of which mitotic event is initially selected.

Mating-competent gene convertants in which the parental disomic state persisted are listed in the columns headed "mitotic recomb." in Table 1. These represent conversions to prototrophy at *leu2* or *his4* accompanied by an additional mitotic exchange that renders the mating-type locus homozygous (**a**/**a** or α/α); the recovered trisomic diploids are clearly identifiable by tetrad analysis.

Diploids which were "partial" aneuploids, *i.e.*, trisomic for the left arm of chromosome III but normal diploid for the right arm, were found in crosses with 6 of the mating-competent convertants ("exceptions" columns in Table 1). Tetrad ratios indicative of trisomy were observed for the left arm markers *his4* and

leu2, implying recovery of both disomic strands, whereas segregations at the mating-type locus and, in 5 instances, at *thr4* and *mal2*, were clearly 2:2, implying recovery of only a single strand from the disome. The formal genetic structure of these partial aneuploids is shown in Table 2. In each case the right arm is continuous with one of the two left arms, though the limited data do not permit unambiguous assignments; the remaining strand might then represent a telocentric in which the genetically marked portion of the right arm is deleted. We judge this structure more likely than one other possibility—that the left arm is tandemly duplicated—because in all six cases one or meiotic tetrads were found (total 13 of 133 asci) in which disomy (**a**/α) for the right arm appeared in invariable association with spore lethality. Typically, spore survival was 2 live:2 dead, and one or both survivors were nonmating (**a**/α). We have never observed segregations of this type in normal diploids. We interpret these tetrads as deriving from meiotic segregations in which both intact chromosomes move at first division to one pole, and the (lethal) telocentric moves to the opposite pole. In all such tetrads, spore lethality segregated at the first meiotic division.

Since only the converted strand is recovered in chromosome loss cases, rigorous conclusions on which allele was converted are not possible. The following indirect analysis, however, may serve to illustrate the nature of the data (Table

TABLE 2

Schematic structures of (n + 1 . n) *aneuploid exceptions*

Class	Inferred structure		Number found
1	+ 4 + + —○— 290 + + 27 —○—	**a** + *mal2*	1
2	+ 4 + + —○— 290 + + 27 —○—	α *thr4* +	2
3	+ 4 + + —○— 290 + + + —○—	α *thr4* +	2
4	+ 4 1 + —○— + + + 27 —○—	α *thr4* +	1

Formal genetic structures of partial aneuploid exceptions were determined by tetrad analyses which indicate that two centromeres were recovered from the disome, but which do not allow clear determination of which left arm is telocentric and which left arm is continuous with the right arm. Only the strand(s) recovered from the disome are indicated; in each case the other complete chromosome constituting the diploid [$2n + 1$ (left) : $2n$ (right)] is that contributed by strain 5037–5A (α) (Class 1) or strain 5037–5B (**a**) (Classes 2, 3, 4). (See MATERIALS AND METHODS).

TABLE 3

Genetic structure of LEU+ *chromosomes recovered after chromosome loss* (n + 1 → n)

Class	Genotype	Number found	Interpretation
1	+ 4 / + + — a + *mal2*	3	2–1 → +
2	290 + / + + — *α thr4* +	11	2–27 → +
3	290 + / + + — **a** + *mal2*	19	(a) 2–1 → +; I (b) 2–27 → +; II

Region

(*n* + 1)

I II

+ *his4-4* *leu2-1* + — **a** + *mal2*

his4-290 + — + *leu2-27* — *α thr4* +

3). If the 33 *leu*+ convertants recovered after chromosome loss and analyzed in diploids, 3 (all **a**) carried the *his4-4* allele from the disome, and 30 (11α, 19**a**) carried the *his4-290* allele. Inspection of the original disomic marker configuration (Figure 1 and Table 3) suggests that the 3 *his4-4* **a** chromosomes could derive from conversion *leu2-1* → + and recovery of the converted strand intact; similarly, the 11 *his4-290* α chromosomes are consistent with conversion *leu2-27* → + and recovery of the converted strand intact. The 19 *his4-290* **a** chromosomes may be explained by invoking an additional exchange in the disome. There are two possibilities: (a) conversion *leu2-1* → +, plus a reciprocal exchange in the distal *leu2-his4* interval (region I); and (b) conversion *leu2-27* → +, plus a reciprocal exchange in the proximal *leu2*-centromere interval (region II). [Formally, this exchange could occur anywhere in the *leu2*-mating-type locus interval. We consider exchange on the right arm unlikely, however, in view of the close relation between conversion and adjacent marker exchange (HURST and FOGEL 1964; FOGEL and HURST 1967; HURST, FOGEL and MORTIMER 1972).] Since both exchange categories probably contribute to this phenotypic class, it is appropriate to enquire whether an approximate partition is possible from other data.

Tetrad data from the recovered 2*n* + 1 diploids are applicable here. Since one chromatid from each sister pair is recovered in these cases, direct inferences on which disomic allele was converted may be made. These data could be considered relatively unbiassed, since the only recovery condition is a reciprocal exchange on the other arm. Of the 15 *leu*+ convertants isolated in 2*n* +1 diploids that could be analyzed, 10 were converted *leu2-1* → +, and 5 were converted *leu2-27* → +. Four of the *leu2-1* convertants, but none of the *leu2-27* convertants, were also recombined at *his4* (genotype *his4-290/his4-290*). Hence, for these diploids conversion at *leu2-1* is more frequent than conversion at *leu2-27*, and nearly half (4/10) the *leu2-1* conversions are recombined distally for *his4*.

If the latter proportion (4/10) is applicable to the chromosome loss cases (Table 3), the recovery of only 3 nonexchange chromosomes interpreted as conversion *leu2-1* → + implies that possibility (a) (conversion *leu2-1* → +, plus

exchange in the distal interval) can account for only a small minority (approximately 2) of the 19 *his4-290* **a** chromosomes; the majority ($19 - 2 = 17$) must be attributed to possibility (b) (conversion *leu2-27* → +, plus exchange in the proximal interval). On this basis, then, the 33 chromosome loss cases may be provisionally apportioned as: 5 *leu 2-1* → + (3 + 2 distally recombined), and 28 *leu2-27* → + (11 + 17 proximally recombined). Comparison of these tentative assignments with the allelic conversion pattern in $2n + 1$ diploids (10 *leu2-1* → +, 5 *leu2-27* → +) suggests that chromosome loss events are significantly ($P < 0.001$) enriched for *leu2-27* conversions, many of which are also proximally recombined.

The strong inference from this analysis is that *leu2-27* is the proximal allele: the converted segment, which must terminate between the two *leu2* alleles, would then extend proximally toward the centromere in nearly all (28/33) the recovered chromosome loss cases. Although the allele map order at *leu2* has not been unambiguously determined in meiotic tetrads (FOGEL and ROTH 1974), the tentative assignments are consistent with other data. For example, at the *his1* locus (HURST and FOGEL 1964) reciprocal mitotic exchanges in intervals adjacent to the heteroallelic site are notably correlated with conversion of the nearer allele; the same is true for meiotic gene conversion at *his1* (FOGEL and HURST 1967). Clearly, an independent determination of the *leu2* allele order would critically test the validity of this analysis.

Finally, as Table 1 implies, the vast majority of recovered prototrophs were nonmating (**a**/α) and hence disomic. Despite the absence of confirmatory tetrad data, these haploids could still be characterized for recombinant phenotypes. The results are shown in Table 4. Of 3532 nonmating *leu*$^+$ convertants, 446 (12.6 percent) were also recombined at *his4*. Of 3849 *his*$^+$ convertants, 35 (0.9 percent) were also recombined at *leu2*. These recombinants are more probably at-

TABLE 4

Recombinants among nonmating (**a**/α; n + 1) *mitotic gene convertants*

Expt	Among *LEU*$^+$		Total clones	Among *HIS*$^+$		Total clones
	HIS	*HIS*$^+$		*LEU*	*LEU*$^+$	
1	22	1	174	0	4	196
2	86	4	656	5	6	633
3	93	6	755	1	2	855
4	82	9	750	0	5	860
5	34	3	242	7	0	267
6	101	5	955	4	1	1038
Total	418	28	3532	17	18	3849

Recombinants were detected phenotypically in recovered nonmating (**a**/α) gene convertants selected in the disome. Recombinants were not partitioned genotypically. The HIS and LEU categories represent genotypes *his4-290/his4-290* or *his4-4/his4-4*, and *leu2-1/leu2-1* or *leu2-27/leu2-27*, respectively, and probably arose by reciprocal exchange. The HIS+ and LEU+ categories probably arose by mitotic gene conversion at *his4* and *leu2*, respectively. Total clones represent total primary clones tested, corrected for mixed clones and extragenic suppressors (see MATERIALS AND METHODS) and for mating-competent individuals (Table 1).

tributable to reciprocal exchange between the two heteroallelic loci, or less probably to mitotic gene conversion at the second heteroallelic locus. Since the spontaneous reciprocal exchange frequency between the two loci is less than 10^{-4}, and the single-site mitotic conversion frequencies are 4×10^{-5} (*his4*) and 4×10^{-6} (*leu2*) (CAMPBELL, FOGEL and LUSNAK 1975), it is clear that convertant selection at either heteroallelic locus notable enhances the probability of recombination elsewhere on the same chromosome arm. These data corroborate, and extend to disomic haploids, earlier findings suggesting that mitotic recombination in yeast occurs as a result of co-extensive homologue interaction in a small fraction only of the mitotic population (FOGEL and HURST 1963; HURST and FOGEL 1964).

DISCUSSION

The principal results may be summarized as follows: (1) Disomic chromosome loss is markedly enhanced over basal levels among mitotic gene convertants selected to arise near the centromere; no such enhancement is seen among convertants arising comparatively far from the centromere. (2) Chromosome loss appears primarily correlated with conversion of the inferred proximal allele, as determined by indirect analysis, and many of these convertants are reciprocally recombined in the adjacent proximal interval. (3) Aneuploid exceptions exhibiting the formal structure [$2n + 1$ (left):$2n$ (right)] have been found; preliminary meiotic data suggest that these diploids may contain telocentrics in which the right arm is deleted.

The hypothesis underlying this work is that disomic chromosome loss and mitotic recombination near the centromere are functionally related. The data are persuasive of a causal link between these two events and explicitly confirm two aspects of this idea: the correlation is preserved independent of which mitotic event is initially selected, and the correlation obtains only for recombinants arising close to the centromere. Since recombination is necessarily predicated on homologue interaction, it clearly must precede the segregational event(s) leading to chromosome loss. But the data do not allow precise determination of when, before chromosome loss, mitotic recombination occurs, since within the disomic population, recombinant lines can arise in any cell generation. The data suggest, however, that the two events are closely spaced in time, perhaps arising in the same cell generation.

We have previously speculated (CAMPBELL, FOGEL and LUSNAK 1975) that mitotic exchange might potentiate sister centromeres to separate prematurely. Normally, sister strands remain conjoined at their centromeres through metaphase and only then separate and disjoin to opposite poles; if exchange were to alter sister centromere binding, or accelerate strand separation, abnormal segregations leading to chromosome loss might ensue. Meiotic mutants in Drosophila (DAVIS 1971) and in Lycopersicon (CLAYBERG 1959), which exhibit high frequencies of equational nondisjunction as a consequence of precocious sister centromere separation, are consistent with this speculation. On the other hand, GELBART (1974) has described a mutant in Drosophila that causes frequent somatic

chromosome loss without concomitant formation of the trisomic product expected from mitotic (equational) nondisjunction. In the present case, the strand recovered after chromosome loss must necessarily have disjoined from its sister. However, the data do not reveal the fate of the other sister pair, since the putative $n + 2$ mitotic segregant anticipated from nondisjunction, *sensu stricto*, is phenotypically indistinguishable in our system for the original disomic parent.

Most yeast chromosomes, including chromosome III, are genetically metacentric (MORTIMER and HAWTHORNE 1975). Hence, size distribution analyses of yeast nuclear DNA (PETES, *et al.* 1973), suggesting that each chromatid contains a single DNA duplex, could be taken to imply molecular continuity through the centromere. Moreover, synaptonemal complexes in yeast meiotic prophase appear to exhibit end-to-end continuity (BYERS and GOETSCH 1975), and spindle microtubule termination in yeast chromatin with no apparent kinetochore (centromere) differentiation has been reported (KUBAI 1975). By these criteria, then, the yeast centromere appears physically indistinguishable from other chromosomal elements. Yet the clear genetic identity of the centromere (as that locus which invariably segregates at the first meiotic division) requires that it possess properties that differentiate it functionally from the rest of the chromosome.

The centromere could, for example, contain unique informational sequence(s) that are recognized only by the segregational apparatus. The correlation between mitotic recombination near the centromere and chromosome loss might then be understood if such sequences were *not* distinguished from other chromosomal segments by certain DNA-substrate events—for example, genetic recombination. If this were so, genetic recombination involving the centromeric region could occur which, if not completed or resolved before chromosome (chromatid) mobilization, might interfere with proper mitotic disjunction.

That the chromosome-loss recombinants were selected some distance (5.8 cM, meiotic) from the centromere in this study does not exclude centromere involvement, since the cross connection postulated as the recombination intermediate (HOLLIDAY 1964) is potentially free to migrate by rotary diffusion along the paired chromatids (SIGAL and ALBERTS 1972; MESELSON 1972; MESELSON and RADDING 1975), thereby generating a variable-length heteroduplex which could be resolved as a converted segment of some length. The finding that chromosome loss is primarily (28 of 33) associated with single-site conversion of the inferred proximal *leu2* allele is consistent with this view: prototroph production by proximal allele conversion means only that the converted segment must terminate between the two heteroalleles, but does not exclude proximal extension for a considerable distance. The five instances of inferred distal allele conversion need not contradict this argument, since additional converted segments closer to the centromere could arise but remain undetected. The data do not rule out, however, a second, less direct mechanism to account for these cases. These considerations suggest that conditions which might shorten the average length of converted segments should at the same time uncouple chromosome loss from centromere-adjacent exchange (FOGEL and ROTH 1974). Conversely, the mutant described by HABER (1974) as having chronically elevated frequencies of chromosome III

mitotic loss might also be expected to exhibit elevated mitotic recombination.

Finally, the discovery in this work of partial aneuploids, from whose meiotic behavior the presence of functional telocentric chromosomes is provisionally inferred, suggests that yeast centromeres possess features in common with those of other eucaryotes. The idea that telocentrics arise by centromere misdivision (DARLINGTON 1939) and the cytological and functional evidence that some eucaryotic centromeres may be duplicate structures (LIMA-DE-FARIA 1955a,b, 1958; STEINETZ-SEARS 1966) may thus be applicable to yeast. Clearly, more extensive analyses will be necessary to confirm our initial findings and to distinguish, for example, between true telocentrics and isochromosomes. The findings reported here, however, may serve as an initial approach to delimiting possible models of yeast centromere structure and function.

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