

# Construction of telocentric chromosomes in *Saccharomyces cerevisiae*

(chromosomal deletions/meiotic pairing/mitotic stability/fluctuation test)

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**ABSTRACT** We describe a simple method for the construction of large chromosomal deletions in yeast. Diploid yeast cells were transformed with DNA fragments that replace large regions of the chromosomes by homologous recombination. Using this method, we have constructed a telocentric chromosome III in which  $\approx 100$  kilobases (kb) of DNA has been removed from the left arm of the chromosome, so that the centromere is 12 kb from the left telomere. This telocentric chromosome is mitotically stable. Its rate of loss in a diploid strain is  $2.5-7.4 \times 10^{-4}$  per cell division compared to a rate of loss of  $0.36-1.8 \times 10^{-4}$  per cell division for a normal chromosome III. It also segregates  $2^+ : 2^-$  with fidelity during meiosis. The construction of systematic deletions in a chromosome should be useful in determining the essential features for proper chromosomal segregation and replication.

Transformation of *Saccharomyces cerevisiae* by DNA plasmids can occur by the integration of the plasmid into the genome via homologous recombination, giving rise to stable transformants (1). In some of the transformants, the homologous DNA in the genome is replaced by the corresponding plasmid sequence without the integration of the plasmid vector sequence. These substitution transformants result from either gene conversion or double reciprocal recombination. It has further been shown that double-stranded breaks introduced within the yeast sequence in the plasmid stimulate the frequency of integrative transformation (2, 3). Presumably, strand invasion of the chromosomal DNA by the homologous free-ended DNA increases the frequency of recombination and gene conversion. These properties of DNA transformation allow gene alterations in yeast by a variety of simple manipulations such as gene interruption (4, 5), gene transplacement (6), and gene replacement (5). Based on the same principle, it should be possible to construct chromosomal deletions in which large regions of the chromosome are substituted by small DNA sequences on the plasmids. However, such deletions can only be made provided there is no requirement for size parity between the chromosomal and plasmid sequences. The systematic construction of large deletions in a chromosome should be useful for identifying components important for the proper segregation and replication of the chromosome. Furthermore, these deletions should indicate if the overall size of a chromosome or if the distance between the telomeres and the centromere of a chromosome is critical for the proper functioning of the chromosome (7, 8).

We have previously described the isolation of a family of repetitive autonomously replicating sequences (ARSs) located near the telomeres of yeast chromosomes (9). Analysis of these telomeric DNA clones showed that there is a highly conserved, 6.7-kilobase (kb) repetitive sequence,  $Y'$ , and a

less conserved, repetitive sequence,  $X$ , located near most yeast telomeres (10). We used this subtelomeric sequence to construct large deletions that extend from the subtelomeric  $Y'$  sequence to the *LEU2* region or to the centromeric region on the left arm of chromosome III. The latter deletion removes almost all sequences between the centromere and the left telomere, creating a telocentric chromosome.

## MATERIALS AND METHODS

**Bacterial and Yeast Strains.** The *Escherichia coli* strain HB101 (*thr leuB pro hsr hsm recA*) was obtained from M. Carlson. The *S. cerevisiae* strain 8534-8C (*MATa ura3-52 his4 leu2*) was obtained from G. Fink. Strain 320-13B (*MATa ura3-52 trp1 met3 MAL2*) was constructed in this laboratory. The diploid 3285 was made by crossing strain 320-13B with strain 8534-8C.

**Plasmids.** Plasmids YIp5 and YRp10 were obtained from D. Stinchcomb (11). Plasmid YRp131B was constructed by C. Chan (9). The plasmid H4YRB, which contains the entire *HIS4* gene, has been described (12).

**Construction of Plasmids.** Plasmid pLUY-1 was constructed by first cloning the 2.3-kb *Sal I/Xho I* fragment containing the yeast *LEU2* gene into the *Sal I* site of the YIp5 plasmid to form the plasmid pGK8. The 6.7-kb *Sal I* fragment containing the  $Y'$ , or (*Y-131*), sequence from the plasmid YRp131B was inserted into the *Sal I* site of plasmid pGK8 to form plasmid pLUY-1. Plasmid pCUY-1 was constructed by cloning the *BamHI/HindIII* fragment containing *CEN3* (13) into the plasmid YIp5 to form plasmid pCU-1. The 6.7-kb *Sal I* fragment containing the  $Y'$  sequence was inserted into the *Sal I* site of plasmid pCU-1 to form plasmid pCUY-1.

**Construction of the Diploid Strains SUH1 and SUH2.** The *URA3* gene was inserted into the *HIS4* region of the strain 320-13B by the following steps. The 2.3-kb *HindIII* fragment within the *HIS4* gene of the plasmid H4YRB was replaced by the 1.1-kb *HindIII* fragment containing the *URA3* gene isolated from the plasmid YRp10. This resulting plasmid was cleaved with the enzyme *EcoRI* to produce a 4.7-kb DNA fragment that contains the *URA3* gene joined to DNA from the *HIS4* region on either side. This 4.7-kb *EcoRI* fragment was used to transform strain 320-13B to *Ura*<sup>+</sup> prototrophy. Eight of 100 *Ura*<sup>+</sup> transformants examined were also *His*<sup>-</sup>. Two of these *Ura*<sup>+</sup> *His*<sup>-</sup> transformants were analyzed by DNA hybridization to verify that the *URA3* gene had replaced part of the *HIS4* gene on the chromosome. These two transformants were crossed with strain 8534-8C to form the diploid strains SUH1 and SUH2.

**Media and Enzymes.** YEPD medium (2% peptone/1% yeast extract/2% glucose), synthetic complete medium, and complete medium without uracil, have been described (14). 5-Fluoroorotic acid was purchased from PCR Research Chemicals, Gainesville, FL. Complete medium supplement-

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Abbreviation: kb, kilobase(s).

ed with 0.5 mg of 5-fluoroorotic acid per ml was used for the positive selection of orotidylate decarboxylase-defective (*ura3*) strains (15). Media for *E. coli* have been described by Miller (16).

**Fluctuation Analysis.** A single yeast colony isolated from an agar plate containing complete medium without uracil was suspended in water and used as an inoculant. Twenty test tubes each containing 5 ml of YEPD medium were inoculated with  $\approx 100$  cells per tube and were allowed to grow for 4 days to stationary phase ( $2-4 \times 10^8$  cells). These 20 cultures were diluted and plated on 5-fluoroorotic acid and YEPD medium. Colonies that grow on the plates with YEPD medium give an estimation of the cell density of each culture. The number of *Ura*<sup>-</sup> cells in each culture was determined by the number of colonies growing on the 5-fluoroorotic acid plates. The rate of chromosome loss per cell division and the variance for each experiment were calculated as described by Luria and Delbruck (17).

**DNA Preparation and Transformation.** Methods for the preparation of plasmid DNA (18), rapid DNA minipreps (19), and genomic yeast DNA (20) have been described. Transformation of *E. coli* (21) and yeast (1) were carried out as described.

**Other Procedures.** Preparation of nick-translated <sup>32</sup>P-labeled DNA probes (22), transfer of DNA to nitrocellulose (23), and conditions for DNA hybridization (24) were carried out according to published procedures.

## RESULTS

**Construction of Deletions on the Left Arm of Chromosome III.** Two plasmids were made for the construction of deletions on the left arm of chromosome III. Plasmid pLUY-1 (Fig. 1a) contains a selectable marker, *URA3*, the repetitive telomere-associated sequence, *Y'* or (*Y-131*), and the *LEU2* gene of chromosome III. Cleavage of this plasmid by the restriction enzyme *Bst*EII yields a linear fragment of 8.5 kb, which carries the *URA3* gene, with 2.3 kb of the *Y'* sequence at one end and 0.7 kb of the DNA from the *LEU2* region at the other end. The restriction map of this DNA fragment is shown in Fig. 2a (solid bar). Similarly, the plasmid pCUI-1 (Fig. 1b) contains the *URA3* gene, *Y'* sequence, and *CEN3*. Cleavage of this plasmid by the restriction enzyme *Bam*HI yields a linear fragment of 7.6 kb, which carries the *URA3* gene, with 1.1 kb of the *Y'* sequence at one end and 1.6 kb of DNA from the *CEN3* region at the other end. The restriction map of this DNA fragment is shown in Fig. 3a (solid bar). Two hundred micrograms of each of these DNA digests was used to transform diploid strain 3285. Five hundred and forty *Ura*<sup>+</sup> transformants were obtained by using plasmid pLUY-1. Two of the *Ura*<sup>+</sup> transformants, DLT1 and DLT2, were also *His*<sup>-</sup> and *Leu*<sup>+</sup>, suggesting that the plasmid sequence had replaced the *HIS4* gene and all other sequences between the telomeric *Y'* sequence and the *LEU2* gene in one copy of chromosome III (Fig. 1a). Similarly, 410 *Ura*<sup>+</sup> transformants were obtained by using the plasmid pCUI-1. Five of these transformants, DCT1-DCT5, were also *His*<sup>-</sup> and *Leu*<sup>-</sup>, suggesting that the entire left arm from the *Y'* sequence to *CEN3* was replaced in the *His*<sup>+</sup> *Leu*<sup>+</sup> copy of chromosome III (Fig. 1b). All seven of these transformants were *Mal*<sup>+</sup> and behaved as nonmaters, indicating that they still contained information from both copies of the right arm of chromosome III.

If extensive deletions had been made on the left arm of chromosome III, one would expect haploid cells containing such deletion chromosomes to be nonviable. Thus, on sporulation, each of the DLT and DCT transformants should produce two viable spores and two nonviable spores. Diploids DLT1, DLT2, DCT1, DCT2, and DCT4 were sporulated and

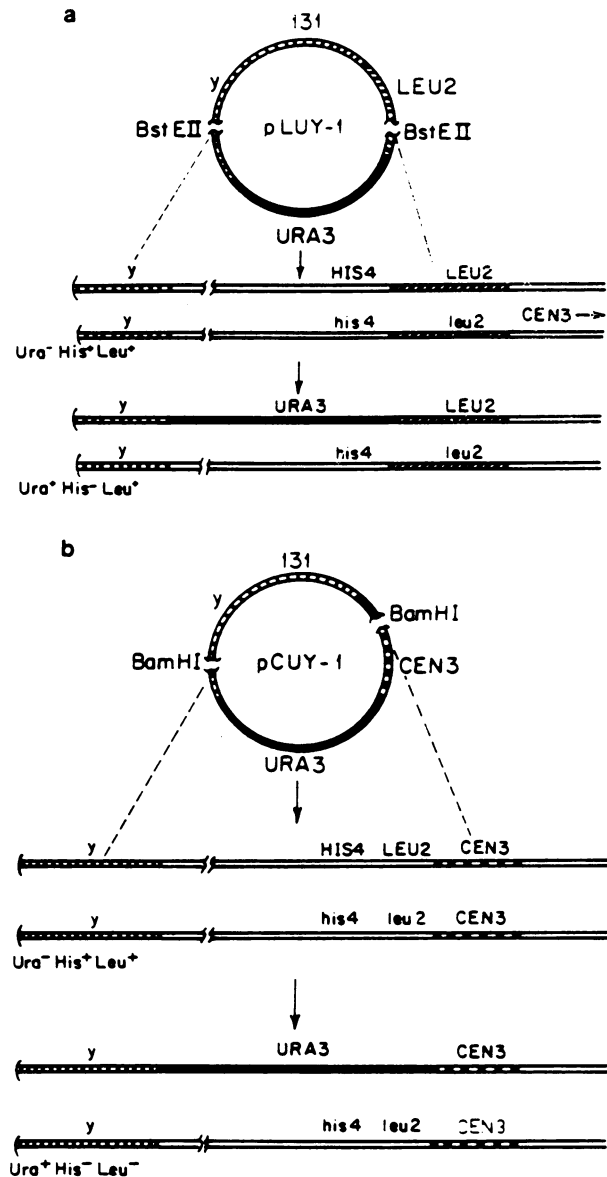


FIG. 1. (a) Construction of a deletion between *LEU2* and the telomere on the left arm of chromosome III. Plasmid pLUY-1 was digested with *Bst*EII and used to transform diploid 3285 to *Ura*<sup>+</sup>. The *His*<sup>-</sup> *Leu*<sup>+</sup> transformants presumably contain a deletion in the copy of chromosome III carrying the *HIS4* and *LEU2* genes. (b) Construction of the deletion between *CEN3* and the telomere on the left arm of chromosome III. Plasmid pCUI-1 was digested with *Bam*HI and used to transform diploid 3285 to *Ura*<sup>+</sup>. The *His*<sup>-</sup> *Leu*<sup>-</sup> transformants presumably have a deletion in the *HIS4 LEU2* copy of chromosome III in which the region between *CEN3* and the telomere has been deleted.

the tetrads were dissected and analyzed (Table 1). At most, two viable spores were obtained from each tetrad. Among the 40 tetrads dissected from each diploid, only one *Ura*<sup>+</sup> spore from strain DLT2 and two from strain DCT1 were obtained. This suggests that *Ura*<sup>+</sup> is very tightly linked to the nonviable phenotype. We also examined the segregation of *LEU2* in tetrads obtained from diploids DLT1 and DLT2. Only six *Leu*<sup>+</sup> spores were obtained from the 80 tetrads dissected, indicating that *LEU2* is also tightly linked to the nonviable phenotype.

**Analysis of Deletion Chromosomes.** To analyze the exact structure of the chromosomal deletion in the diploid DLT1 and to verify that the pBR322 DNA is located near the telo-

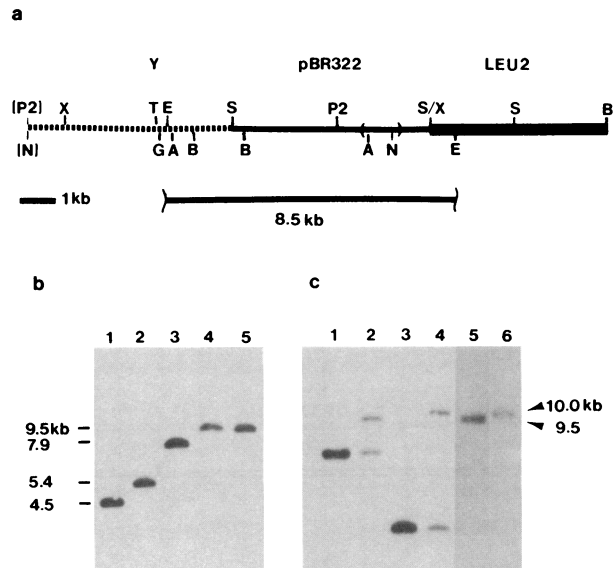


FIG. 2. Hybridization analysis of the deletion on chromosome III in the DLT1 strain. (a) Restriction maps of the rearranged region in the transformant DLT1 and the transforming DNA fragment (shown as solid bar).  $\square$ ,  $Y'$  sequence;  $\text{---}$ , pBR322 DNA;  $\text{---}$ ,  $URA3$  sequence;  $\text{---}$ , sequence from  $LEU2$  region. Restriction enzyme sites shown are as follows: A, *Ava* I; B, *Bam*HI; E, *Bst*EII; G, *Bgl* II; H, *Hind*III; N, *Nco* I; S, *Sal* I; T, *Sst* I; X, *Xho* I. Two micrograms of genomic DNA from the DLT1 strain was digested with different restriction enzymes, separated on an agarose gel, transferred to a nitrocellulose filter, and hybridized with a  $^{32}\text{P}$ -labeled probe. (b) The 2.9-kb *Pvu* II/*Sal* I fragment from pBR322 DNA was used as a hybridization probe. Genomic DNA was digested with the following: lane 1, *Pvu* II/*Ava* I; lane 2, *Pvu* II/*Bgl* II; lane 3, *Pvu* II/*Xho* I; lane 4, *Pvu* II/*Nco* I; lane 5, *Pvu* II. (c) Lanes 1–4, the 1.7-kb *Sal* I/*Bst*EII fragment from the  $LEU2$  region from plasmid pSZ58 (24) was used as hybridization probe. Lane 1, DNA from parent strain 3285 digested with *Bam*HI; lane 2, DLT1 DNA digested with *Bam*HI; lane 3, 3285 DNA digested with *Sst* I; lane 4, DLT1 DNA digested with *Sst* I. Lanes 5 and 6, 1.4-kb *Pvu* II/*Sal* I fragment from pBR322 DNA was used as a hybridization probe for genomic DNA from strain DLT1 digested with *Bam*HI (lane 5) or *Sst* I (lane 6).

mere, we prepared genomic DNA from this diploid. The DNA was cut with the enzyme *Pvu* II, either alone or in combination with a number of enzymes whose restriction sites on the subtelomeric  $Y'$  sequence are known (Fig. 2b). The 2.9-kb *Pvu* II/*Sal* I fragment of the pBR322 DNA was used to probe this DNA (Fig. 2a). From these hybridization results, a restriction map of the region immediately centromere-distal to the pBR322 DNA was constructed (Fig. 2a). This restriction map is identical to that of the  $Y'$  sequence on plasmid pLUY-1 from the  $Y'$ -pBR322 junction to  $\approx 5.5$  kb from the junction. Here, we observed apparent *Pvu* II, *Sma* I, and *Nco* I restriction sites that are not present in any of the cloned copies of the  $Y'$  sequence. These sites coincide with the region where the chromosome ends in some  $Y'$  sequences (10). We believe that these apparent restriction sites represent the end of the chromosome (Fig. 2a).

If the pBR322 sequence located near the telomere is joined to the  $LEU2$  region of chromosome III, we should be able to show that probes from the pBR322 DNA and the  $LEU2$  region hybridize to the same junction fragment on the rearranged chromosome III. Genomic DNA from the diploid DLT1 and its parent strain 3285 was cut with the enzyme *Bam*HI (Fig. 2c, lanes 1, 2, and 5) and the enzyme *Sst* I (Fig. 2c, lanes 3, 4, and 6). The 1.7-kb *Sal* I/*Bst*EII fragment from the  $LEU2$  region hybridized to a single *Bam*HI fragment (7.1 kb; lane 1) in the parent strain and to two *Bam*HI fragments (9.5 and 7.1 kb; lane 2) in the deletion strain. Thus, the 9.5-

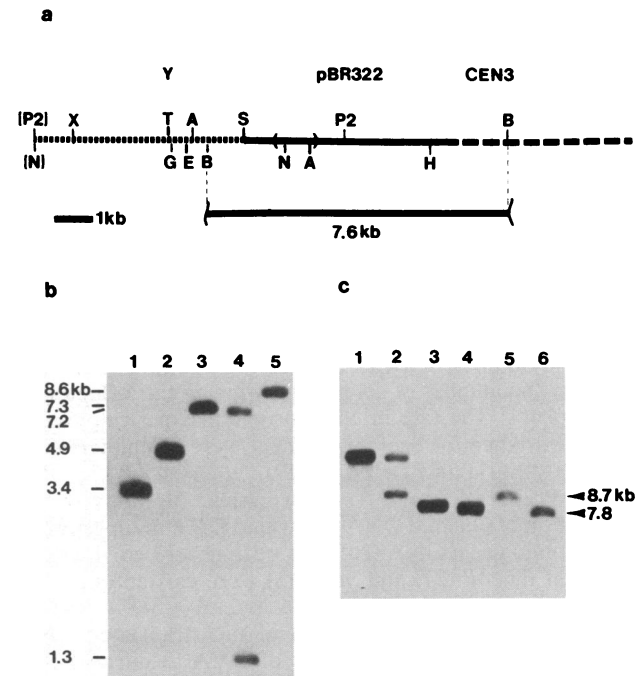


FIG. 3. Hybridization analysis of deletion chromosome in the DCT4 strain. (a) Restriction maps of rearranged region in the transformant DCT4 and the transforming DNA fragment (solid bar).  $\square$ ,  $CEN3$  sequence; other symbols are as in Fig. 2. Hybridization analysis was carried out as described in Fig. 2 legend. (b) Genomic DNA from strain DCT4 was hybridized with the 1.4-kb *Pvu* II/*Sal* I fragment from pBR322 DNA. Genomic DNA was digested with the following: lane 1, *Pvu* II/*Bam*HI; lane 2, *Pvu* II/*Bgl* II; lane 3, *Pvu* II/*Xho* I; lane 4, *Pvu* II/*Nco* I; lane 5, *Pvu* II. (c) Lanes 1–4, the 2.6-kb *Bam*HI/*Eco* RI fragment from the  $CEN3$  region of plasmid pYe46B2 (13) was used as the hybridization probe. Lane 1, DNA from parent strain 3285 was digested with *Pvu* II; lane 2, DCT4 DNA digested with *Pvu* II; lane 3, 3285 DNA digested with *Bam*HI; lane 4, DCT4 DNA digested with *Bam*HI. (The hybridizing band in lane 4 corresponds to a doublet of 8.0 and 7.8 kb.) Lanes 5 and 6, the 2.9-kb *Pvu* II/*Sal* I fragment from pBR322 DNA was used as a hybridization probe for genomic DNA from strain DCT4 digested with *Pvu* II (lane 5) or *Bam*HI (lane 6).

kb fragment in the deletion strain corresponds to the new junction fragment in the deletion chromosome. When the 1.4-kb *Pvu* II/*Sal* I fragment from pBR322 DNA was used as a hybridization probe, it hybridized to this same 9.5-kb junction fragment (lane 5). We were also able to show that the same two hybridization probes hybridized to a single 10-kb junction fragment in the *Sst* I genomic digest of the deletion strain (lanes 4 and 6). Thus, the pBR322 DNA and the  $URA3$  gene have been joined to the  $LEU2$  region on the chromo-

Table 1. Spore viability in tetrads derived from diploids with chromosome III deletions

Diploid	Number of tetrads dissected	Number of viable spores per tetrad				
		4	3	2	1	0
DLT1	40	0	0	35	5	0
DLT2	40	0	0	36	4	0
DCT1	40	0	0	36	2	2
DCT2	40	0	0	36	4	0
DCT4	40	0	0	38	2	0
3285	10	9	1	0	0	0

Diploids were grown in YEPD to a concentration of  $8 \times 10^7$  cells per ml and were then sporulated in 1% potassium acetate (pH 6.7). Tetrads were dissected by micromanipulation and spore viability was determined.

some. Experiments using DNA from the diploid DLT2 produced identical results. Hence, in strains DLT1 and DLT2, we have deleted  $\approx 80$  kb of DNA from the left arm of one chromosome III. The end of this chromosome is 12 kb from the *LEU2* gene and 32 kb from the centromere (25).

Similar experiments were carried out with diploids DCT1, DCT2, and DCT4. Genomic DNA from the diploid DCT4 was digested with various restriction enzymes and probed with the 1.4-kb *Pvu* II/*Sal* I fragment from pBR322 DNA (Fig. 3b). A restriction map of the region centromere-distal to the pBR322 DNA was constructed (Fig. 3a). This map is identical to that of the *Y'* sequence on the plasmid pCUY-1 for  $\approx 5.5$  kb from the *Y'*-pBR322 junction. Again, the apparent coincident *Pvu* II, *Sma* I, and *Nco* I restriction sites indicate that the rearranged chromosome ends within the *Y'* sequence  $\approx 5.5$  kb from the *Y'*-pBR322 DNA junction. We were also able to show that probes from the pBR322 DNA and the *CEN3* region hybridized to the same junction fragments—i.e., the 8.7-kb *Pvu* II fragment and the 7.8-kb *Bam*HI fragment, on the rearranged chromosome III (Fig. 3c). The same results were obtained when DNA from the diploids DCT1 and DCT2 were used. Thus, in diploids DCT1, DCT2, and DCT4, we deleted  $\approx 100$  kb of DNA from the telomeric *Y'* sequence to the centromere in chromosome III. The end of the deleted chromosome is 12 kb from *CEN3* (Fig. 3a).

**Stability of Deletion Chromosomes.** To detect slight changes in the mitotic stability of a chromosome, we used the fluctuation analysis described by Luria and Delbruck (17). The recent finding that mutants defective in orotidylate decarboxylase (*ura3* mutant strains) are resistant to fluoroorotic acid (15) allows us to select directly for chromosome loss events. Since the deletion chromosome in each of our deletion strains contains the only copy of the *URA3* gene in the genome inserted at the site of the deletion, it is possible to directly select for *Ura*<sup>-</sup> strains that have lost the deletion chromosome. As a control, the *URA3* gene was inserted into the *HIS4* gene of a normal chromosome III in wild-type diploid strains, SUH1 and SUH2, homozygous for *ura3*. The rate of loss of this normal chromosome III carrying the *URA3* gene was also determined.

The results of the fluctuation analysis of the rate of chromosome loss in the DLT strains (deletion from *LEU2* to the telomere), the DCT strains (deletion from centromere to telomere), and the SUH strains (normal chromosome III) are summarized in Table 2. To distinguish between *Ura*<sup>-</sup> strains that have lost the deletion chromosome and those that have resulted from mitotic recombination, other markers on the right arm of chromosome III were also examined. *Ura*<sup>-</sup> cells that are also *Mal*<sup>-</sup> and  $\alpha$ -maters have lost the chromosome III carrying the *MATa* allele. The rate of loss of the deletion chromosome in the DLT strains (DLT1 and DLT2) is  $1.4$ – $1.9 \times 10^{-4}$  per cell division. In comparison, the rate of loss of the deletion chromosome in the DCT strains (DCT1, DCT2, and DCT4) is  $2.5$ – $7.4 \times 10^{-4}$  per cell division. The ratio of the growth rates of the DCT4 strain to the derivative strain of DCT4 that has lost the deletion chromosome is 1.06 per generation (data not shown). Thus, the bias contributed to these numbers by the differential growth rates of the diploid versus monosomic strain is no more than a factor of 2. The rate of chromosome loss for the normal chromosome III in the SUH1 and SUH2 strains is  $0.36$ – $1.8 \times 10^{-4}$  per cell division. These rates are  $\approx 35\%$  lower than those obtained for the DLT strains and lower than those obtained for the DCT strains by a factor of 4.5. Thus, removal of almost all sequences from the left arm of chromosome III has little effect on the stability of the chromosome.

If we score the number of *Ura*<sup>-</sup> cells that are *Leu*<sup>-</sup>, *Mal*<sup>+</sup> and nonmaters as mitotic crossover events that have occurred between the centromere and the *LEU2* gene, then the

Table 2. Rate of chromosome loss and mitotic crossover in wild-type and deletion strains

Strain	Rate of chromosome loss	Variance	Mitotic crossover
SUH1	$3.6 \times 10^{-5}$	12	$2.2 \times 10^{-5}$
SUH2	$1.8 \times 10^{-4}$	64	$8.6 \times 10^{-5}$
DLT1	$1.9 \times 10^{-4}$	27	$8.0 \times 10^{-5}$
DLT2	$1.4 \times 10^{-4}$	4.7	$1.7 \times 10^{-4}$
DCT1	$2.5 \times 10^{-4}$	144	$<10^{-6}$
DCT2	$4.6 \times 10^{-4}$	409	$<10^{-6}$
DCT4	$7.4 \times 10^{-4}$	1000	$<10^{-6}$

Value shown for each strain is average of 20 independent cultures.

rate of mitotic crossover for the DLT strains is  $0.8$ – $1.7 \times 10^{-4}$  per cell division and that for the SUH strains is  $2.2$ – $8.6 \times 10^{-5}$  per cell division. In contrast, all *Ura*<sup>-</sup> cells are also *Mal*<sup>-</sup> and  $\alpha$ -maters in the DCT strains, indicating that mitotic crossover is nondetectable between the *URA3* marker and the centromere. This is expected because the *URA3* marker in this case is tightly linked to the centromere.

**Meiotic Pairing and Segregation of the Deletion Chromosomes.** To find out if the deletion chromosomes paired and segregated properly during meiosis, we examined segregation of the *Ura*<sup>+</sup> marker to the viable spores. No *Ura*<sup>+</sup> spore was observed in any of the 40 tetrads dissected for the DLT1, DCT2, and DCT4 strains. However, 2 *Ura*<sup>+</sup> spores were obtained from 1 of the 40 tetrads derived from the diploid DCT1. Both of these spores behaved as nonmaters in mating tests with *MATa* or *MATa* tester strains. This result suggests that these spores contain both the normal and deletion copy of chromosome III resulting from nondisjunction of these chromosomes in meiosis I. A *Ura*<sup>+</sup> spore was also obtained from one of the tetrads derived from the diploid DLT2. This spore mated as a *MATa* cell in mating tests and presumably did not result from nondisjunction of the deletion chromosome. We also examined the linkage between *MATa* and *MAL2*, two markers on the right arm of chromosome III. The frequency with which *MATa mal2* and *MATa MAL2* spores were obtained indicates a normal amount of recombination between the two markers.

## DISCUSSION

We have described the construction of chromosomal deletions by the transformation of diploid yeast cells with DNA fragments bearing, at each end, homology to the chromosomal DNA. Two large chromosomal deletions were constructed. In one deletion,  $\approx 80$  kb of DNA between the *LEU2* gene and the telomere on the left arm of chromosome III has been removed. In another deletion, a telocentric chromosome III was constructed by the removal of  $\approx 100$  kb of DNA between the centromere and the left telomere. The replacement of these large chromosomal sequences by the small sequences of the transforming DNA fragments could have arisen by double-reciprocal recombination between the DNA fragments and chromosome III. Alternatively, these deletion chromosomes could have been generated by a single recombination event between the *LEU2* or *CEN3* sequences on the DNA fragments and the chromosome, but derived their telomeres from any chromosome via gene conversion. It has been shown that most yeast telomeres contain the highly conserved *Y'* sequence (10). Linear plasmids, restriction-cut within a *Y'* element, regain the missing *Y'* DNA by a recombination event (29). Even though all of the deletion chromosomes analyzed appear to have identical telomeric sequences, these sequences may not necessarily be derived from the left telomere of the normal chromosome III.

Our ability to construct systematic deletions in a chromosome should be useful not only in identifying the functional

components of a chromosome, but also in studying the spatial relationship required for the proper function of these components on the same chromosome. The stability of the telocentric chromosome was comparable to that of a normal chromosome III. This suggests that no sequence, including the subtelomeric *X* and part of the *Y'* sequence, on the left arm of chromosome III is required for proper mitotic stability. This result also indicates that the decrease in the size of the chromosome and the decrease in the distance between the centromere and telomere, at least on one arm, do not greatly affect the mitotic stability. The meiotic pairing and segregation of the telocentric chromosome appear to occur with fidelity. While no nondisjunction events were observed in any of the 40 tetrads examined for strains DCT2 and DCT4, there is 1 meiosis I nondisjunction event observed among the 40 tetrads analyzed in the DCT1 strain. This frequency is considerably higher than the meiosis I nondisjunction frequency of  $0.64 \times 10^{-4}$  per viable spore reported for the normal chromosome V (26). This difference may simply reflect the dissimilar nondisjunction frequencies between different chromosomes. Alternatively, and more likely, these data suggest that the meiotic pairing and segregation of the telocentric chromosome may be slightly impaired. The method described in this paper for the construction of large deletions in chromosomes is not restricted to deletions with endpoints in telomeric sequences. This procedure can be used to delete any region of the chromosome provided that sequences at the endpoints of the deletion are available (unpublished data).

In this paper, we used 5-fluoroorotic acid resistance as a positive selection for the loss of chromosome III by the insertion of the *URA3* gene into the chromosome III of a diploid strain homozygous for *ura3*. This method can be used to determine the rate of loss of any chromosome provided the resulting monosomic strain is viable. Our measurement for the rate of loss of the telocentric chromosome is  $2.5\text{--}7.4 \times 10^{-4}$  per generation and that for the normal chromosome III is  $0.36\text{--}1.8 \times 10^{-4}$  per generation. The number obtained for the normal chromosome III is similar to that reported by Campbell *et al.* (27) for the loss of disomic chromosome III and that reported by Hartwell *et al.* (28) for disomic chromosome V and VII.

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