RESOLUTION OF DICENTRIC CHROMOSOMES BY TY-MEDIATED RECOMBINATION IN YEAST

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ABSTRACT

We have integrated a plasmid containing a yeast centromere, *CEN5*, into the *HIS4* region of chromosome *III* by transformation. Of the three transformant colonies examined, none contained a dicentric chromosome, but all contained a rearranged chromosome *III*. In one transformant, rearrangement occurred by homologous recombination between two Ty elements; one on the left arm and the other on the right arm of chromosome *III*. This event produced a ring chromosome (ring chromosome *III*) of about 60 kb consisting of *CEN3* and all other sequences between the two Ty elements. In addition, a linear chromosome (chromosome *IIIA*) consisting of sequences distal to the two Ty elements including *CEN5*, but lacking 60 kb of sequences from the centromeric region, was produced. Two other transformants also contain a similarly altered linear chromosome *III* as well as an apparently normal copy of chromosome *III*. These results suggest that dicentric chromosomes cannot be maintained in yeast and that dicentric structures must be resolved for the cell to survive.—The meiotic segregation properties of ring chromosome *III* and linear chromosome *IIIA* were examined in diploid cells which also contained a normal chromosome *III*. Chromosome *IIIA* and normal chromosome *III* disjoined normally, indicating that homology or parallel location of the centromeric regions of these chromosomes are not essential for proper meiotic segregation. In contrast, the 60-kb ring chromosome *III*, which is homologous to the centromeric region of the normal chromosome *III*, did not appear to pair with fidelity with chromosome *III*.

DICENTRIC chromosomes are chromosomes that contain two centromeres. Cytological and genetic studies of dicentric chromosomes in the endosperm tissue of maize showed that they are highly unstable (McCLINTOCK 1939, 1941). Dicentric chromosomes can be formed when a chromosome with a broken arm is replicated and the broken ends are fused. A sequence of events, known as the bridge-breakage-fusion cycle is then initiated by the movement of the two centromeres to opposite poles during mitosis resulting in a chromatin bridge between the two centromeres. When the tension is sufficient, the bridge is broken and a chromosome with one broken arm is introduced into each sister nucleus where this cycle of events is repeated. In the zygote, a broken chromosome can be repaired by a process termed healing, which permanently stabilizes the broken chromosome.

In yeast, dicentric linear and circular chromosomes have also been found to be unstable in mitosis (HABER, THORBURN and ROGERS 1984). Genetic analysis

of these dicentric chromosomes showed that, as a result of rearrangement, they give rise to a variety of distinct structures. MANN and DAVIS (1983) showed that autonomously replicating plasmids containing two centromeres are unstable in yeast. Analysis of transformants containing these dicentric plasmids indicated that plasmid rearrangements resulting in the deletion of one or both of the centromeres had taken place. It was speculated that attachment of the mitotic spindle apparatus to the two centromeres followed by the separation of the two sequences to opposite poles resulted in mechanical breakage of the plasmid. Exonuclease action followed by ligation produced the deletion plasmid. In this paper we have studied at the molecular level, the sequence of events that result from the formation of dicentric chromosomes in yeast. These studies are facilitated by the isolation of yeast centromeres (CLARKE and CARBON 1980; FITZGERALD-HAYES *et al.* 1982; STINCHCOMB, MANN and DAVIS 1982; PANZERI and PHILIPPSEN 1982; MAINE, SUROSKY and TYE 1984) and a transformation system that allows the integration of DNA sequences by homologous recombination (HINNEN, HICKS and FINK 1978).

MATERIALS AND METHODS

Strains and plasmids: *E. coli* strain HB101 (*thr leuB hsr hsm recA*) (BOYER and ROULLAND-DUSSOIX 1969) was used as the host for plasmid amplification. Yeast strains TD1 (*MATα ura3-52 his4-38 trp1-289*) and TD3 (*MAT***a** *ura3-52 his4-519 leu2-3, −112*) were obtained from T. DONAHUE. Yeast strain K201 (*MAT***a** *his4 leu2 cdc10 thr4 ade6 lys2 cry1 MAL2*) was obtained from A. KLAR. Plasmids used and their sources are as follows: R16, G. FINK; pSZ57-E9, MAINE, SUROSKY and TYE (1984); p109, J. ABRAHAM; YRp7, STRUHL *et al.* (1979); pGT23, TSCHUMPER and CARBON (1982); pRG8-8, GABER and CULBERTSON (1982); A5H and G4B, C. NEWLON; pYe46B2, CLARKE and CARBON (1980).

Media and enzymes: YEPD and synthetic complete medium (SHERMAN, FINK and LAWRENCE 1974) and *E. coli* media (MILLER 1972) have been described. Restriction enzymes and other chemicals were used as described (CHAN and TYE 1980).

DNA preparation and transformation: Purified plasmid DNA was prepared by the method described by BIRNBOIM and DOLY (1979). Rapid DNA minipreps were prepared by the boiling technique (HOLMES and QUIGLEY 1981). Total yeast DNA was isolated as described (CRYER, ECCLESSHALL and MARMUR 1975). Circular DNA from yeast was prepared by the method of DEVENISH and NEWLON (1982). Transformation of *E. coli* (MORRISON 1977) and yeast (HINNEN, HICKS and FINK 1978) were carried out as described.

Other procedures: The preparation of nick-translated ^{32}P-labeled DNA probes (RIGBY *et al.* 1977), transfer of DNA to nitrocellulose (SOUTHERN 1975) and hybridization conditions (CHAN and TYE 1980) have been described. Stability assays have been described by MAINE, SUROSKY and TYE (1984). Meiotic tetrad analysis was performed as described (SHERMAN, FINK and LAWRENCE 1974).

RESULTS

Transformation and initial characterization: Our approach was to construct dicentric chromosomes by the introduction of the centromere from chromosome *V, CEN5*, into a specific location on chromosome *III* and examine the effects on the chromosome. We chose to study chromosome *III* because DNA sequences covering a large part of chromosome *III* have been cloned, so that rearrangements in chromosome *III* could be easily detected. We have attempted to construct dicentric chromosomes by transforming yeast with a plasmid that contains *CEN5*, the *HIS4* gene from chromosome *III* and the *URA3* gene from chromosome *V*. Since the plasmid does not contain an autonomously replicating

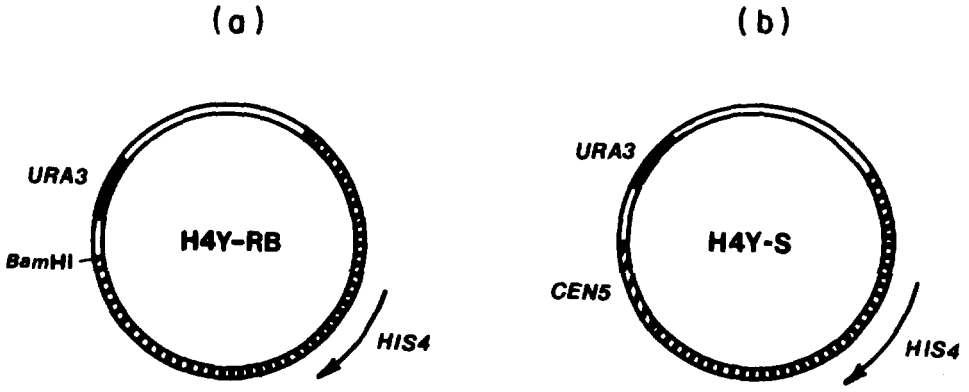

FIGURE 1.—Plasmids used in yeast transformation. a, H4Y-RB. This plasmid contains an 11-kb fragment from the *HIS4* region inserted into the *Bam*HI/*Eco*RI sites of YIp5. This plasmid was constructed by integration of the plasmid R16 into the genome followed by plasmid eviction as described by WINSTON, CHUMLEY and FINK (1983). Plasmid R16, which contains a 3.2-kb *Eco*RI fragment from the 5′ end of the *HIS4* gene cloned into the *Eco*RI site of plasmid YIp5, was integrated at the *HIS4* locus of the yeast genome. Genomic yeast DNA prepared from this strain was cut with *Bam*HI, ligated and used to transform *E. coli* strain HB101 to ampicillin resistance. Plasmids from the transformants were isolated and one plasmid, H4Y-RB, had the structure as shown. b, H4Y-S. The 1.5-kb *Bam*HI fragment from the plasmid pSZ57-E9 containing the functional *CEN5* sequence was cloned into the *Bam*HI site of plasmid H4Y-RB.

sequence, the His^+ Ura^+ transformants must have resulted from the integration of the plasmid into the chromosome.

Yeast strain TD1 was transformed to histidine and uracil prototrophy using plasmid H4Y-S, which carries *CEN5*, or a similar plasmid H4Y-RB, which does not contain *CEN5* (Figure 1). Fifty-three transformants were produced using 400 μg of plasmid H4Y-S. Seventy-nine transformants were obtained using 20 μg of plasmid H4Y-RB. The integrating plasmid containing *CEN5* produced transformants at a frequency 30-fold lower than the plasmid without the centromere, suggesting that some event other than, or in addition to, the integration of the plasmid may be required to produce transformants.

We first determined whether the plasmid H4Y-S had integrated into a chromosome and, if so, where it had integrated. Genomic DNA was prepared from 14 transformants, separated on an agarose gel and examined by gel-blotting analysis using ^{32}P-labeled pBR322 DNA. In all cases, the pBR322 DNA probe hybridized to the high molecular weight chromosomal DNA band, suggesting that the plasmid had integrated into chromosomal DNA in each of these transformants (data not shown). To examine the *CEN5* sequences in the transformants (ICH strains), DNA from the transformants was cut with the enzyme *Bam*HI and probed with the 1.5-kb *Bam*HI fragment from plasmid H4Y-S which contains *CEN5* (Figure 2). The *CEN5* DNA probe hybridized to one band in DNA from strain TD1 and two bands in DNA from each transformant. The 3.7-kb DNA band in the DNA from the transformants corresponds to the chromosome *V* copy of *CEN5* present in the TD1 strain. The 1.5-kb DNA band corresponds to the copy of *CEN5* derived from plasmid H4Y-S. To determine whether the plasmid had integrated at the *HIS4* region, the DNA from these transformants

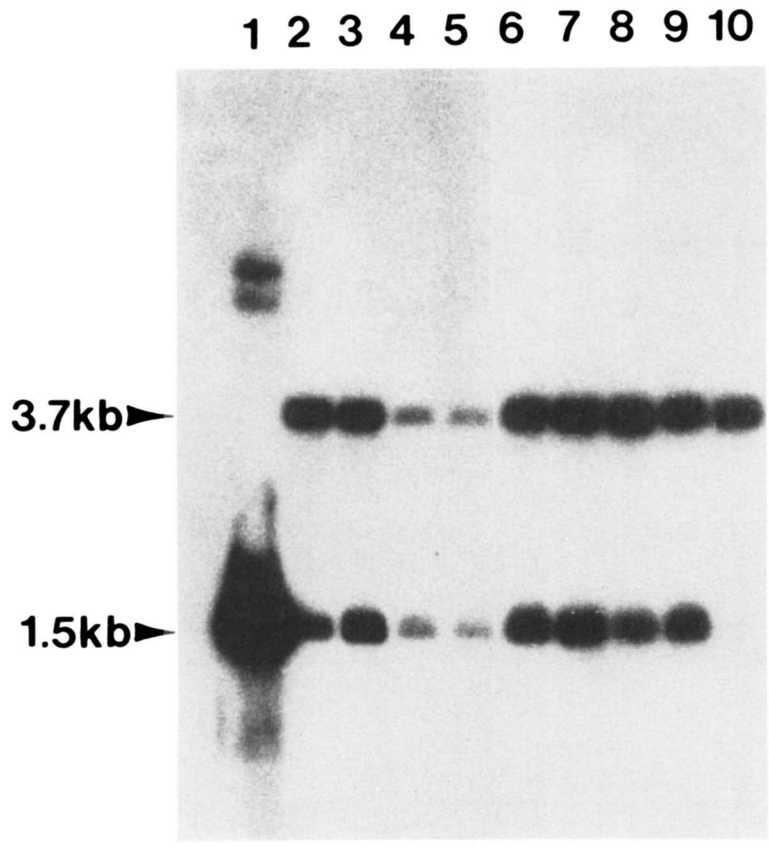

FIGURE 2.—The ICH strains contain two copies of *CEN5*. Fifty nanograms of plasmid H4Y-S and 2 μg of genomic yeast DNA from the ICH strains and TD1 strain were digested with *Bam*HI, separated on a 0.7% agarose gel, transferred to a nitrocellulose filter and hybridized with the ^{32}P-labeled 1.5-kb *Bam*HI fragment containing *CEN5*. Lane 1, Plasmid H4Y-S; lane 2, ICH1; lane 3, ICH2; lane 4, ICH5; lane 5, ICH9; lane 6, ICH10; lane 7, ICH11; lane 8, ICH12; lane 9, ICH13; lane 10, TD1. The higher molecular weight hybridizing fragments in lane 1 result from partial digestion of the plasmid.

was cut with different restriction enzymes and probed with ^{32}P-labeled DNA from either plasmid pBR322 or the *HIS4* region. Nine of the 14 transformants analyzed produced the hybridization pattern expected for an integration of unrearranged plasmid H4Y-S at *his4* (data not shown).

Three of the nine transformants, ICH2, ICH5 and ICH13, were analyzed in further detail as will be described in the remainder of the RESULTS. Using genetic and genomic hybridization analyses, we determined that the transformants had undergone chromosome rearrangements and located the breakpoints of the rearranged chromosomes. In one transformant, ICH2, chromosome *III* which is approximately 350 kb has been resolved into two fragments. One fragment is a ring chromosome of approximately 60 kb containing *CEN3, LEU2* and *SUF16* (Figure 3b). The second fragment, chromosome *IIIA*, is linear and contains *CEN5* and sequences distal to the Ty elements on both arms of the chromosome.

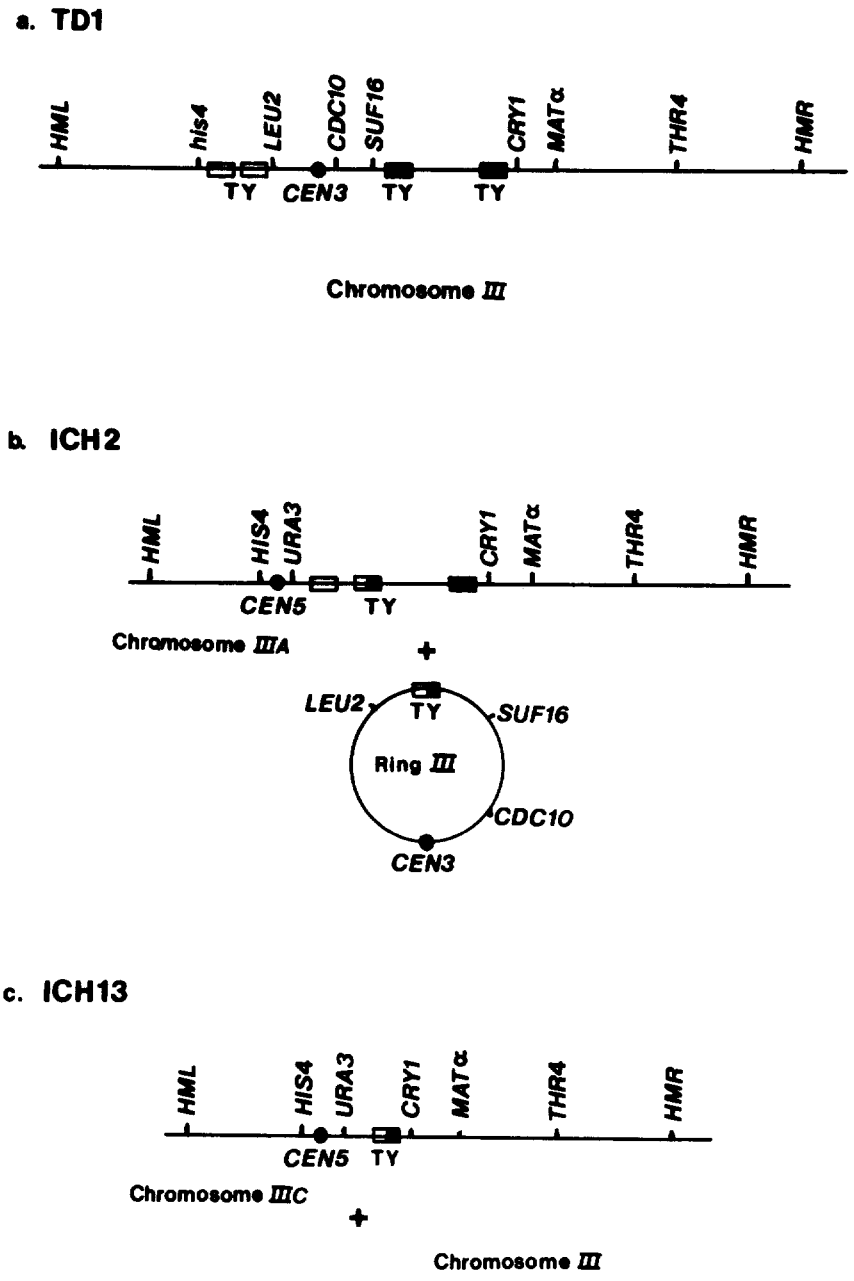

FIGURE 3.—Structure of rearranged chromosomes in ICH strains. a, Map of chromosome *III* in strain TD1. ▭ and ▬ indicate locations of Ty elements. The map distances are not drawn to scale. b, Strain ICH2 contains a ring chromosome (ring chromosome *III*) of approximately 60 kb containing *CEN3, LEU2, CDC10* and *SUF16*. Also present is a linear chromosome (*CIIIA*) consisting of H4Y-S plasmid sequences of *CEN5, URA3, HIS4* and sequences from the distal portions of the left and right arms of chromosome *III*. c, Strain ICH13 contains a normal copy of chromosome *III* and a linear chromosome (chromosome *IIIC*) consisting of H4Y-S plasmid sequences and sequences from the distal portions of the left and right arms of chromosome *III*.

TABLE 1

Marker loss in diploids

Diploid	No. screened	Phenotype	No. obtained
I2K2	5,200	Leu$^-$, TS	18
		His$^-$, Thr$^-$, Cry^r, a-mater	2
		Leu$^-$, His$^-$, TS	1
I5K2	8,000	His$^-$	14
		Leu$^-$, TS	1
I13K2	10,600	His$^-$	40
		Leu$^-$, TS	1
T1K2	6,000		0

Diploids were made by crossing the ICH strains (*MATα his4-38 ura3-52 trp1-289* (*HIS4, URA3*)) with strain K201 (*MAT***a** *his4 leu2 cdc10 cry1 thr4 ade6 lys2 MAL2*). After approximately 15 generations of nonselective growth, the diploids were plated on nonselective medium and replica-plated onto selective media to identify auxotrophs. Diploid I2K2 was constructed by crossing strain ICH2 with K201; diploid I5K2 was constructed by crossing strain ICH5 with K201; diploid I13K2 was constructed by crossing strain ICH13 with K201; diploid T1K2 was constructed by crossing strains TD1 and K201. TS, temperature sensitive for growth at 35°; Cry^r, resistant to cryptopleurine; **a**-mater, mates with a *MATα* tester.

Transformants ICH5 and ICH13 contain a normal copy of chromosome *III* and a rearranged chromosome *III* (chromosome *IIIB* and chromosome *IIIC*, respectively), similar in structure to chromosome *IIIA* (Figure 3c).

New linkage of chromosome III *markers in ICH strains*: In maize, the region between the two centromeres in a dicentric chromosome is unstable due to the cycle of breakage and fusion that occurs in this region. We examined the region in chromosome *III* containing the integrated *CEN5* by determining the stability as well as the linkage of the *HIS4, URA3* and *LEU2* genes (see Figure 3a for the genetic map of chromosome *III*). Chromosome loss or extensive chromosomal rearrangements that might have been occurring in the ICH strains would be lethal in haploids. To determine whether such events were occurring at a high frequency, diploids were made by crossing the ICH strains with strain K201. Chromosome *III* of the K201 strain contained multiple recessive markers on the left (*his4, leu2*) and on the right (*cdc10, cry1, MAT***a**, *thr4* and *MAL2*) arms of the chromosome. The loss of all or part of chromosome *III* from the ICH strains during mitosis in the diploid would uncover these recessive markers. After 15 generations of nonselective growth, 18 Leu$^-$ and two His$^-$ colonies were obtained from 5200 colonies of the diploid I2K2, made by crossing strains ICH2 and K201 (Table 1). No Leu$^-$ or His$^-$ auxotrophs were obtained from 6000 colonies of the wild-type diploid T1K2 (Table 1). All of the Leu$^-$ auxotrophs were temperature sensitive for growth but were His$^+$, Thr$^+$, Cry^s and nonmaters. The fact that the *cdc10* mutation is a temperature-sensitive mutation suggests that the *LEU2* and *CDC10* genes from chromosome *III* of the ICH2 strain are physically

linked to each other but unlinked to *HIS4* or any sequences from the distal portion of the right arm. The two His^- auxotrophs were Leu^+, Thr^-, Cry^r and mated as *MAT***a** cells. Cryptoplurine resistance determined by the *cry1* allele is recessive and would result from the loss of the *CRY1* allele on chromosome *III* of the ICH2 strain. Similarly, diploids would mate as *MAT***a** cells if the *MATα* information on chromosome *III* of the ICH2 strain was lost. It appeared that in these diploids *HIS4* was unlinked to *LEU2* but linked to markers on the distal portion of the right arm of chromosome *III*.

The auxotrophs isolated from diploids I5K2 and I13K2 made by crossing strains ICH5 and ICH13, respectively, with strain K201 were similar to each other (Table 1). Most auxotrophs were simply His^-. One colony isolated from the I5K2 diploid and one from the I13K2 diploid was Leu^- and temperature sensitive for growth. Again, results from these diploids suggest that *HIS4* and *LEU2* are no longer physically linked. These results provided the initial evidence that some rearrangement was occurring in the diploid or had occurred in the haploid transformant such that new linkage groups were produced.

Genetic analysis of the ICH strains: To examine the rearrangements of chromosome *III*, the ICH strains were analyzed by genetic crosses. These ICH strains [*MATα trp1 his4 ura3* (*HIS4, URA3*)] were crossed with the strain TD3 (*MAT***a** *his4 ura3 leu2*) and tetrads obtained after sporulation of the diploids were dissected and analyzed. From this analysis we could examine (1) the linkage between *HIS4* and *URA3*, (2) the linkage between *HIS4* and *LEU2* and (3) the segregation of *HIS4* and *LEU2* with respect to the centromere-linked marker, *trp1*.

Analysis of the complete tetrads obtained from the diploid I2T3 made by crossing strains ICH2 and TD3 showed that *HIS4, URA3* and *LEU2* were all very tightly centromere linked when scored against the centromere-linked marker *trp1* (Table 2). For example, in 22 of the 23 tetrads (96%) *HIS4* showed first division segregation. This percentage is much higher than in wild-type strains in which *HIS4* shows first division segregation in 64% of the tetrads (MORTIMER and SCHILD 1980). In contrast to the lack of physical linkage suggested by mitotic segregation, *HIS4* and *LEU2* showed extremely tight linkage among 23 complete tetrads (Table 3). However, only about 20% of the asci dissected were complete tetrads, compared to the 90% of four-spore tetrads obtained from a control diploid made by crossing strains TD1 and TD3. When spores from tetrads that produced only one or two viable spores were analyzed, *HIS4* and *LEU2* did not cosegregate (Table 3). This suggests that *HIS4* and *LEU2*, which may be associated with different chromosomes, must cosegregate in order to obtain four viable spores. As expected, *HIS4* and *URA3* were tightly linked.

Analysis of the 26 tetrads from diploid I2T3 that produced two viable spores showed that 43 (83%) of the 52 spores were Leu^+His^-, whereas none was Leu^-His^+ (Table 3). This suggests that one of the two rearranged chromosomes, *i.e.*, the one carrying the *HIS4* marker, pairs with and disjoins from the normal chromosome *III* with fidelity. This point will be discussed in greater detail in the DISCUSSION.

Tetrads from diploids I5T3 and I13T3, made by crossing the ICH5 and

TABLE 2

Genetic analysis of ICH strains

Cross	Marker pair	FD (%)	SD	PD	NPD	T
ICH2 × TD3	*his4–trp1*	22 (96)	1			
	ura3-trp1	23 (100)	0			
	leu2-trp1	23 (100)	0			
	his4-leu2			22	0	1
	his4-ura3			22	0	1
ICH5 × TD3	*his4-trp1*	24 (96)	1			
	ura3-trp1	25 (100)	0			
	leu2-trp1	21 (84)	4			
	his4-leu2			12	9	4
	his4-ura3			24	0	1
ICH13 × TD3	*his4-trp1*	25 (100)	0			
	ura3-trp1	25 (100)	0			
	leu2-trp1	22 (88)	3			
	his4-leu2			9	12	4
	his4-ura3			25	0	0
Expected ratio	*his4-cen3*	2	1			
	leu2-cen3	10	1			
	his4-leu2			100	1	50
	trp1-cen4	100	1			

Diploids were made by crossing the ICH strains [*MATα his4-38 ura3-52 trp1-289* (*HIS4 URA3*)] with strain TD3 (*MAT***a** *his4-519 ura3-52 leu2-3, 112*). The diploids were sporulated and the tetrads were dissected and analyzed. FD, first division segregation; SD, second division segregation. These segregations are determined by examination of the segregation of the marker relative to *trp1*, a known centromere-linked marker. PD, parental ditype; NPD, nonparental ditype; T, tetratype. Expected ratios were calculated from results presented by MORTIMER and SCHILD (1980).

TABLE 3

Segregation of the HIS4 *and* LEU2 *markers in tetrads from diploid I2T3*

Experiment no.	Tetrads	No. analyzed	No. of spores of the phenotype			
			Leu^+His^-	Leu^+His^+	Leu^-His^-	Leu^-His^+
1	0-spore	1				
	1-spore	5	4	1	0	0
	2-spore	26	43	1	8	0
	3-spore	8	4	8	11	1
	4-spore	10	0	20	20	0
2	4-spore	23	1	45	45	1

Diploid I2T3 was made by crossing strain ICH2 with strain TD3. In experiment 1 a total of 50 tetrads were dissected and all of the spores were analyzed. In experiment 2 a total of 115 tetrads were dissected, but only the 23 (20%) tetrads with four viable spores were analyzed.

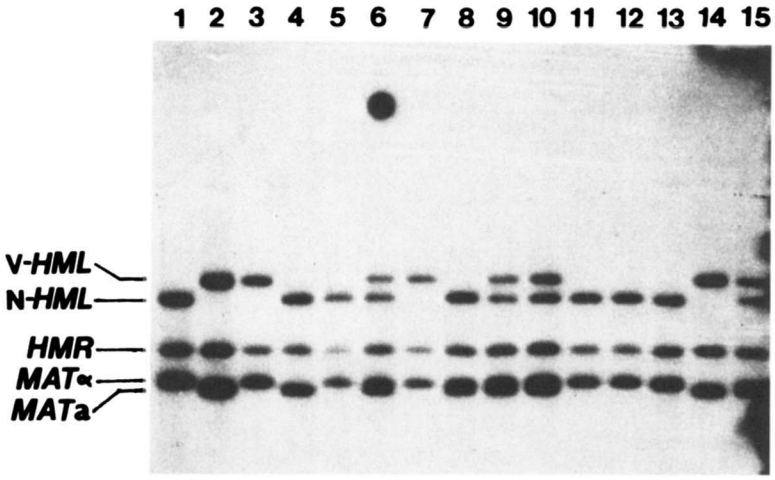

FIGURE 4.—Analysis of mating loci in spores derived from crosses with ICH strains. Genomic yeast DNA was prepared from the cells and digested with *Hin*dIII. The digested DNA was fractionated on a 0.7% agarose gel, transferred to nitrocellulose and hybridized with a ^{32}P-labeled 4.2-kb *Hin*dIII fragment containing *MAT***a** from plasmid p109. Lanes 1–4, Spores I2T3-1A to −1D from a tetrad derived from diploid I2T3; lanes 5–8, spores I5T3-14A to −14D from a tetrad derived from diploid I5T3; lanes 9–12, spores I13T3-9A to −9D from a tetrad derived from diploid I13T3; lane 13, TD1, lane 14, TD3; lane 15, T1T3, a diploid made by crossing TD1 with TD3. V-*HML*, variant of *HMLα*; N-*HML*, normal *HMLα*.

ICH13 strains, respectively, to strain TD3, were also analyzed. In contrast to the linkage studies in wild-type strains, *HIS4* is tightly linked to *URA3* and to a centromere but appears to be completely unlinked to *LEU2*. The results from the genetic analysis of the ICH strains suggest that (1) *HIS4* and *URA3* are very close to a functional centromere as evidenced by the tight centromere linkage of these markers and (2) *HIS4* and *LEU2* have rearranged in such a way that these markers no longer appear linked.

Aberrant segregation of chromosomal III *markers*: Tetrads derived from the I5T3 and I13T3 strains give rise to some spores that are nonmaters. This nonmating phenotype is correlated with the His phenotype of the spore. Only His$^+$ spores that should have been *MAT***a** (based on the phenotype of the other two or three spores in the tetrad) displayed this behavior. His$^-$ spores always mated as *MAT***a** or *MATα* and His$^+$ spores that were *MATα* mated well with the *MAT***a** tester. In contrast, in all tetrads derived from the I2T3 strain, the *MATα* and *MAT***a** alleles segregated properly.

One possible explanation for the nonmating phenotype of the His$^+$ spores from the I5T3 and I13T3 strains was they they contained both functional *MAT***a** and *MATα* information. To investigate this possibility, total DNA was prepared from tetrads derived from diploids I2T3, I5T3 and I13T3. This DNA was digested with *Hin*dIII and probed with a ^{32}P-labeled fragment containing *MAT***a**. The *MAT***a** probe also hybridizes to *MATα*, *HMLα* and *HMR***a** due to homology between the mating type cassettes (NASMYTH and TATCHELL 1980) (Figure 4).

Genomic digests of DNA from the TD1 strain (lane 13) showed a 4.3-kb hybridizing fragment that corresponds to *MATα*. The TD3 strain (lane 14) showed a 4.2-kb hybridizing fragment that corresponds to *MAT***a**. Of the four spores from tetrad I2T3-1 derived from diploid I2T3 (lanes 1–4), two contain *MATα* and two contain *MAT***a** as expected. Hybridization analyses of tetrads derived from diploids I5T3 and I13T3 are shown in lanes 5–8 and 9–12, respectively. Spores I5T3-14B, -14D (lanes 6 and 8) and I13T3-9A, -9B (lanes 9 and 10) show bands that correspond to both *MATα* and *MAT***a** as in the diploid T1T3 (lane 15). All four of these spores were His^+ and were nonmaters in the mating test. Ten other nonmating spores derived from diploids I5T3 or I13T3 also contained *MAT***a** and *MATα* mating type cassettes (data not shown). Thus, in these tetrads there are four copies of *MATα*, two of which appear linked to *HIS4*.

This hybridization analysis also showed that the TD3 strain contains a variant of the *HMLα* *Hind*III fragment with a size of 6.7 kb rather than the normal 6.1-kb *HMLα* *Hind*III fragment observed in strain TD1. The presence of this variant allowed us to distinguish between *HMLα*'s from the ICH strains and those from the TD3 strain. Segregation of the *HMLα*'s suggested a duplication of the normal *HMLα* allele in tetrads derived from diploid I5T3 and I13T3. In the tetrad derived from diploid I13T3, there are two bands corresponding to the variant form of *HMLα* contributed by the TD3 strain and four bands corresponding to the normal form of *HMLα* contributed by the ICH13 strain. In the tetrad derived from diploid I5T3, there are two bands corresponding to the variant form and three bands corresponding to the normal form of *HMLα*. The fact that there are only three bands corresponding to the normal *HMLα* suggests that meiotic recombination may have occurred, resulting in the cosegregation of two copies of the normal *HMLα* to one of the spores. Densitometer measurements confirm that the I5T3-14D spore indeed contains two copies of the normal *HMLα* (Figure 4, lane 8). Thus, these tetrads appear to have four copies of *MATα* and four copies of *HMLα* from the ICH strains. Genetic analysis of the *THR4* marker in strains ICH2, ICH5 and ICH13 also indicate that there are two copies of the *THR4* gene in tetrads derived from the ICH2 strain but *THR4* exists in four copies in tetrads derived from the ICH5 and ICH13 strains.

To determine what parts of chromosome *III* were duplicated and whether these duplications were present in the haploid ICH strains, quantitative hybridization experiments were carried out on the ICH2, ICH5, ICH13 and TD1 strains. *Eco*RI-digested DNA from these strains was probed with ^{32}P-labeled fragments containing part of the *HIS4* region, part of the *LEU2* gene and the *TRP1* gene. The *TRP1* gene, which is located on chromosome *IV*, was used as an internal control. In a second experiment, DNA from the four strains was cut with the enzyme *Hind*III and probed with ^{32}P-labeled fragments containing the *MAT***a** allele, the *ARG4* gene and part of the *SUF16* region. The *ARG4* gene, which is located on chromosome *VIII*, was used as an internal control. In the ICH2 strain, all sequences examined were present in single copies except for the sequence from the *HIS4* region which was duplicated (Table 4). This duplication results from the integration of the plasmid H4Y-S containing *HIS4*. In the ICH5

TABLE 4

Copy number of chromosome III *regions in the ICH strains*

Region	ICH2	ICH5	ICH13	TD1
HMLα	1.1	1.9	2.1	1
HIS4	2.4	3.5	3.3	1
LEU2	1.0	1.0	1.3	1
SUF16	1.0	0.8	1.1	1
MATα	0.7	1.8	2.1	1
*HMR***a**	1.0	1.9	1.9	1

Total yeast DNA from ICH2, ICH5, ICH13 and TD1 strains was cut with *Eco*RI and probed with the following ^{32}P-labeled fragments. (1) A 4.1-kb *Eco*RI fragment from a region 3′ to the *HIS4* gene from plasmid H4Y-RB. (2) A 1.0-kb *Eco*RI/*Sal*I fragment containing the 3′ end of the *LEU2* gene from plasmid G4B. (3) A 1.4-kb *Eco*RI fragment containing the *TRP1* gene and *ARS1* from plasmid YRp7. In a second experiment, DNA from the four strains was cut with the enzyme *Hin*dIII and probed with the following ^{32}P-labeled fragments. (1) A 4.2-kb *Hin*dIII fragment containing *MAT***a** from plasmid p109. (2) A 2.1-kb *Hin*dIII fragment containing *ARG4* from plasmid pGT23. (3) A 2.0-kb *Hin*dIII fragment from the *SUF16* region from plasmid pRG8-8. Experiments were done in duplicates, and the autoradiograms were scanned by a densitometer to determine the copy number. Values for ICH strains were calculated relative to the parental strain TD1.

and ICH13 strains the *LEU2* and SUF16 regions were present in single copies whereas the *HMLα, MATα* and *HMR***a** regions were present in two copies and *HIS4* in three copies. Thus, in ICH5 and ICH13 the centromere-proximal sequences of chromosome *III* were present in single copies, whereas the centromere distal sequences on both arms were duplicated.

Breakpoints on the left arm of chromosome III *in the ICH strains*: The loss of linkage between *HIS4* and *LEU2* and the duplication of the centromere-distal markers on chromosome *III* but not *LEU2* and *SUF16* suggests that the region where the rearrangement might have occurred is between *HIS4* and *LEU2*. To locate the exact position of the breakpoint on the chromosome, the entire region of approximately 30 kb between *HIS4* and *LEU2* was examined for changes in restriction pattern using cloned DNA fragments as probes. No alterations were observed except in a region containing two Ty elements adjacent to *LEU2*.

There are at least two families of Ty elements, Tyl (CAMERON, LOH and DAVIS 1979) and Ty917 (ROEDER *et al.* 1980), identified among the approximately 35 copies of Ty elements in the yeast genome. These two families share homologies in about one-third of their sequences. In the TD1 strain there is a Ty element similar in restriction pattern to Ty917 located 500 base pairs from the centromere-distal end of the *LEU2* gene (KINGSMAN *et al.* 1981; ANDREADIS *et al.* 1982) (Figure 5a). Less than 2 kb from the centromere-distal end of this Ty element lies a second Ty element similar to Tyl.

In the ICH2 strain, the rearrangement has occurred within the Ty element closest to the *LEU2* region. This was shown by a series of genomic hybridization

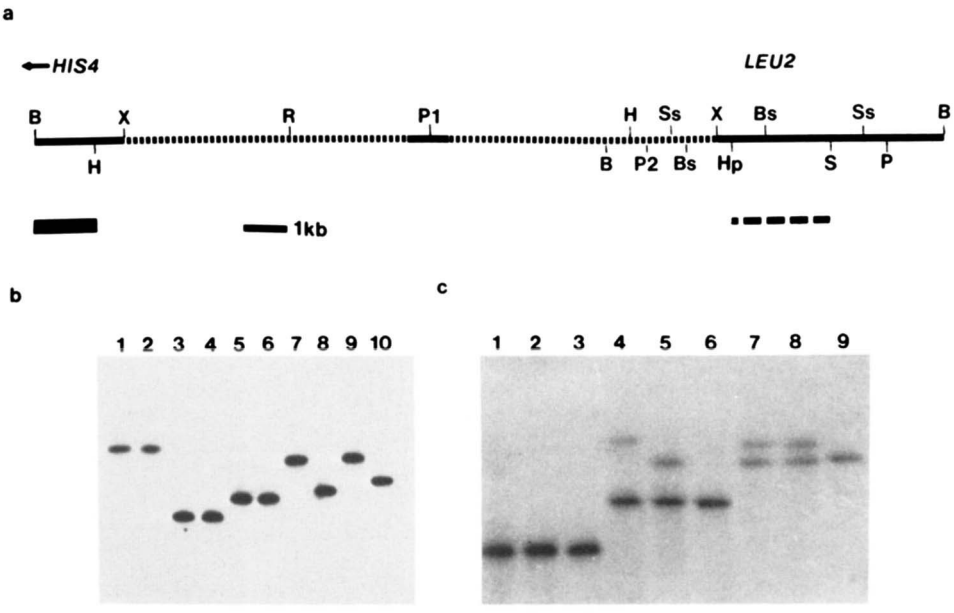

FIGURE 5.—Rearrangements on the left arm of chromosome *III* in the ICH strains. a, The restriction map of part of the region between *HIS4* and *LEU2* in TD1 ▮▮▮▮. indicates locations of the Ty elements. Restriction enzyme sites shown are B, *Bam*HI; H, *Hin*dIII; X, *Xho*I; R, *Eco*RI; P1, *Pvu*I; P2, *Pvu*II; Ss, *Sst*I; Bs, *Bst*EII; Hp, *Hpa*I; S, *Sal*I; P, *Pst*I. b, Breakpoint on the left arm of chromosome *III* of the ICH2 strain. Genomic yeast DNA from strains ICH2 and TD1 was digested with restriction enzymes, separated on a 0.7% agarose gel, transferred to a nitrocellulose filter and hybridized with the ^{32}P-labeled 2.0-kb *Hpa*I/*Sal*I fragment indicated by ▮▮▮. Lanes 1–2, Genomic DNA of the strains ICH2 and TD1, respectively, digested with *Bst*EII; lanes 3–4, digested with *Sst*I; lanes 5–6, digested with *Pst*I/*Pvu*II; lanes 7–8, digested with *Pst*I/*Hin*dIII; lanes 9–10, digested with *Pst*I/*Bam*HI. c, Breakpoints on the left arm of chromosome *III* of ICH5 and ICH13 strains. Total yeast DNA from strains ICH5, ICH13 and TD1 was digested with restriction enzymes, separated on a 0.7% agarose gel, transferred to a nitrocellulose filter and hybridized with the ^{32}P-labeled 1.1-kb *Bam*HI/*Hin*dIII fragment indicated by ▮. Lanes 1-3, ICH5, ICH13 and TD1 DNA digested with *Eco*RI/*Bam*HI; lanes 4-6, ICH5, ICH13 and TD1 DNA digested with *Pvu*I/*Bam*HI; lanes 7-9, ICH5, ICH13 and TD1 DNA digested with *Bam*HI.

experiments using the 2.0-kb *Hpa*I/*Sal*I fragment containing the *LEU2* gene as probe from plasmid G4B (Figure 5b). There was no difference in the hybridization patterns between the genomic digests of the TD1 and ICH2 strains when the restriction enzymes *Bst*EII, *Sst*I or *Pst*I/*Pvu*II were used. However, a change in the hybridization patterns was observed when the combination of restriction enzymes *Pst*I/*Hin*dIII or *Pst*I/*Bam*HI were used.

Restriction mapping of the DNA sequence on the other side of the breakpoint shows that there are four restriction sites, *Cla*I, *Kpn*I, *Eco*RI and *Pst*I, with map positions characteristic of Tyl elements (Figure 6). This suggests that part of a Tyl-like element had been joined to the Ty917-like element adjacent to *LEU2*. In this analysis, the rearrangement is observed in the region between the *Hin*dIII and *Pvu*II sites, although recombination may have occurred anywhere in the homologous region around the common *Pvu*II sites. Genomic hybridization experiments using the 1.1-kb *Hin*dIII/*Bam*HI fragment (Figure 5a) located on

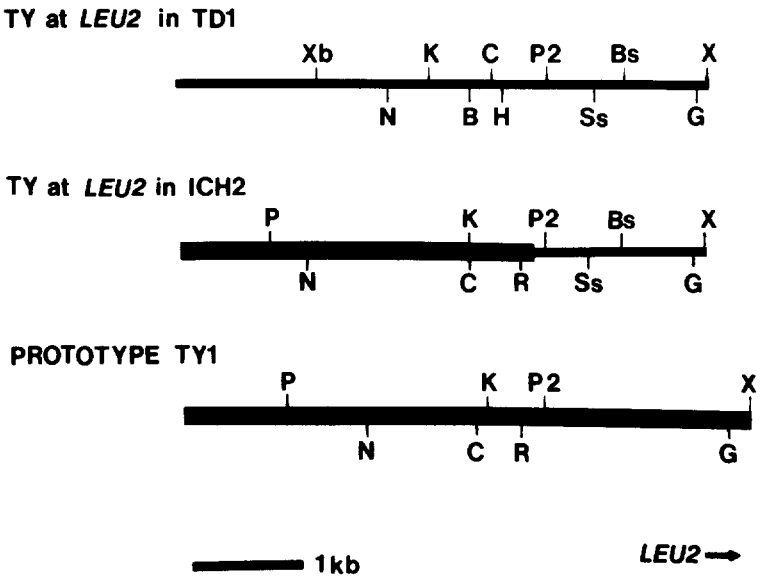

FIGURE 6.—Partial restriction maps of the Ty element adjacent to *LEU2* in the TD1 and ICH2 strains and a prototype Tyl element. The restriction map of the prototype Tyl element is a composite map of eight Tyl elements (R. T. SUROSKY, unpublished data). The Ty elements were aligned at the *Pvu*II site. In the ICH2 strain, the thin line represents DNA present in the parental strain TD1; the thick line represents new DNA. Restriction enzyme sites shown are Xb, *Xba*I; N, *Nru*I; K, *Kpn*I; B, *Bam*HI; C, *Cla*I; H, *Hin*dIII; P2, *Pvu*II; Ss, *Sst*I; G, *Bgl*II; X, *Xho*I; R, *Eco*RI; Bs, *Bst*EII; P, *Pst*I.

the *HIS4* side of the Ty elements from plasmid A5H confirmed that rearrangement had indeed taken place within this region in the ICH2 strain (data not shown).

To examine the breakpoints in strains ICH5 and ICH13, restriction digests of DNA from strains ICH5, ICH13 and TD1 were probed with the 1.1-kb *Hin*dIII/*Bam*HI fragment and their hybridization patterns were compared. When the genomic DNA was cleaved with the enzyme *Bam*HI in combination with other restriction enzymes that cleave within the Tyl element, no alterations were observed in the ICH strains (Figure 5c). However, when genomic DNA was cleaved with the combination of enzymes *Bam*HI/*Pvu*I or with *Bam*HI alone, two bands could be seen in the ICH strains. One of the two bands corresponded in size to that seen in the TD1 strain, whereas the second band, although different in size in each case, was larger than that observed in the TD1 strain. From the size of the *Bam*HI/*Pvu*I fragment, it appears that the *Pvu*I site is located within the unique region between the Ty elements. In the ICH5 and ICH13 strains, recombination may have occurred at the very end of the Ty element or in the unique region between the Ty elements. However, a more likely explanation is that, as in the ICH2 strain, part of the Ty element has become joined to another Tyl element. If this were the case, the identity of such a recombinant Ty element would be obscured by the restriction site homogeneity between Tyl elements. The rearrangement would only become apparent when we examined restriction fragments with end points outside of the hybrid Ty element.

We also used the *Hpa*I/*Sal*I fragment from the *LEU2* side of the Ty element to examine the region in the ICH5 and ICH13 strains. Only bands identical with those seen in the TD1 strain were observed even when fragments that spanned this entire region were examined (data not shown). These hybridization results from strains ICH5 and ICH13 can be explained as follows. The two bands observed using the probe from the *HIS4* side of the Ty elements correspond to a fragment from the normal chromosome *III* and a fragment from the rearranged chromosome *IIIB* or *IIIC*. Chromosome *IIIB* and *IIIC* do not contain sequences centromere proximal to the Ty element where the recombinant occurred. Thus, when the probe from the *LEU2* side of the Ty element was used, only a normal sized band from chromosome *III* was observed.

Breakpoints on the right arm of chromosome III: The existence of the hybrid Ty element in the ICH2 strain suggested that recombination between Ty elements might have occurred. It was possible that the other Ty element involved in the recombination was located on the right arm of chromosome *III*. Two Tyl-like elements are known to be present on the right arm of chromosome *III*, one approximately 5 kb centromere distal to the *SUF16* gene (KINGSMAN *et al.* 1981; GABER and CULBERTSON 1982) and the other 8 kb centromere proximal to *CRY1* (C. NEWLON, unpublished data). These two Ty elements and the two Ty elements adjacent to *LEU2* are all in the same orientation. When DNA from the ICH2 and TD1 strains was cut with the combination of restriction enzymes *Hin*dIII/*Pst*I or *Hin*dIII/*Cla*I and probed with a 1.7-kb *Eco*RI fragment from the unique region on the centromere-proximal side of the Ty element adjacent to *SUF16*, no differences in the hybridization were observed (Figure 7). When *Hin*dIII-digests were probed with the *Eco*RI fragment, the two strains produced fragments of different sizes. The *Hin*dIII site located within the Ty element is approximately 1 kb centromere distal to the *Cla*I site. Hence, rearrangement must have occurred between the *Hin*dIII and *Cla*I sites. The region in which the rearrangement occurred within this Ty element corresponds well to the site of rearrangement mapped within the Ty element adjacent to *LEU2* and corresponds to the homologous region between the two Ty elements.

If homologous recombination between these Ty elements had indeed occurred, it would bring the *LEU2* gene close to the *SUF16* gene, separated by a hybrid Tyl element. There is no *Hin*dIII site between the breakpoint and the *LEU2* gene on the left arm of chromosome *III* and none between the breakpoint and the *Eco*RI fragment on the right arm. Hence, probing of a *Hin*dIII digest of DNA from the ICH2 strain with either the *Hpa*I/*Sal*I fragment from *LEU2* or the *Eco*RI fragment from the *SUF16* region should hybridize to the same *Hin*dIII fragment. This was confirmed by the hybridization to a single band of approximately 35 kb when either fragment was used as a probe (Figure 8).

Using similar procedures, we found the Tyl element near *CRY1* to be the site of recombination in strain ICH13. When a probe from the unique region centromere distal to the Ty element near *CRY1* was used, two hybridizing fragments were observed in strain ICH13 (data not shown). One fragment is identical in size with that observed in the parental strain TD1. When a probe from the unique region centromere proximal to the Ty element was used, strains

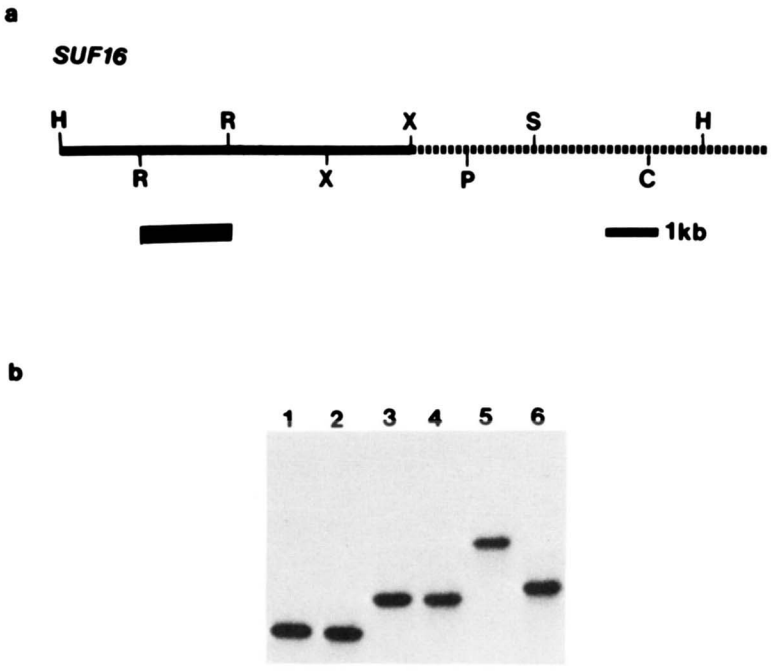

FIGURE 7.—Breakpoints on the right arm of chromosome *III* in the ICH2 strain. a, Restriction map of *SUF16* region in TD1. ▭ indicates the location of the Ty element. The restriction enzyme sites shown are H, *Hin*dIII; R, *Eco*RI; X, *Xho*I; P2, *Pvu*II; P, *Pst*I; S, *Sal*I; C, *Cla*I. b, Genomic yeast DNA from the ICH2 or TD1 strains were digested with restriction enzymes and separated on a 0.7% agarose gel. The DNA was transferred to a nitrocellulose filter and probed with the ^{32}P-labeled 1.7-kb *Eco*RI fragment from the *SUF16* region as indicated by ▬. Genomic DNA of the ICH2 and TD1 strains, respectively, digested with: lanes 1-2, *Pst*I/*Hin*dIII; lanes 3-4, digested with *Cla*I/*Hin*dIII; lanes 5-6, digested with *Hin*dIII.

ICH13 and TD1 produced identical hybridizing fragments. These results and the results obtained from the analysis of the breakpoint on the left arm of chromosome *III* suggest that the ICH13 strain contains a normal copy of chromosome *III* in addition to a rearranged copy of the chromosome (chromosome *IIIC*). No breakpoints could be determined on the right arm of chromosome *III* in the ICH5 strain. It is possible that the recombination occurred at the Ty element near the *SUF16* gene. The lack of a probe from the unique region centromere distal to this Ty element prevented us from testing for such a rearrangement. It is also possible that the recombination occurred at a Ty element of unknown location on chromosome *III*.

Ring chromosome analysis of the ICH strains: If recombination between the Ty elements on the left and right arm of a chromosome has taken place, then the end products of this event would be a circular and a linear chromosome. To examine the ICH strains for ring chromosomes, we isolated DNA from the ICH2, ICH5, ICH13 and TD1 strains using an extraction procedure that enriches for circular DNA (DEVENISH and NEWLON 1982). DNA isolated by this

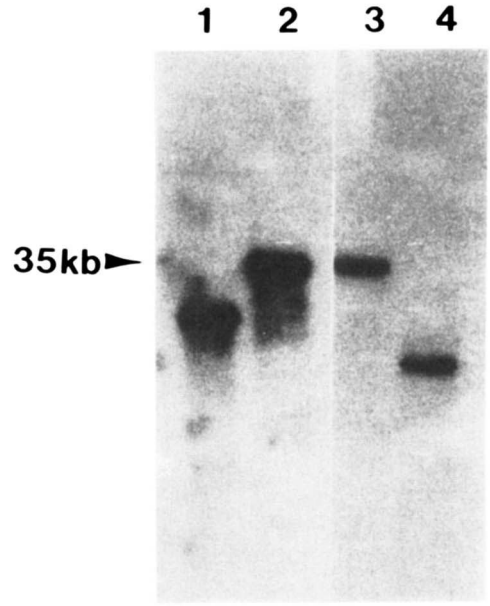

FIGURE 8.—In strain ICH2, DNA probes from the *LEU2* and *SUF16* region hybridize to the same restriction fragment. Genomic yeast DNA was digested with *Hin*dIII and separated on a 0.7% agarose gel. The DNA was transferred to a nitrocellulose filter and probed with the ^{32}P-labeled probe. Lane 1, TD1 DNA probed with the 2.0-kb *Hpa*I/*Sal*I fragment from the *LEU2* region; lane 2, ICH2 DNA probed with the 2.0-kb *Hpa*I/*Sal*I fragment from the *LEU2* region; lane 3, ICH2 DNA probed with the 1.7-kb *Eco*RI fragment from the *SUF16* region; lane 4, TD1 DNA probed with the 1.7-kb *Eco*RI fragment from the *SUF16* region.

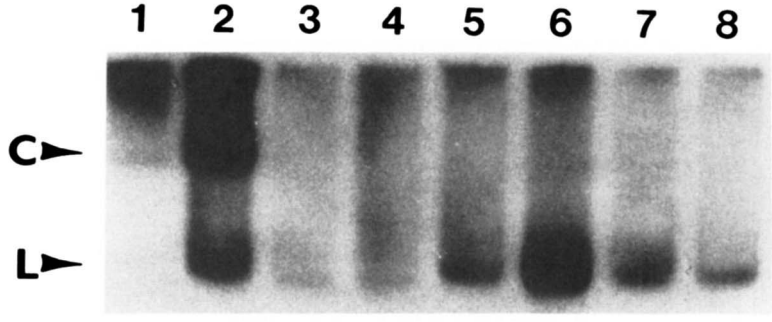

FIGURE 9.—Analysis of ring chromosome in strain ICH2. DNA was prepared from different yeast strains by the method of DEVENISH and NEWLON (1982). The DNA was separated on a 0.7% agarose gel, transferred to a nitrocellulose filter and probed with a ^{32}P-labeled 2.0-kb *Hpa*I/*Sal*I fragment containing *LEU2*. Lane 1, ICH2 DNA; lane 2, ICH2 × TD3 diploid DNA; lane 3, ICH5 DNA; lane 4, ICH5 × TD3 diploid DNA; lane 5, ICH13 DNA; lane 6, ICH13 × TD3 DNA; lane 7, TD1 DNA; lane 8, TD3 DNA. C, Hybridization band corresponding to circular chromosomal DNA; L, hybridization band corresponding to linear chromsomal DNA.

procedure was probed with the 2.0-kb *Hpa*I/*Sal*I fragment from the *LEU2* region (Figure 9). In the ICH2 strain, the *LEU2* DNA probe hybridized to the band corresponding to circular chromosomal DNA which migrates slower than the linear chromosomal DNA (DEVENISH and NEWLON 1982). In the TD1, ICH5

TABLE 5

Determination of sequences present on ring chromosome in strain ICH2

Region	ICH2	ICH5	ICH13	TD1
HIS4	L	L	L	L
LEU2	C	L	L	L
CEN3	C	L	L	L
SUF16	C	L	L	L
MATα, *HMLα* and *HMR***a**	L	L	L	L

Probes prepared from DNAs of different regions of chromosome *III* were hybridized to DNA from ICH2, ICH5, ICH13 and TD1 strains. The probes used were the 4.1-kb *Eco*RI fragment from the *HIS4* region from plasmid H4Y-RB, the 1.0-kb *Eco*RI/*Sal*I fragment from the *LEU2* region from plasmid G4B, the 2.6-kb *Bam*HI/*Eco*RI fragment containing *CEN3* from plasmid pYe46B2, the 2.0-kb *Hin*dIII fragment from the *SUF16* region from plasmid pRG8-8, the 4.2-kb *Hin*dIII fragment containing *MAT***a** from plasmid p109. C, hybridization band corresponding to circular chromosomal DNA; L, hybridization band corresponding to linear chromosomal DNA.

and ICH13 strains, this probe hybridized to the linear chromosomal DNA. The DNA from these four strains was also probed with fragments from other regions of chromosome *III* (Table 5). In the ICH2 strain the probes from the centromere-proximal regions such as *CEN3* and *SUF16* hybridized to the band corresponding to circular chromosomal DNA. The *HIS4* and *MAT***a** probes hybridized to the band corresponding to linear chromosomal DNA. In the TD1, ICH5 and ICH13 strains all probes hybridized to the linear chromosomal DNA. These results confirm our interpretation of the previous results that chromosome *III* of the ICH2 strain is broken into two chromosomes, a ring chromosome and a linear chromosome *IIIA*. In the ICH5 and ICH13 strains, a similar event has taken place except that the ring chromosome has been lost leaving behind the linear chromosome *IIIB* or chromosome *IIIC* in addition to the normal chromosome *III*.

DISCUSSION

We attempted to create dicentric chromosomes by integrating *CEN5* at the *HIS4* region on chromosome *III* in a haploid yeast strain. In the transformants that we analyzed, no dicentric chromosomes were present. Instead, it seemed that Ty-mediated rearrangements had played a role in the resolution of these presumptive dicentric chromosomes. The presence of repetitive Ty elements plus their fortuitous positioning and orientation on chromosome *III* resulted in the occurrence of Ty-mediated rearrangments in at least three of the nine transformants in which *CEN5* had been integrated at *his4*. This analysis represents only a small sampling of the total transformants obtained. Other events, such as chromosome breakage and repair, may occur in addition to Ty-Ty recombination to give rise to the transformants. The efficiency of transformation of the nonreplicating *CEN* plasmid is only 30-fold lower than that of the nonreplicating plasmid without a centromere. One possible explanation for the apparently high frequency of transformation of the nonreplicating *CEN* plasmid is that the dicentric chromosome, once formed, may be maintained for several

generations. MANN and DAVIS (1983) observed, after 25 generations, unrearranged plasmid in a population of cells transformed with a dicentric plasmid. The ability of the cell to maintain the dicentric chromosome through several mitotic divisions would increase the probability of obtaining some kind of stable rearrangement.

In the ICH2 strain, the rearrangement was generated by a recombination between a Ty element adjacent to *LEU2* and a Ty element near *SUF16*. This event resulted in the production of a ring chromosome *III* and an altered linear chromosome *IIIA*. The 60-kb ring chromosome contains all sequences between the Ty elements including *CEN3*. Chromosome *IIIA* contains *HIS4, URA3, CEN5* and all chromosome *III* sequences distal to the Ty elements. Rearrangements in the ICH5 and ICH13 strains occurred by a similar mechanism. In the ICH13 strain, a recombination between the second Ty element adjacent to *LEU2* and a Ty element in the *CRY1* region has occurred. The recombination event in the ICH5 strain involved the same Ty element at *LEU2* as in the ICH13 strain, but we have been unable to determine which Ty, if any, on the right arm is involved due to the lack of an appropriate probe.

The recombination events that occurred in the ICH5 and ICH13 strains should have produced a ring chromosome, but no ring chromosome was observed in these strains. Instead, these strains contain an apparently normal copy of chromosome *III*. One explanation for the presence of both of these linear chromosomes is that the rearrangement occurred after S phase when the chromosomes were duplicated but prior to or during mitosis (Figure 10). This would produce a cell with one normal chromosome *III*, an altered linear chromosome *III* and a ring chromosome *III*. In mitosis, the altered chromosome *III* could segregate independently of either the ring or the normal chromosome *III*. In the case of the ICH5 and ICH13 strains, chromosome *IIIB* and *IIIC*, respectively, segregated with the normal chromosome. The ICH2 strain might have also contained all three structures at one point, but instead of segregating with the normal copy of chromosome *III*, chromosome *IIIA* segregated with the ring.

Although we were never able to observe a dicentric chromosome, it is possible that a dicentric chromosome did exist in the ICH strains at some point. We were unable to determine whether Ty-mediated recombination occurred before or after the integration of the centromere-containing plasmid. If integration of the plasmid occurred prior to recombination, then a short-lived dicentric chromosome was generated (Figure 11). This structure was then resolved into the products that were observed in the transformants. If recombination occurred prior to integration, then integration of *CEN5* resulted in the rescue of an unstable acentric linear fragment.

Recombination between Ty elements occurs in normal growing cells. In the DEL1 strain of yeast, the clustered genes *CYC1, OSM1* and *RAD7* are flanked by two Ty elements in the same orientation (LIEBMAN, SHALIT and PICOLOGLOU 1981). These markers are lost at a frequency of 10^{-5}–10^{-6} due to recombination between the two Ty elements, resulting in a deletion of the region between the elements. Fink and coworkers have shown that His^+ revertants of a Ty element insertion mutation at *his4* contained chromosomal translocations, deletions or

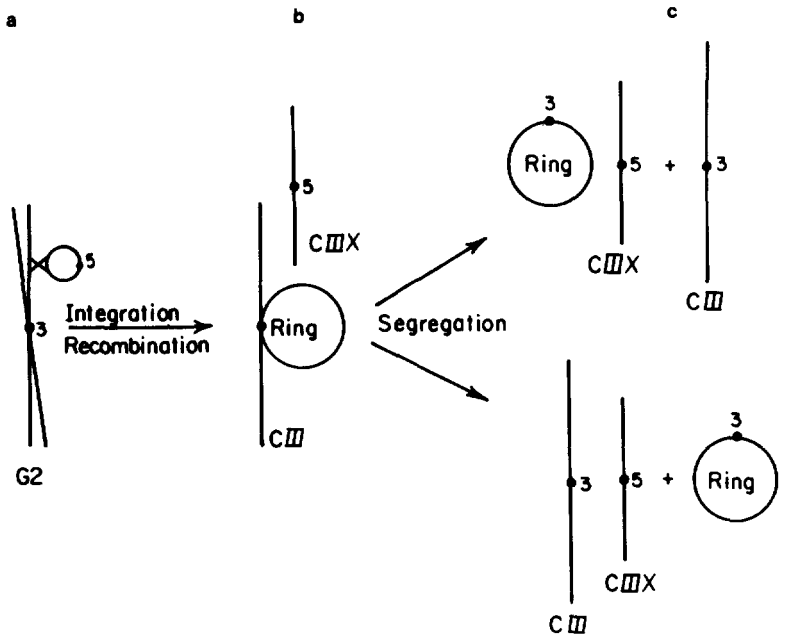

FIGURE 10.—Model to explain the absence of ring chromosome in the ICH5 and ICH13 strains. a, Integration of *CEN5* and recombination between Ty elements occur in G2, after DNA replication but before mitosis. b, After rearrangement, the cell will contain a normal copy of chromosome *III*, ring chromosome *III* and chromosome *IIIX*. c, Chromosome *IIIX* can segregate with ring *CIII* or the normal copy of chromosome *III*.

inversions associated with the Ty element (CHALEFF and FINK 1980; Roeder and Fink 1980, 1982).

Recombination can occur between repetitive sequences other than Ty elements. Homologous recombination has been observed between the mating cassettes, *MAT, HMR* and *HML*, to produce large deletions as well as ring chromosomes (HAWTHORNE 1963; STRATHERN *et al.* 1979; KLAR *et al.* 1983). Recombination events between homologous sequences of nonhomologous chromosomes have also been observed (SCHERER and DAVIS 1980; MIKUS and PETES 1982; POTIER, WINSOR and LACROUTE 1982; SUGAWARA and SZOSTAK 1983). Any properly oriented homologous sequence should be capable of mediating the types of rearrangements we observed in the ICH strains. Thus, using other homologous sequences, it should be possible to construct yeast chromosomes *in vivo*. The first step in this procedure would be to provide direct homology between the region of the chromosome that is of interest. This could be achieved by the directed integration (ORR-WEAVER, SZOSTAK and ROTHSTEIN 1981) of the appropriate sequences in the proper orientation. The second step would be the integration of a centromere between the direct repeats to select for recombinants. Recombination between the direct repeats would produce the ring chromosome with the desired sequences.

The ability to isolate chromosomal rearrangements allows us to determine

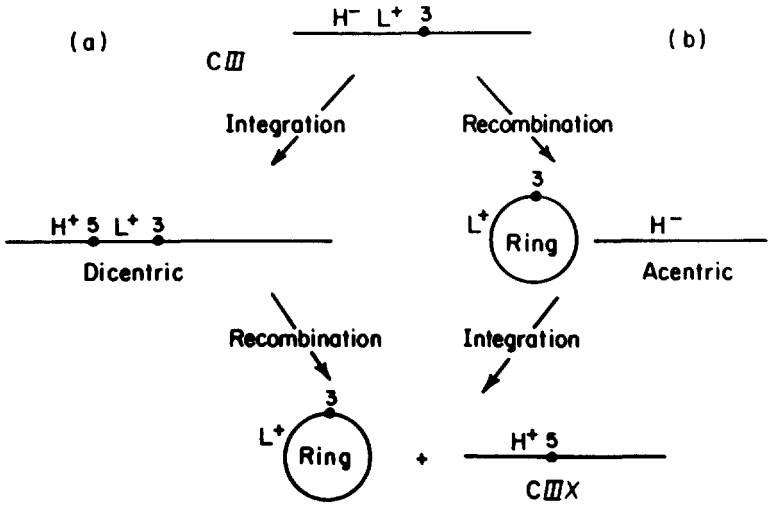

FIGURE 11.—Two possible pathways for generating the rearrangements observed in the ICH2 strains. In pathway a, integration of the *CEN5*-containing plasmid occurs prior to recombination between Ty elements. In pathway b, recombination between the Ty elements occurs prior to integration of *CEN5*. H, *HIS4*; L, *LEU2*; 3, *CEN3*; 5, *CEN5*.

what features are important for chromosome stability. In diploids made by crossing strain ICH2 to a haploid containing a normal chromosome *III*, the ring chromosome *III* and the linear chromosome *IIIA* were lost at a frequency of 1.9×10^{-3} and 1.8×10^{-4} per cell division, respectively (R. T. SUROSKY, unpublished data). The ring chromosome *III* is about 20-fold more stable than a plasmid containing an *ARS* and *CEN3* (CLARKE and CARBON 1980). The increased stability of the ring may be due to the fact that it is larger than most of the plasmids tested or that it contains multiple replication origins. The stability of chromosome *IIIA* is similar to the stability of chromosome *III* (10^{-4} chromosome loss per division) in diploids (SUROSKY and TYE 1985). The meiotic behavior of ring chromosome *III* and linear chromosome *IIIA* is also quite different in the ICH2 × TD3 diploids (Table 3). Four-spore tetrads are produced as a result of the disjunction of both the ring chromosome (*LEU2*) and the linear chromosome *IIIA* (*HIS4*) from the normal chromosome *III*. Other patterns of segregation will produce tetrads with less than four viable spores. The large number (83%) of Leu^+His^- spores from tetrads that produced only two viable spores indicates that the ring chromosome frequently cosegregates with the normal chromosome. In contrast, the absence of Leu^-His^+ spores indicates that the linear chromosome *IIIA* and normal chromosome *III* pair and segregate properly. The absence of *CEN3* and 60 kb of DNA around the centromere and the presence of *CEN5* at *HIS4* in chromosome *IIIA* does not appear to interfere with this pairing. This result corroborates the finding of CLARKE and CARBON (1983) that substitution of *CEN3* by *CEN11* does not alter the meiotic behavior of chromosome *III*. In fact, this study shows that extensive rearrangements of chromosome *III*, including substitution and displacement of the centromere coupled with a large deletion at the centromeric region, had little effect on the meiotic pairing of the chro-

mosome provided there is extensive homology between the homologs. The absence of pairing between the ring chromosome and chromosome *III* could be due to insufficient homology between the two chromosomes. Alternatively, the ring chromosome *III* is unable to compete with linear chromosome *IIIA* in the pairing with chromosome *III*. Further analysis of these rearranged chromosomes and others generated by similar mechanisms may indicate what features are important for the proper mitotic and meiotic behavior of a chromosome.

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