MEIOTIC AND MITOTIC BEHAVIOR OF DICENTRIC CHROMOSOMES IN *SACCHAROMYCES CEREVISIAE*

JAMES E. HABER,[1] PATRICIA C. THORBURN AND DAVID ROGERS[2]

Rosenstiel Basic Medical Sciences Research Center and Department of Biology, Brandeis University, Waltham, Massachusetts 02254

Manuscript received June 14, 1983
Revised copy accepted October 20, 1983

ABSTRACT

Meiotic recombination between a circular and a linear chromosome in *Saccharomyces cerevisiae* has been investigated. The circle was a haploid-viable derivative of chromosome *III* constructed by joining regions near the two chromosome ends via a recombinant DNA construction: (*HMR/MAT-URA3*-pBR322-*MAT/HML*) and was also deleted for *MAL2* (which therefore uniquely marks a linear chromosome *III*). Recombination along chromosome *III* was measured for eight intervals spanning the entire length of the circular derivative. Only 25% of all tetrads from a ring/rod diploid contained four viable spores. These proved to be cases in which there was either no recombination along chromosome *III* or in which there were two-strand double crossovers or higher order crossovers that would not produce a dicentric chromosome.——At least half of the tetrads with three viable spores included one Ura$^+$ Mal$^+$ spore that was genetically highly unstable. The Ura$^+$ Mal$^+$ spore colonies gave rise to as many as seven genetically distinct, stable ("healed") derivatives, some of which had lost either *URA3* or *MAL2*. Analysis of markers on chromosome *III* suggests that dicentric chromosomes frequently do *not* break during meiosis but are inherited intact into a haploid spore. In mitosis, however, the dicentric chromosome is frequently broken, giving rise to a variety of genetically distinct derivatives. We have also shown that dicentric ring chromosomes exhibit similar behavior: at least half the time they are not broken during meiosis but are broken and healed during mitosis.——The ring/rod diploid can also be used to determine the frequency of sister chromatid exchange (SCE) along an entire yeast ring chromosome. We estimate that an unequal number of SCE events occurs in approximately 15% of all cells undergoing meiosis. In contrast, the mitotic instability (and presumably SCE events) of a ring chromosome is low, occurring at a rate of about 1.2×10^{-3} per cell division.

THE pioneering studies of MCCLINTOCK (1939, 1941) established that broken chromosomes are mitotically unstable. MCCLINTOCK argued that the broken ends of a chromosome were highly recombinogenic and promoted a series of different chromosomal rearrangements. She observed that broken chromosomes could undergo a "breakage-fusion-bridge" (BFB) cycle, in which replicating broken ends would fuse to form a dicentric linear chromosome whose two centromeres would be pulled to opposite poles during mitosis, resulting in an anaphase bridge. The mechanical stress induced by pulling the

[1] To whom correspondence should be addressed.
[2] Present address: Genetics Institute, 225 Longwood Avenue, Boston, Massachusetts 02115.

dicentric chromosome in two directions often ruptured the bridge, resulting in the inheritance of partially deleted or duplicated broken chromosomes into two daughter nuclei. Each broken chromosome could then, in turn, continue the BFB cycle, giving rise to further genetically distinct, mitotically unstable daughter cells. Although the BFB cycle apparently could continue nearly indefinitely in somatic tissue, mitotically stable cells could be generated in several ways. In some cases, the bridge would become trapped at mitosis and apparently lost, creating aneuploid daughter cells. In other cases, especially in gametophytic tissue, these broken chromosomes could become "healed". These healing events sometimes appeared to result from translocations between the broken chromosome end and another chromosome. In other cases, apparently terminally deleted chromosomes were generated by the formation or acquisition of a new chromosome end (telomere). McCLINTOCK's studies argued that the normal chromosome end was essential to maintain chromosomal stability.

The fate of broken chromosomes is not the same in all organisms. In Drosophila, which can tolerate much less aneuploidy than maize, products of dicentric chromosomes have not been recovered, although indirect evidence for their formation and breakage has been presented (MORGAN 1933; NOVITSKI 1955).

We have been interested in extending these studies to a simpler eukaryote, the yeast, *Saccharomyces cerevisiae*, which has several advantages for such studies. In addition to having a well-defined "classical" genetic system, yeast can be readily transformed by recombinant DNA to create novel chromosomal constructions. Moreover, yeast will apparently tolerate extensive aneuploidy, so that stable products of broken chromosomes can be recovered and analyzed.

We have already shown that broken chromosomes can be generated and studied in *S. cerevisiae*. Just as McCLINTOCK (1951) showed that the moveable controlling element, Ds, would frequently cause chromosome breakage at or near itself, we showed that transposition events involving the switching of the yeast mating-type (*MAT*) locus also sometimes involved the formation of a chromosome break at *MAT* (WEIFFENBACH and HABER 1981; McCUSKER and HABER 1981). Chromosome breaks were apparently lethal in haploid cells but could be studied in diploids containing an intact homologous chromosome. The broken acentric chromosome segment distal to *MAT* was lost, whereas the centromere-containing broken chromosome fragment could become healed (that is, mitotically stable). An analysis of these healing events showed that most often a broken chromosome became stable by an homologous recombination event between the broken chromosome end and its intact homologue. However, in about 10% of these healing events, the broken chromosome was recovered as a stable apparently terminally deleted chromosome, hemizygous for markers distal to, and including *MAT*. In some cases the deleted chromosome *III* was also missing markers proximal to the position of the original chromosome break at *MAT* (McCUSKER and HABER 1981; J. H. McCUSKER, P. S. LIU and J. E. HABER, unpublished results). These shorter healed chromosomes may have arisen by a BFB cycle, or they may have occurred by exonucleolytic shortening of the broken chromosome.

Our studies of chromosomes broken initially at *MAT* could not show whether a BFB cycle did occur in yeast. Specifically, we could not show whether dicentric chromosomes either formed or were broken. To address this question we adopted the experimental approach used by L. V. MORGAN (1933) to generate dicentric chromosomes during meiosis by recombination between a linear and a circular chromosome. As shown in Figure 1A, a meiosis involving a single crossover between a circular (ring) chromosome and its linear (rod) homologue would yield a tetrad containing a dicentric chromosome plus an intact ring and an intact rod. The two meiotic segregants inheriting the ring or rod chromosome would be viable. The viability of the other two, nonsister segregants would depend on the fate of the dicentric chromosome: (1) If the dicentric chromosome were trapped during meiotic anaphase, the entire dicentric might fail to be included in either spore, and both segregants would die, lacking chromosome *III*. (2) If the dicentric chromosome broke at a random point along the bridge between the two centromeres, one of the two spores would inherit a broken chromosome carrying all of the essential sequences along chromosome *III* plus a duplication of part of one arm. This segregant would be expected to be viable, so long as the broken chromosome was not lost. (3) If the dicentric chromosome did not break during meiosis, it might be inherited intact into one spore. The segregant might be expected to survive if the dicentric chromosome were subsequently broken and healed during mitosis. Again, this segregant would be expected to be mitotically unstable. The other spore, lacking chromosome *III*, would die. Thus, we would expect to find tetrads containing two or three viable spores arising from meioses involving a ring and a rod chromosome.

Analysis of the behavior of dicentric chromosomes in yeast has several advantages over previous studies in maize or Drosophila, in which the meiotic events had to be deduced from the single surviving product of female meiosis. First, all of the viable products of a single meiosis can be recovered, so that it is possible to determine directly the likely number of crossings over that have occurred along chromosome *III* to generate a dicentric chromsome. Second, the dicentric chromosomes or their broken fragments are recovered in haploid spores, so that there are no intact normal homologues present with which broken chromosomes might recombine. Third, a large number of mitotically stable derivatives of a single spore containing a dicentric chromosome can be recovered and analyzed by both genetic and biochemical means. We have used this approach to demonstrate that dicentric chromosomes in yeast are often inherited without breakage during meiosis but that they do break frequently during mitosis. Colonies containing broken chromosomes are mitotically unstable and give rise to a wide variety of healed, stable chromosomes.

We have also shown that dicentric ring chromosomes can be inherited meiotically and give rise to the same pattern of mitotic instability as we have observed with dicentric linear chromosomes.

Sister-chromatid exchange (SCE) during meiosis: The use of a ring and a rod chromosome also allowed us to estimate the extent of SCE that occurs along chromosome *III* during meiosis. This approach, first used by MORGAN (1933),

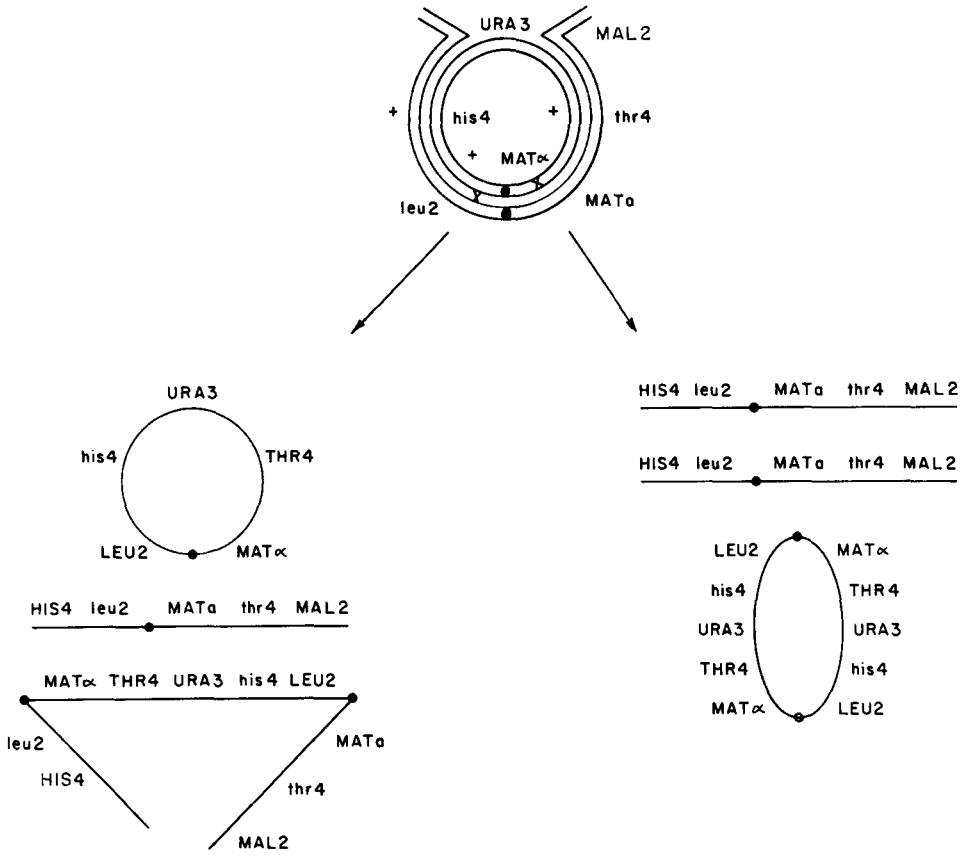

FIGURE 1.—Effect of crossing over involving a circular chromosome *III* during meiosis. A, Exchange between a circular chromosome and a linear homologue leads to the formation of a tandemly repeated dicentric chromosome. The dicentric chromosome may break, in which case one spore may receive a fragment containing more than a complete chromosome *III*, whereas the other spore would receive a partially deleted chromosome *III* and be inviable. Alternatively, the dicentric might be trapped during meiosis, in which case two spores would be inviable. More complex exchange events between a ring and a rod, *e.g.*, a three-strand double crossover, may lead to a more complicated structure, a tricentric chromosome. The subsequent inheritance of this structure into spores will lead to inviability of probably two (or three) spores. Formation of dicentric (or tricentric) chromosomes does not selectively eliminate either circular (Ura^+) or linear (Mal^+) chromosomes. B, Exchange between circular sister chromatids leads to the formation of a dicentric circular chromosome. Again, depending on whether the dicentric breaks and is inherited into a spore, one or two spores of the tetrad will be inviable. In this case, there is a selective loss of circular (Ura^+) chromosomes, as SCE with a linear chromosome does not create dicentric chromosomes. The extent of SCE can, therefore, be assessed from the excess of linear (Mal^+) over circular (Ura^+) chromosomes (MORGAN 1933).

examines the preferential survival of linear chromatids over circular chromatids during meiosis. SCE events between two linear chromatids cannot be detected; however, SCE events between circular sister chromatids result in the formation of a dicentric circular chromosome, which should be either lost or broken during meiosis (Figure 1B). In either case, at least one of two spores that should have inherited a circular chromosome will not be viable. Our

TABLE 1

Comparison of meiotic tetrads between a diploid with two linear chromosomes III *and a diploid containing a circular and a linear chromosome*

A. Spore viability

No. of viable spores per tetrad	No. of tetrads with indicated no. of viable spores	
	Circle/line[a]	Line/line[b]
4	46	84
3	55	16
2	74	5
1	33	2
0	8	3

B. Minimum number of crossovers on chromosome III *among tetrads with four viable spores*

No. of apparent crossovers	No. of tetrads with indicated no. of crossovers	
	Circle/line	Line/line
0	19	2
1	0	2
2	15	18
2-strand d.c.o.[c]	15	5
3-strand d.c.o.	0	5
4-strand d.c.o.	0	8
3	2	22
4	9	13
5	1	5
Average no. of exchanges	1.7	2.8

[a] Tetrads were analyzed from both diploids PT136 and PT139. Diploid PT136 had the genotype

circle	(*HIS4*	*leu2*	*CRY1*	*MAIα*	*THR4*	*HMR/MAT***a**-*URA3-MAT/HMLα*)		
linear	*HMLα*	*HIS4*	*leu2*	*cry1*	*MAT***a**	*thr4*	*HMR***a**	*MAL2*
	ura3	*adel*	*lys2*	+	*can1*			
	ura3	*adel*	+	*leu1*	+			

Diploid PT139 had the genotype

circle	(*HIS4*	*leu2*	*CRY1*	*MATα*	*THR4*	*HMR/MAT***a**-*URA3-MAT/HMLα*)		
linear	*HMLα*	*his4*	*LEU2*	*cry1*	*MAT***a**	*thr4*	*HMR***a**	*MAL2*
	ura3	*ade1*	*lys2*	+	*can1*			
	ura3	*ade1*	+	*leu1*	+			

[b] Tetrads from diploid PT148 were dissected and analyzed.

HMLα-URA3-pBR322-*HMLα*	*HIS4*	*leu2*	*CRY1*	*MATα*	*THR4*	*HMR***a**	*MAL2*	*ura3*	+	+	+
HMLα	*his4*	*LEU2*	*cry1*	*MAT***a**	*thr4*	*HMR***a**	*mal2*	*ura3*	*leu1*	*ade1*	*lys2*

[c] d.c.o. = double crossovers.

results show that, in yeast, SCE events occur about 5% as often as nonsister exchanges.

MATERIALS AND METHODS

Genetic methods: The strains used in this work are listed in the legend of Table 1. Cells were generally grown and sporulated at 30°. Standard genetic techniques, described by SHERMAN, FINK

and LAWRENCE (1970) were used. Cells were grown either on YEPD medium or on defined synthetic medium lacking one or more nutrients at 30°. To score *MAL2* (maltose utilization) unambiguously, we have used a method developed by J. H. McCusker (unpublished results) in which colonies were replica-plated to YEPMAL (2% yeast extract, 1% peptone, 2% maltose) agar plates that were incubated in a vented chamber with a water trap to which 100 g of crushed dry ice were added to replace air with a nearly pure CO_2 atmosphere. The anaerobic atmosphere allowed clear scoring of maltose (or other carbon source) utilization.

Biochemical analysis: DNA was isolated as described previously (HABER, ROGERS and MCCUSKER 1980). SOUTHERN (1975) blots of restriction endonuclease fragments were probed with ^{32}P-labeled plasmid. Plasmid pJH3 carries an *Eco*RI-*Hind*III fragment containing the yeast mating-type (*MAT***a**) locus (HABER, ROGERS and MCCUSKER 1980).

RESULTS

Construction of a haploid-viable circular chromosome III: The strategy for constructing a haploid-viable circular chromosome *III* is shown in Figure 2. The

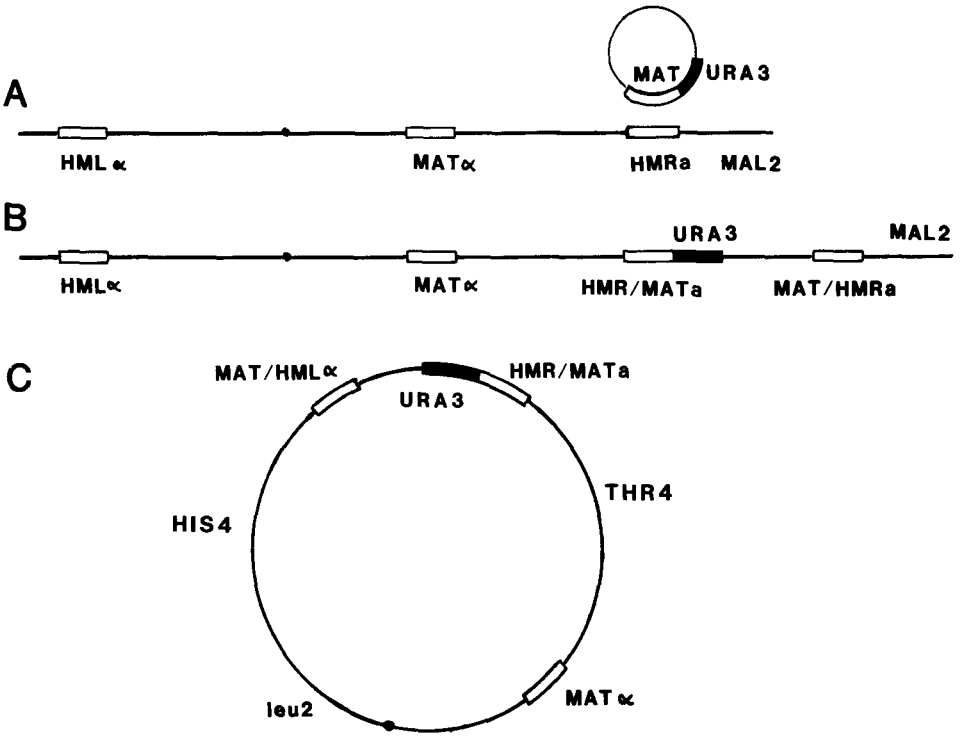

FIGURE 2.—Formation of a haploid-viable circular chromosome *III*. A pBR322 plasmid (pJH2) containing *MAT***a** and *URA3* was integrated into *HMR***a** of chromosome *III* (A) (HABER and ROGERS 1982). The integration (B) led to the expression of the *MAT/HMR***a** fusion as well as of *MATα*, causing the haploid cell to be nonmating. In the strain actually used in this study, (DR204) the plasmid integration created a tandem duplication of the plasmid sequences. The transformed strain was nonmating, expressing both α information from *MATα* and **a** from the *HMR***a**::pJH2 integration. When this nonmater was conjugated with an **a** mating haploid, an α-mating Ura$^+$ derivative of the nonmater was selected. The derivative was a haploid-viable circular chromosome in which homologous *MAT* sequences in the integrated plasmid recombined with *HMLα* (C). Neither the *MAT/HMLα* nor *HMR/MAT***a** sequences are strongly expressed, so that the mating type of strains with the circular chromosome is solely determined by *MATα* (or *MAT***a**). Note also that the circle has lost *MAL2* (distal to *HMR***a**) but is uniquely marked by *URA3* and pBR322 sequences.

ura3 strain DB745 was transformed by the integrative plasmid pJH2, containing the *Eco*RI-*Hind*III *MAT***a** fragment and the 1.1-kb *Hind*III *URA3* sequence (HABER and ROGERS 1982). The integration of the plasmid at *HMR***a** created a tandem duplication of mating-type genes: *HMR/MAT***a**-*URA3*-pBR322-*MAT/HMR***a** in which the normal repression of the silent copy gene *HMR***a** had been disrupted. The *MAT/HMR***a** fusion is analogous to so-called Hawthorne deletions (HAWTHORNE 1963; STRATHERN *et al.* 1979) and expresses *MAT***a** functions. The *HMR/MAT***a** fusion is analogous to the *SAD1* chromosomal rearrangement (HOPPER and MACKAY 1980; KASSIR, HICKS and HERSKOWITZ 1983), which has been shown to express **a** functions only very weakly. These transformants (Figure 2B), therefore, expressed α genes from *MAT*α and **a** genes from *MAT/HMR***a** within the tandem duplication; consequently, they had a nonmating phenotype (HABER and ROGERS 1982).

By crossing these nonmaters with a *ura3 MAT***a** strain, we selected α-mating Ura$^+$ derivatives that no longer expressed **a** sequences from the *MAT/HMR* region. Matings between the nonmating *URA3 HIS4 leu2 THR4 MAL2* strain DR204 (HABER and ROGERS 1982) and the *MAT***a** *ura3 his4 LEU2 thr4 mal2* strain A145 occurred at a frequency of about 10^{-6}. We recovered a Ura$^+$ Mal$^-$ colony, DR204/A145-G, that was nonmating and prototrophic for *his4, leu2, thr4* but appeared to have lost the *MAL2* gene from strain DR204. When this diploid was sporulated and dissected, only about 25% of the tetrads yielded four viable spores. Among the complete tetrads, all of the nutritional markers in the cross showed 2+:2− segregation. The low spore viability is expected for a diploid containing a circular chromosome and a linear homologue. These results were consistent with the formation of a circular chromosome (Figure 2C) in which the distal segments beyond *HML* and *HMR* (including *MAL2*) were lost, and the two silent copy genes were bridged by the plasmid sequences: *HMR/MAT***a**-*URA3*-pBR322-*MAT/HML*α.

One Ura$^+$ segregant, DR204/A145-G1B, was selected for further analysis. DNA from this segregant, as well as from the parent strains, was isolated, cleaved with the restriction endonuclease *Bam*HI and analyzed by a Southern blot, using the pBR322 plasmid containing the *MAT***a** sequence as a probe. The results of that analysis, shown in Figure 3, showed that this segregant (lane C) did not contain the *Bam*HI fragment containing *HML*α nor that containing *HMR***a**; rather, there were two new bands, as expected from the structure shown in Figure 2C. An 8.2-kb fragment (U) proved to contain *HMR/MAT***a** and *URA3*, whereas the other 9.5-kb segment (P) contained most of the pBR322 sequences and *MAT/HML*α. Further confirmation of the structure came from a *Hind*III digest (data not shown) that also lacked the *HML*α and *HMR***a** bands but contained bands at 7.4 and 4.7 kb that are the expected sizes for such fusion bands based on the known restriction sites surrounding *HML*, *MAT* and *HMR* (STRATHERN *et al.* 1980; NASMYTH and TATCHELL 1980; ASTELL *et al.* 1981; HABER and ROGERS 1982). As a final demonstration of the structure, we screened subclones of DR204/A145-G1B to obtain a Ura$^-$ derivative that subsequent Southern blot analysis showed did not contain pBR322 sequences and had only two *Bam*HI fragments complementary to a *MAT*-containing probe: one *MAT*α band and one *HML/HMR*α fusion band (Figure 3,

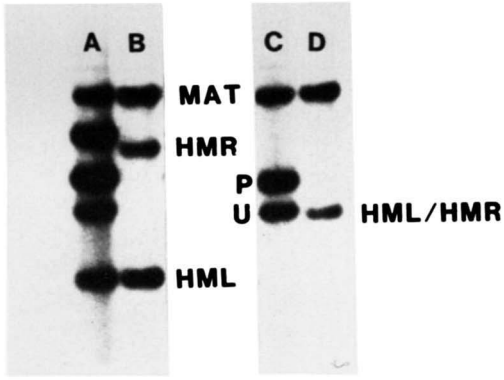

FIGURE 3.—Southern blot of *Bam*HI-digested DNA from strains showing the construction of a haploid-viable circular chromosome. Strain DB745 contains three fragments homologous to a ^{32}P-labeled pBR322 plasmid containing *MAT***a** (lane B). The integration of pJH2 into *HMR***a** created a nonmating transformant (DR204) lacking *HMR***a** but containing two new bands (lane A). The circular derivative (lane C) lacks both *HML* and *HMR* but has two bands (U and P) expected for the *HMR/MAT***a**-*URA3*-pBR322 *MAT/HMLα* structure bridging *HMR* and *HML*. Finally, a Ura$^-$ derivative of strain G1B, carrying a circular chromosome *III* containing an *HMR/HMLα* fusion, is shown in lane D.

lane D). These results would be expected from the excision of the plasmid by homologous recombination between the *HMR/MAT***a** and *MAT/HMLα* sequences flanking *URA3* and pBR322. All of these observations are consistent with the formation of a circular derivative of chromosome *III* which is apparently haploid-viable despite being deleted for sequences distal to *HML* and *HMR* (including *MAL2*). A haploid-viable circular chromosome *III*, but without the pBR322-*URA3*-*MAT* sequences, has been described previously by KLAR (1983).

Mitotic stability of a circular chromosome III: The circular (ring) chromosome *III* strain, designated G1B, grows well as a haploid strain. Subclones of this strain were surveyed to demonstrate that the integrated plasmid was quite stable. Among 4614 subclones, only one colony was Ura$^-$. The Southern blot analysis shown in Figure 3 confirmed that in this Ura$^-$ colony the duplication *HMR/MAT***a**-*URA3*-pBR322-*MAT/HMLα* had been excised. In contrast, when the ring chromosome carrying *HIS4 leu2 MATα THR4 URA3* was crossed with a normal strain carrying a linear chromosome *III* marked with *his4 LEU2 MAT***a** *thr4 MAL2*, the resulting diploid was significantly mitotically unstable. In standard mating tests, diploids carrying a circle and a linear chromosome were weakly **a** mating, and further analysis showed that the **a** mating cells had also lost *URA3*, *THR4* and *HIS4* (that is, they had lost *MATα* and all of the dominant markers carried on the circular chromosome *III*). Among 6356 subclones, 23 were found to be *MAT***a** His$^-$ Thr$^-$ and Ura$^-$. Fluctuation analysis of subclones from eight independent colonies indicated that the circular chromosome was lost at a rate of 1.2×10^{-3} per cell division. Either by patch testing or by subcloning, there were no cases in which chromosome loss appeared to involve the linear (Mal$^+$) homologue.

Behavior of a ring chromosome during meiosis: The ring chromosome *III* is

uniquely marked by *URA3* and pBR322 sequences not normally found on chromosome *III*. Conversely, a linear chromosome *III* carrying *MAL2* is also unequivocally marked, as this sequence was entirely deleted in forming the ring. We constructed two diploids with one ring and one linear chromosome *III* (PT136 and PT139; Table 1). The two chromosomes were also heterozygous for *his4*, *cry1*, *MAT* and *thr4*, as well as hemizygous for *URA3*-pBR322 and *MAL2*. PT139 was also heterozygous for *leu2*. Thus, we could directly measure recombination in six intervals along the circular chromosome *III*: *URA3-his4*, *his4-leu2*, *leu2-cry1*, *cry1-MAT*, *MAT-thr4* and *thr4-URA3* (Figure 4A). In addition, we could determine first division segregation of the centromere of chromosome *III* by following the segregation of *leu1*, which is very closely linked to the centromere of chromosome *VII*. Thus, we could also divide the *leu2-cry1* interval into two regions: *leu2*-centromere, centromere-*cry1*.

To examine the effect of a circular chromosome *III* on meiotic recombination and segregation, we also needed a nearly isogenic diploid with two linear chromosomes, marked over the same genetic distance. To this end, we constructed another derivative of strain DB745 in which *URA3* and pBR322 sequences were integrated at *HML* (Figure 4B). In this case, we transformed strain DB745 with plasmid pJH24, containing the 5.0-kb *Eco*RI-*Bam*HI *HMLα* fragment inserted into the *URA3*-pBR322 plasmid YIp5 (S. STEWART and J. E. HABER, unpublished results). The integration of this plasmid at *HMLα* created a tandem duplication of *HMLα-URA3*-pBR322-*HMLα* in which neither copy of *HMLα* is expressed (data not shown). When this strain was crossed

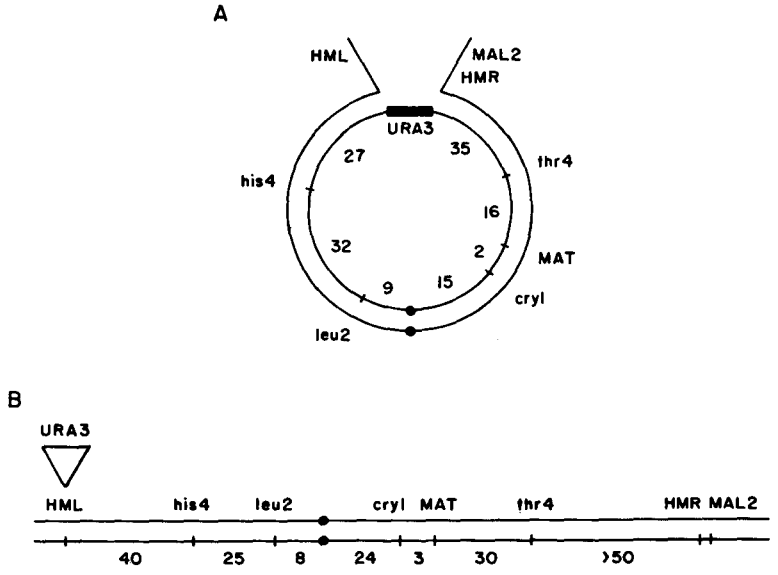

FIGURE 4.—A, Genetic map constructed from tetrads with four viable spores between a circular and a linear chromosome *III*. Distances in centiMorgans (cM) are based on 46 tetrads. B, Equivalent genetic map based on tetrads with four viable spores between two linear chromosomes *III*, one of which contains *URA3* inserted by integration of a transforming pBR322-*URA3-HMLα* plasmid (pJH5) at *HMLα*.

with strain PT40-4B (diploid PT148), it was heterozygous for all of the markers along the length of chromosome *III*, from *HML* (marked by *URA3*) to *HMR* (marked by *MAL2*), which is effectively the length of the circular chromosome in which *HML* and *HMR* are joined by the plasmid containing *URA3* and pBR322.

Three diploids, PT136 and PT139 carrying the ring and linear chromosomes and PT148 with two linear chromosomes, were sporulated for tetrad analysis. As shown in Table 1A, there was a marked difference in spore viability between the two types of diploids. As expected, the control diploid with two linear homologues had very good spore viability, with 84 of 110 tetrads having four viable spores. In contrast only 22% (46 of 216) of the tetrads from the ring/rod diploids PT136 and PT139 had four viable spores. More than 50% had two or fewer viable spores. Thus, the presence of a ring chromosome significantly diminished spore viability.

Analysis of tetrads with four viable spores: For all of the 46 tetrads from diploids PT136 and PT139 that had four viable spores, we determined the number of apparent recombination events that occurred along chromosome *III*. A genetic map was constructed to confirm that these diploids did indeed contain a circular chromosome. Furthermore, because all four members of each meiosis were recovered, we could also determine whether multiple crossovers along the chromosome involved two, three or four chromatids. A similar analysis was performed on 62 of the tetrads from the control diploid, PT148.

The 62 tetrads analyzed from the control diploid PT148 gave a genetic map (Figure 4B) that agrees quite well with the more detailed genetic map assembled by MORTIMER and SCHILD (1980). By examining all of the crossover events in a single tetrad, we could also determine the minimum number of exchange events that occurred during meiosis. In each case we could determine whether there had been no crossovers (E_0), one crossover (E_1), etc. along chromosome *III*. The results from the control cross, summarized in Table 1B, showed that the minimum number of exchange events appeared to be normally distributed, with an average number of exchange events of 2.8 crossovers per meiosis. Only two of the 62 tetrads analyzed apparently had no crossovers (E_0) along chromosome *III*.

We carried out a similar analysis on tetrads with four viable spores from the ring/rod diploids, PT139 and PT136. Among the 46 tetrads involving a ring (marked by *URA3*) and a rod (marked by *MAL2*), these two markers always segregated 2 Ura^+ Mal^-:2 Ura^- Mal^+. Thus, these complete tetrads included two ring and two rod chromosomes, which always segregated to different spores. Southern blot analysis confirmed this conclusion for one tetrad (data not shown). From the 46 complete tetrads, we could also determine the minimum number of exchange events along chromosome *III* required to account for each complete tetrad. The data for the ring/rod diploids are also presented in Table 1B. Nearly all of the 46 cases in which two ring and two rod chromosomes were recovered involved either no apparent crossovers along these homologues (E_0) or else two crossovers (E_2). All 15 E_2 tetrads were, in fact, two-strand double crossovers, as we would predict. Only two-strand double crossovers will restore two intact rings and two intact rods; three- or four-

TABLE 2

Analysis of tetrads containing two viable spores (diads) from diploids containing a circular and a linear chromosome[a]

	Phenotypes of viable spores		
	1 Ura$^+$ Mal$^-$ 1 Ura$^-$ Mal$^+$	2 Ura$^+$ Mal$^-$	2 Ura$^-$ Mal$^+$
No. of diads	35	1	15
	1 Ura$^+$ Mal^{+b} 1 Ura$^-$ Mal$^+$	1 Ura$^+$ Mal^{+b} 1 Ura$^+$ Mal$^-$	2 Ura$^+$ Mal^{+b}
No. of diads	11	5	8

[a] These data include diads from diploids PT139 and from PT136.

[b] Approximately 50% of the Ura$^+$ Mal$^+$ colonies were visibly sectored for Ura$^+$/Ura$^-$ and/or Mal$^+$/Mal$^-$.

strand double crossovers would generate dicentric chromosomes and render some spores inviable. This is in contrast to the control cross, in which only 28% (5/18) of the E_2 events were two-strand double crossovers. The two E_3 cases were both three-strand triple crossovers in which, again, all four chromatids could be recovered intact. There were no cases of a complete tetrad with only one apparent exchange event along chromosome *III* (E_1). This supports the expectation that any tetrad with a single exchange event between a ring and a rod would form a dicentric chromosome, so that not all four spores would be viable. Thus, the only tetrads that contained four viable spores resulted from meioses in which no dicentric chromosomes would be expected to form.

As expected, the map from the ring/rod diploids is circular, with *URA3* linked to both *HIS4* and *THR4* (Figure 4A). The average number of exchange events along chromosome *III* was 1.7 in the ring/rod diploids compared with 2.8 for the linear control. The 20–30% reduction in map distances of the ring/rod in Figure 4 probably results from the fact that only a fraction of all recombination events was counted as tetrads in which one or more recombination events led to the formation of a dicentric product would have been excluded.

Analysis of asci with two viable spores; evidence of the formation of dicentric chromosomes: If dicentric chromosomes that arose during meiosis could not be recovered in viable spores, a single recombination event between a ring and rod chromosome during meiosis would be expected to yield only two viable spores: one ring and one rod. When we examined those tetrads from diploids PT136 and PT139 with only two viable spores, we found that about 35 of 74 such asci with two viable spores did, in fact, contain one Ura$^+$ Mal$^-$ and one Ura$^-$ Mal$^+$ segregant (Table 2). These might represent cases in which a dicentric chromosome was formed between the other two chromatids and in which no viable spores containing all or part of this structure were recovered. On the other hand, the remaining 39 diads must have arisen in other ways. There were 15 cases in which both viable spores were Ura$^-$ Mal$^+$, but only one case

in which both spores were Ura$^+$ Mal$^-$. These data suggested that the loss of both circular chromatids occurred much more frequently than the loss of both linear chromatids. One explanation of these results is that the two circular chromatids were sometimes eliminated by SCE.

The remaining 24 asci with two viable spores contained at least one segregant that was visibly sectored for Ura$^+$/Ura$^-$ or, less frequently, for Mal$^+$/Mal$^-$. We did not find any such mitotically unstable segregants in the 46 tetrads containing four viable spores; consequently, this mitotic instability appeared to be related to the failure to recover all four viable meiotic products. We did not attempt to analyze these cases further, as more definitive data could be obtained by examining the large group of tetrads containing only three viable spores.

Analysis of asci with three viable spores; evidence for the recovery of all or part of a dicentric chromosome: A recombination event between a circular chromatid and its linear homologue would be expected to yield two viable spores, one ring and one rod, unless all or part of the dicentric chromosome was recovered in a haploid-viable form. Evidence that such products of dicentrics could indeed be recovered was implicit in the finding that 25% (55/216) of the tetrads from the ring/rod diploids contained three viable spores. Some of these could be accounted for by the fact that not every tetrad, even in a control cross, yields four viable spores, but a closer inspection of these triads made it evident that few of these arose by random spore inviability (Table 3A). For example, there were 22 cases in which there were two Ura$^-$ Mal$^+$ segregants and one Ura$^+$ Mal$^-$ spore but only seven cases in which two Ura$^+$ Mal$^-$ and one Ura$^-$ Mal$^+$ segregants were recovered. These data again suggested that ring chromosomes were less frequently recovered than rods. Much more striking was the fact that half (25/55) of the triads contained one Ura$^+$ Mal$^+$ segregant in addition to a Ura$^+$ Mal$^-$ (ring) and a Ura$^-$ Mal$^+$ (rod) segregant. In about half of these cases, the Ura$^+$ Mal$^+$ segregant was mitotically unstable and was visibly sectored for *ura3*, *his4*, *leu2* or *thr4*. The recovery of both Ura$^+$ and Mal$^+$ phenotypes in a single spore suggested that the fourth spore had failed to inherit a chromosome *III* and that the Ura$^+$ Mal$^+$ segregant contained all or part of both homologues.

We have examined 14 instances from strain PT139 in which there were three viable spores and in which one segregant was Ura$^+$ Mal$^+$. Five representative cases are presented in Table 3B. In three of the five cases (PT139-72, PT139-73 and PT139-76), it appears that the Ura$^+$ Mal$^+$ spore arose in an E_1 tetrad in which the two other segregants still have the parental configuration of markers. One very striking feature of the Ura$^+$ Mal$^+$ segregants in Table 3B (and, in fact, in 12 of the 14 cases) was that these Ura$^+$ Mal$^+$ segregants were at least partially His$^+$, Leu$^+$ and Thr$^+$, although colonies were sometimes sectored for one or more of these markers. In view of the linkage of these markers in the parent chromosomes (*cf.* Figure 1A) it was unlikely that the Ura$^+$ Mal$^+$ segregants were hemizygous and wild type for *HIS4*, *LEU2* and *THR4*. Such phenotypes would be expected if the segregants had inherited all or part of a tandemly duplicated dicentric chromosome and were, therefore, heterozygous for these regions of the chromosome. The presence of sectors

TABLE 3

Analysis of tetrads containing three viable spores (triads) from diploids PT136 and PT139, containing a circular and a linear chromosome

A. Segregation of URA3 *and* MAL2

	Phenotypes of viable spores		
	2 Ura^+ Mal^- 1 Ura^- Mal^+	1 Ura^+ Mal^- 2 Ura^- Mal^+	1 Ura^+ Mal^- 1 Ura^- Mal^+ 1 Ura^+ Mal^+[a]
No. of triads	7	22	26

B. Segregation of chromosome III *markers in five triads with Ura^+ Mal^+ segregants*[b]

Segregant	*ura3*	*mal2*	*his4*	*leu2*	*cry1*	*MAT*	*thr4*
PT139-29A	+	+	+	+	R	**a**	+
PT139-29B	−	+	−	+	S	α	+
PT139-29C	+	−	+	−	R	**a**	−
PT139-72A	+	+	+	+/−	R	**a**	+
PT139-72B	−	+	−	+	R	**a**	−
PT139-72C	+	−	+	−	S	α	+
PT139-73A	+	+	+	+	R	**a**	+
PT139-73B	+	−	+	−	S	α	+
PT139-73C	−	+	−	+	R	**a**	−
PT139-76A	+	−	+	−	R	**a**	+
PT139-76B	+	+	+	+	S	α	+/−
PT139-76C	−	+	−	+	R	**a**	−
PT139-98A	+	−	+	−	R	α	+
PT139-98B	+	+	+	+	R	**a**	+
PT139-98C	−	+	−	−	S	α	−

[a] Ura^+ Mal^+ colonies were frequently sectored when picked colonies were streaked on YEPD plates and then replicated to various nutritional "drop out" plates; such sectoring is a clear indication of mitotic instability. The high degree of instability is shown more completely in Table 4.

[b] Cells resistant to cryptopleurine were designated R; those sensitive, S. Sectored colonies were marked +/−.

for various chromosome *III* nutritional markers also suggested that these colonies were variegated and contained cells of several different phenotypes.

To demonstrate that these segregants gave rise to a variety of mitotic derivatives, we subcloned the five Ura^+ Mal^+ His^+ Leu^+ Thr^+ colonies listed in Table 3B. Among 100 subclones, we recovered various colonies that were auxotrophic for nearly every marker, in different combinations (Table 4). In some cases, all of the 100 subclones were auxotrophic for one marker (*e.g.*, *his4* in strain PT139-73A or *leu2* in strain PT139-76B) even though the original colony contained sufficient numbers of His^+ or Leu^+ cells to grow in the absence of these amino acids. These results provide strong evidence that the initial Ura^+ Mal^+ spore was mitotically unstable and gave rise to a number of phenotypically distinct derivatives. For example, in strain PT139-72A, we found subclones that were Leu^+ Thr^+, Leu^- Thr^-, Leu^+ Thr^- and Leu^- Thr^+.

TABLE 4

Phenotypes of stable derivatives from five unstable Ura$^+$ Mal$^+$ spores

Colonies	*HIS4*	*LEU2*	*MAT*[a]	*THR4*	*MAL2*	*URA3*	No. of subclones
A. Strain PT139-29A							
A.	−	+	**a**	−	+	−	82
B.	−	+	N	−	+	−	7
C.	+	−	α	−	−	+	1
D.	−	+	N → **a**	−	+	+	8
E.	−	+	N → α	+	+	−	2
B. Strain PT139-72A							
A.	+	+	**a**	−	+	−	31
B.	+	−	**a**	−	+	−	17
C.	+	+	**a**	+	+	+	3
D.	+	−	N	+	+	+	31
E.	+	+	N → **a**	+	+	+	8
F.	+	−	N → **a**	+	+	+	2
G.	+	−	α	+	−	+	2
C. Strain PT139-73A							
A.	−	+	α	+	+	+	82
B.	−	+	N	+	+	+	2
C.	−	+	α	−	+	−	2
D.	−	+	α	+	−	+	2
E.	−	+	N	+	+	−	12
D. Strain PT139-76B							
A.	+	−	α	+	+	−	22
B.	+	−	α	−	+	−	11
C.	+	−	α	+	+	+	64
D.	+	−	α	+	+	−	1
E.	+	−	α	+	−	+	1
F.	−	−	α	+	+	+	1
E. Strain PT139-98B							
A.	+	+	**a**	−	+	−	43
B.	+	+	**a**	+	+	+	2
C.	+	+	N	+	+	+	55

[a] N → **a** and N → α indicate essentially nonmating colonies that exhibit weak mating 1 day after mating tests are normally scored.

Both Ura$^+$ Mal$^-$ and Ura$^-$ Mal$^+$ derivatives were also recovered. Within a colony there were also a variety of mating types: some subclones expressed *MAT***a**, others were *MAT*α and others were nonmating (that is, expressing both *MAT***a** and *MAT*α). There were also some that showed the same weak mating behavior of the original ring/rod diploids. These data suggest that the Ura$^+$ Mal$^+$ His$^+$ Leu$^+$ Thr$^+$ segregants inherited all of the regions found on a dicentric tandemly duplicated chromosome.

Behavior of dicentric ring chromosomes: We also have examined the meiotic and mitotic behavior of diploids containing two different ring chromosomes *III*, in

TABLE 5

Spore viability among tetrads from diploids PT246 and PT247 containing two different circular chromosomes III

A. Spore viability

No. of viable spores per tetrad	No. of tetrads with indicated number of viable spores
4	17
3	41
2	56
1	43
0	41

B. Minimum number of crossovers on chromosome III *among tetrads with four viable spores*

No. of apparent crossovers	No. of tetrads with indicated number of crossovers
0	1
1	0
2	9
2-strand d.c.o.[a]	9
3-strand d.c.o.	0
4-strand d.c.o.	0
3	3
4	3
5	1
Average no. of exchanges	2.6

[a] d.c.o. = double crossovers.

which a single crossing over between nonsister chromatids would generate a dicentric circular chromosome. Diploids PT246 and PT247 were constructed by crossing ring chromosome-containing (Ura^+ Mal^-) segregants from diploid PT139 with the Ura^- ring chromosome described earlier (Figure 3). These diploids were heterozygous for *his4*, *leu2 cryl*, *MAT*, *thr4* and *URA3* along chromosome *III*. Spore viability from these ring/ring diploids (Table 5A) was similar to that observed for the ring/rod diploids (Table 1). Among tetrads with four viable spores (Table 5B), there were no E_1 tetrads or E_2 tetrads with three- or four-strand double crossovers. There was also no apparent reduction in the average number of meiotic exchanges in these diploids, compared with the rod/rod diploid PT148.

About 20% of the tetrads had three viable spores. In 21 of 41 cases, one segregant was visibly sectored for one or more of the heterozygous markers along chromosome *III*, as one might expect for a segregant inheriting all or part of a dicentric chromosome initially heterozygous for these markers. Three examples are presented in Table 6A. Just as we had observed for the variegated segregants generated from ring/rod diploids, most of these variegated segregants from ring/ring diploids (15/21) were completely or partially wild type for all of the other nutritional markers along chromosome *III*. Furthermore, when some of these colonies were subcloned, we recovered a variety of genetically distinct mitotic derivatives. Examples of such subcloning are given

TABLE 6

Analysis of asci containing three viable spores (triads) from diploid PT246[a]

	HIS4	*LEU2*	*MAT*	*THR4*	*URA3*	No. of subclones
A. Phenotypes of triads[b]						
PT246-12A	−	+	**a**	−	+	
12B	+	−	**a**	−	−	
12C	+/−	+/−	α	+	+	
PT246-15A	+	+/−	N	+	+	
18B	−	+	**a**	−	+	
18C	−	−	α	−	−	
PT246-64A	+	+	**a**	+/−	+	
64B	+	−	**a**	−	−	
64C	−	+	α	+	+	
B. Phenotypes of subclones						
PT246-12C						
A.	−	−	α	+	+	16
B.	−	+	α	+	+	23
C.	−	−	α	+	−	2
D.	−	+	α	+	−	3
E.	+	+	α	+	−	2
F.	+	−	α	+	−	10
G.	+	+	α	+	+	45
PT246-15A						
A.	+	−	N	+	+	42
B.	+	−	α	+	−	17
C.	+	+	N	+	+	154
PT246-64A						
A.	−	+	**a**	−	−	9
B.	−	+	**a**	+	−	5
C.	+	+	α	−	+	4
D.	+	−	**a**	−	+	43
E.	+	−	**a**	+	+	3
F.	+	+	N	+	+	47

[a] Diploids PT246 and PT247 both had the genotypes:

circle [*HIS4 leu2 CRY1 MAT*α *THR4* *HMR/HMLα*] *ura3 ade1*

circle [*his4 LEU2 cry1 MAT***a** thr4 HMR/MAT**a**-*URA3-MAT/HMLα*] *ura3 ade1*

[b] Sectored colonies are designated +/− for nutritional markers. Nonmating and very weakly mating colonies (which contain both *MAT***a** and *MAT*α) are designated N.

in Table 6B. Thus, segregant PT246-64A was initially sectored for *THR4/thr4* and His^+ Leu^+ and Ura^+. From 218 subclones were recovered six genetically distinct derivatives. These derivatives were heterozygous for all of the markers that were initially distributed along the two ring chromosomes, indicating that the original spore must have been heterozygous for all of these regions.

DISCUSSION

During meiosis, recombination between a ring and a rod chromosome leads to the formation of a dicentric, tandemly duplicated chromosome that can apparently be inherited into a yeast spore. Our data are compatible with the notion that the original Ura$^+$ Mal$^+$ His$^+$ Leu$^+$ Thr$^+$ spore colonies contained either all or a broken fragment of a dicentric chromosome (heterozygous for *his4*, *leu2*, *MAT* and *thr4*), and that the different stable derivatives of this colony resulted from the subsequent breakage and healing of the dicentric. These data are not compatible with an alternative possibility that the Ura$^+$ Mal$^+$ segregants were simply disomic for a ring and a rod parental chromosome. From our examination of the mitotic stability of diploids carrying a ring and a rod, we would have expected the *URA3*-containing ring chromosome to be lost in fewer than 1% of the subclones and to lose simultaneously all of the other wild-type alleles on the ring chromosome. In contrast, each Ura$^+$ Mal$^+$ segregant gave rise at high frequencies to a variety of phenotypically different mitotic subclones (Table 4). Furthermore, in four of five cases the *MAL2* marker (associated with the linear parent) was lost in 1–2% of the subclones. These results were in sharp contrast to those we found studying the mitotic stability of a ring/rod diploid.

Dicentric chromosomes are often inherited into spores without breakage: One striking conclusion we draw from this study is that yeast dicentric chromosomes generated in meiosis are frequently inherited *without* chromosome breakage into a haploid meiotic spore. We base this conclusion on several observations. First, in nearly all of the tetrads with three viable spores, the Mal$^+$ Ura$^+$ segregant was always also heterozygous for all other markers along chromosome *III* (although never heterozygous for markers on other chromosomes). The exact arrangement of these markers in a dicentric will vary, depending on the site of crossing over between the ring and the rod, but to be heterozygous for both the linked markers (*his4 LEU2*)/(*HIS4 leu2*) and *thr4*/*THR4* requires that regions on both sides of both original centromeres must have been inherited into the spore (*cf.* Figure 1A). These conclusions are further substantiated by Southern blot analysis of various healed derivatives in the accompanying paper (HABER and THORBURN 1984) that show that the Ura$^+$ Mal$^+$ colonies also inherited both *HML* and *HMR* (the distal regions of both arms of a dicentric chromosome).

If a dicentric chromosome had broken in meiosis, we would have expected the two broken, monocentric fragments to have been segregated to different meiotic poles. Under those circumstances, *HML*- and *HMR*-containing regions should not have been included in the same spore. Although it is possible that the two broken fragments nevertheless segregated to the same pole, it is more reasonable to conclude that the entire, intact dicentric was inherited by a single meiotic spore in these Ura$^+$ Mal$^+$ colonies.

We do not exclude the possibility that breakage of a dicentric yeast linear chromosome does sometimes occur during meiosis. Approximately half of the asci with three viable spores did not contain a Ura$^+$ Mal$^+$ segregant; most of these had two Ura$^-$ Mal$^+$ and one Ura$^+$ Mal$^-$ segregants. Possibly one member

of these triads inherited a broken chromosome that had ruptured during meiosis and did not include the (*HMR/MAT***a**-*URA3*-pBR322-*MAT/HMLα*) bridge. Alternatively, these could simply represent cases in which subsequent mitotic breaking of an intact dicentric led to a population of healed derivatives none of which retained the *URA3*-containing region. An alternative explanation—that these triads lacking a Ura^+ Mal^+ spore resulted from an SCE event—will be considered later.

Our results are distinctly different from those in maize, in which McClintock (1941) concluded that such dicentric linear chromosomes broke during meiotic anaphase and that broken, monocentric chromosomes were recovered from pollen or eggs. Similarly, although no viable offspring containing derivatives of a broken dicentric chromosome have been recovered in Drosophila, an analysis of the change in proportions of offspring led Novitski (1955) to conclude that dicentric linear chromosomes broke during meiosis.

It also appears that, in yeast, dicentric ring chromosomes, generated by crossing over between two nonsister circular homologues, were also inherited into meiotic spores. In 15 of 21 cases, a variegated colony, sectored for one nutritional marker, was also heterozygous for all of the other chromosome *III* markers. These data again argue that, during meiosis, dicentric ring chromosomes frequently segregate into spores without breaking to form monocentric fragments. In mitotic cells, these chromosomes apparently broke and rearranged to form a variety of genetically distinct derivatives. The behavior of dicentric ring chromosomes in yeast is different from what has been observed in Drosophila, in which Novitski (1955) concluded that dicentric ring chromosomes were trapped during meiosis and, unlike linear dicentrics, were not broken and inherited in functional gametes. In maize, McClintock (1938) observed that dicentric rings, presumably arising by SCEs, sometimes broke during meiosis but often appeared to be trapped and lost at anaphase.

Breakage and healing of dicentric chromosomes: In mitotic cells, dicentric chromosomes apparently undergo breakage and healing. It should be pointed out that, in our analysis, we only recovered cells that carried all of the essential genes found on chromosome *III*, so that a cell in which a broken chromosome was healed to produce a terminally deleted deficiency chromosome *III* would not have been viable. It is possible that such a cell might be transiently viable and be rescued by mating with another cell within the spore colony. We have shown previously that cells containing broken, deficiency chromosomes could be rescued in this manner (Weiffenbach and Haber 1981; McCusker and Haber 1981). Such matings to form diploid cells from an initially haploid spore could also occur between two cells in which breakage of a dicentric chromosome yielded one **a**-mating and one α-mating daughter cell. Indeed, among the subclones we have analyzed, some of the nonmating cells have proven to be diploid (Haber and Thorburn 1984).

It is clear that we can recover a wide variety of genetically distinct, stable derivatives from a single unstable Ura^+ Mal^+ cell containing a dicentric chromosome. A detailed analysis of the types of stable healed derivatives is presented in our accompanying paper (Haber and Thorburn 1984). Briefly, we

have found two distinct types of events. In the majority of instances, a broken chromosome end apparently recombined with an homologous region on another segment derived from the dicentric (the original dicentric chromosome is, in essence, a tandem duplication of nearly all of chromosome *III*). Both new circular and new linear chromosomes *III*, with different arrangements of markers, were recovered. In a smaller number of cases, we recovered stable derivatives containing apparently novel healing events. These were cases in which a broken chromosome end apparently acquired a new telomere—either by a translocation event or possibly by a *de novo* creation or addition of new telomeric sequences. These novel chromosomes contained partial duplications of one arm of chromosome *III*, all linked to one centromere. Both of these types of healing events are similar to those we found when we rescued chromosomes broken at the *MAT* locus (MCCUSKER and HABER 1981; WEIFFENBACH and HABER 1981).

Recombination involving circular chromosomes: One advantage of following recombination in yeast is that all four products of meiosis can be recovered. We have examined the pattern of crossover events along virtually the entire length of yeast chromosome *III* (excluding the regions distal to *HML* and *HMR*, which contain no essential genes). Among those tetrads with four viable spores, there was approximately a 25% difference in the genetic map distances along chromosome *III* in diploids containing two linear chromosomes, *vs.* those with one linear and one circular homologue. This observation confirms that of STRATHERN *et al.* (1980) who measured the *his4-MAT* interval in a diploid carrying a linear chromosome *III* and a haploid inviable small circular derivative of chromosome *III* joining *MAT* and *HML*. The decrease in map distances can probably be explained entirely by the failure to include tetrads where crossovers led to the formation of a dicentric (all E_1 and ¾ of E_2 tetrads). Thus, it appears that recombination is not significantly impaired between ring chromosomes and their linear homologues in yeast.

Extent of SCE in circular chromosomes: As we mentioned in the introduction of this paper, MORGAN (1933) first used ring chromosomes in meiosis as a means of measuring SCE. In a ring/rod cross, SCEs would selectively eliminate two ring chromatids during meiosis by the formation of a dicentric circular chromosome. SCEs between two linear chromosomes would not affect chromosome structure or segregation. From the data in Table 2 we had found that approximately 20% of the tetrads with two viable spores (15/75) had two viable linear chromosomes, whereas there was only a single example of a tetrad with only two viable ring chromosomes. These data can be explained by SCEs that eliminated the two circular (Ura^+) chromatids. To estimate the frequency with which SCE occurs, we also assume that none of the 44 tetrads with four viable spores had experienced an unequal number of SCE events between the sister ring chromatids. Thus, we can calculate that at least 12.5% [15/(75 + 44)] of these meioses included at least one SCE between the two sister ring chromatids. This value is probably an underestimate of SCE, for several reasons. First, we have not included the possibility of having two (rather than zero) SCE events in tetrads that have not eliminated the circular chromatids. Second, we have

not included corrections for cases in which SCE events would have been missed because of concomitant nonsister exchanges leading to more complex tricentric chromosomes. Even including such corrections, the frequency of SCE events along a ring chromosome *III* would appear to be approximately 15%.

Further evidence for SCE events between sister ring chromatids can be found by examining the genotypes of asci with three viable spores. In tetrads with no nonsister exchange events, an SCE between ring chromatids would be expected to yield two parental linear chromosomes and a dicentric ring chromosome that would be inherited into a third spore. Indeed, there were 22 triads with two Ura^- Mal^+ and one Ura^+ Mal^- segregants (Table 3), of which 15 had entirely parental arrangements of chromosome *III* markers. In five more cases, the arrangement of markers could be explained by a two-strand double crossover between a ring and a rod, as well as an SCE between the ring chromatids. Thus, most of these triads had genotypes consistent with the formation of a dicentric ring chromosome by SCE.

The estimate that about 15% of tetrads from a ring/rod diploid contained an SCE along chromosome *III* is considerably higher than the estimate of 3% SCE along the *X* chromosome in Drosophila (NOVITSKI 1955). One might expect the frequency of SCE to be higher in yeast, however, given the fact that the total genetic length of the ring chromosome *III* is approximately three times the genetic length of the ring *X* chromosome studied in Drosophila.

The frequency of SCE along chromosome *III* during meiosis (0.15 event) is much lower than the frequency of exchange between *nonsister* chromatids (1.7 events, shown in Table 1). We can compare these results with the previous measurements of SCE (PETES 1979, 1980; SZOSTAK and WU 1980) for exchanges within the repeated cluster of ribosomal DNA genes in yeast. Ribosomal DNA genes exhibit a very strong suppression of non-SCE during meiosis. The cluster of rDNA genes is more than 900 kb, or approximately twice the length of chromosome *III* (NEWLON, DEVENISH and LIPCHITZ 1982); however, only about 5% of all tetrads exhibit reciprocal exchanges between nonsister chromatids (PETES 1979). However, SCE (measured as *unequal* SCE between genes in this highly repeated cluster) was estimated to occur in 10% of the tetrads (PETES 1980). Thus, it appears that SCE occurs at a similar frequency along both repeated rDNA and the essentially unique DNA sequences of chromosome *III*.

We wish to thank JEFFERY HALL for first introducing us to ring/rod chromosome studies. JOHN MCCUSKER, MICHAEL LICHTEN and HEIDI HOROWITZ offered many helpful comments. This work was supported by National Institutes of Health grant GM20056 and National Science Foundation grant PCM-8110633.

LITERATURE CITED

ASTELL, C. R., L. AHLSTROM-JONASSON, M. SMITH, K. TATCHELL, K. A. NASMYTH and B. D. HALL, 1981 The sequence of the DNAs coding for the mating type loci of *Saccharomyces cerevisiae*. Cell **27:** 15–23.

HABER, J. E. and D. T. ROGERS, 1982 Transposition of a tandem duplication of yeast mating-type genes. Nature **296:** 768–770.

HABER, J. E., D. T. ROGERS and J. H. MCCUSKER, 1980 Homothallic conversions of yeast mating-type genes occur by intrachromosomal recombination. Cell **22:** 277–289.

HABER, J. E. and P. C. THORBURN, 1984 Healing of broken linear dicentric chromosomes in yeast. Genetics **106:** 207–226.

HAWTHORNE, D. C., 1963 A deletion in yeast and its bearing on the structure of the mating type locus. Genetics **48:** 1727–1729.

HOPPER, A. K. and V. L. MACKAY, 1980 Control of sporulation in yeast: SAD—a mating-type specific, unstable alteration that uncouples sporulation from mating-type control. Mol. Gen. Genet. **180:** 301–314.

KASSIR, Y., J. B. HICKS and I. HERSKOWITZ, 1983 *SAD* mutation of *Saccharomyces cerevisiae* is an extra **a** cassette. Mol. Cell. Biol. **3:** 871–880.

KLAR, A. J. S., 1983 Efficient production of a ring derivative of chromosome III by the mating-type switching mechanism in *Saccharomyces cerevisiae.* Mol. Cell. Biol. **3:** 796–802.

MCCLINTOCK, B., 1938 The production of homozygous deficient tissue by means of the aberrant mitotic behavior of ring-shaped chromosomes. Genetics **23:** 315–376.

MCCLINTOCK, B., 1939 The behavior of successive nuclear divisions of a chromosome broken at meiosis. Proc. Natl. Acad. Sci. USA **25:** 405–416.

MCCLINTOCK, B., 1941 The stability of broken ends of chromosomes in *Zea Mays.* Genetics **26:** 234–282.

MCCLINTOCK, B., 1951 Chromosome organization and gene expression. Cold Spring Harbor Symp. Quant. Biol. **16:** 13–17.

MCCUSKER, J. H. and J. E. HABER, 1981 Evidence of chromosomal breaks near the mating-type locus of *Saccharomyces cerevisiae* that accompany *MATα* × *MATα* matings. Genetics **99:** 383–403.

MORGAN, L. V., 1933 A closed *X* chromosome in *Drosophila melanogaster.* Genetics **18:** 250–283.

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

NASMYTH, K. A. and K. TATCHELL, 1980 The structure of transposable yeast mating type loci. Cell **19:** 753–764.

NEWLON, C. S., R. J. DEVENISH and L. R. LIPCHITZ, 1982 Mapping autonomously replicating segments on a circular derivative of chromosome III. Rec. Adv. Yeast Mol. Biol. **1:** 52–68.

NOVITSKI, E., 1955 Genetic measure of centromere activity in *Drosophila melanogaster.* J. Cell. Comp. Physiol. **45:** 151–169.

PETES, T. D., 1979 Meiotic mapping of yeast ribosomal DNA on chromosome XII. J. Bacteriol. **138:** 185–192.

PETES, T. D., 1980 Unequal meiotic recombination within tandem arrays of yeast ribosomal DNA genes. Cell **19:** 765–774.

SHERMAN, F., G. FINK and C. LAWRENCE, 1979 *Methods in Yeast Genetics.* Cold Spring Harbor Laboratories, Cold Spring Harbor, N.Y.

SOUTHERN, E. M., 1975 Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **98:** 503–517.

STRATHERN, J., E. SPATOLA, C. MCGILL and J. B. HICKS, 1980 Structure and organization of transposable mating type cassettes in Saccharomyces yeasts. Proc. Natl. Acad. Sci. USA **77:** 2839–2843.

SZOSTAK, J. and R. WU, 1980 Unequal crossing over in the ribosomal DNA of *Saccharomyces cerevisiae.* Nature **284:** 426–430.

WEIFFENBACH, B. and J. E. HABER, 1981 Homothallic mating type switching generates lethal chromosomal breaks in *rad52* strains of *Saccharomyces cerevisiae.* Mol. Cell. Biol. **1:** 522–534.

Corresponding editor: D. BOTSTEIN