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Genetics

Structure and organization of transposable mating type cassettes in *Saccharomyces* yeasts

(transposon/heteroduplex/hybridization/recombination)

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ABSTRACT Cell type in *Saccharomyces* yeasts is regulated by two transposable blocks of DNA, the a and α cassettes. There are three loci where either cassette can exist. At the *HML* and *HMR* loci the cassettes are not expressed. The cassette at the *MAT* locus is expressed and controls the cell type. Changes of cell type involve transposition-substitution of cassettes from *HML* or *HMR* into *MAT*. We recently reported the molecular cloning of the α cassette at the *HML* locus, *HMLα*, and showed that it contained sequences homologous to *HMR* and *MAT*. Using *HMLα* as a hybridization probe, we have isolated *HML*a, *HMRα*, *HMR*a, *MATα*, and *MAT*a. Heteroduplex analysis and restriction endonuclease mapping studies indicate that the a and α cassettes differ by a substitution corresponding to about 750 base pairs in α and about 600 base pairs in a. The *HML*, *HMR*, and *MAT* loci have regions of homology flanking the position of the a versus α substitution. We have used specific chromosome rearrangements fusing *MAT* and *HML* and *MAT* with *HMR* to orient the cloned sequences on the genetic map and have found that all three genes have the same left-to-right polarity on the chromosome.

The genes that control cell type in *Saccharomyces* yeasts reside on transposable genetic elements, the a and α cassettes (1), that occupy three different loci on chromosome III. At *HML* and *HMR* the cassettes are normally silent. The cassette residing at *MAT* is expressed and controls the cell type by activating a and α specific mating functions. The rapid interconversion of cell type during vegetative growth of homothallic strains represents the alternate transposition of a and α cassettes to *MAT* from *HML* or *HMR* catalyzed by an unlinked gene, *HO*. This transposition occurs in an orderly fashion in the cell pedigree and is not reciprocal (2, 3). That is, the cassettes at *HML* and *HMR* are not altered or consumed by the process (4–6).

The cassette model for mating type interconversion was supported by genetic results obtained in several laboratories (7–11). We have recently reported the molecular cloning of the *HMLα* cassette (12) and have used the cloned segment to confirm the basic features of the model by physical means. Sequences contained in the *HMLα* probe were found to be present at three sites in the genome. The restriction fragments corresponding to these sites were genetically mapped and identified as *HML*, *HMR*, and *MAT*. We found that the a and α alleles of each locus were correlated with a size difference in the appropriate restriction fragment indicating that the α cassette is 150 base pairs (bp) longer than the a cassette. Furthermore, we showed that the rapid interconversion of *MAT*a and *MATα* catalyzed by the *HO* gene correlated with a change in the restriction pattern expected for the substitution of a *MATα* cassette for a shorter *MAT*a cassette. In this report we define the structure of the homology among the cassettes as well as their orientation with respect to the genetic map of chromosome III.

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MATERIALS AND METHODS

Yeast Strains. The strains used are given in Table 1.

Vectors and Cloning. Genomic sequences carrying the various mating type genes were isolated by plaque-filter screening (14) of libraries of yeast DNAs carried in λgtWES (15) for homology to an *HMLα* clone (12). *In vitro* packaging of the recombinant phage libraries was done by the method of Enquist and Steinberg (16).

Restriction Analysis. Yeast DNA was isolated by the method of Cryer *et al.* (17). Restriction endonuclease digests of total yeast DNAs were displayed on horizontal agarose slab gels and were transferred to nitrocellulose and probed for homology to labeled cloned DNAs by the method of Southern (18). DNA labeled for hybridization was prepared by the method of Maniatis *et al.* (19).

Electron Microscopy. Heteroduplex DNA molecules were prepared in formamide (20, 21) and visualized on a Philips 201 electron microscope. Length calculations were done on a Numomics electronic planimeter using as internal standards portions of the vector pBR322 (22).

S1 Nuclease. The lengths of heteroduplex regions were also determined by S1 nuclease digestion of the single-stranded regions followed by agarose electrophoresis as described by Shenk *et al.* (23) combined with the technique of Southern (18). Size standards were restriction endonuclease fragments of pBR322 (22).

RESULTS

Isolation of *HMLα*, *HMR*a, *HMRα*, *MAT*a, and *MATα*

The cross-homology between the *HMLα* clone and genomic *HML*a, *HMR*a, *HMRα*, *MAT*a, and *MATα* sequences (1) enabled us to use the *HMLα* cloned gene as a probe to screen phage or plasmid libraries of yeast DNA for clones carrying each of the other mating type genes. Clones corresponding to each genetic locus were identified by comparing the sizes of cloned restriction fragments with those we had identified for each gene in digests of whole yeast DNA. The α version of each locus was recovered from a recombinant library of DNA from strain JH204.1 (*HMLα MATα HMRα*). The corresponding a genes were similarly cloned from strain JH210 (*HML*a *MAT*a *HMR*a). Fig. 1 summarizes our observations based on the physical analysis of these cloned genes to provide the reader with an orientation for the actual experiments that follow.

Heteroduplex analysis of mating type genes

In order to examine the nature of the homology between *HML*a, *HMLα*, *HMR*a, *HMRα*, *MAT*a, and *MATα*, electron microscopic analyses of heteroduplexes formed between cloned DNA fragments corresponding to each of these loci were made. Molecules to be hybridized were prepared either directly from the phage in which they were originally isolated or from plas-

Abbreviation: bp, base pair(s).

Table 1. Yeast strains used in this study

Strain	Genotype	Ref.
JH204.1	*HML*α *MAT*α *HMR*α	12
JH210	*HML***a** *MAT***a** *HMR***a**	12
XG1 no. 24	*HML*α *MAT***a** *HMR***a**/α-lethal ring	11
XG1 no. 24c	*HML*α *MAT***a** *HMR***a**/minus α ring	11
DC101	*HML*α *MAT*α *HMR***a**	This paper
DC102	*HML*α *MAT*α *HMR***a**/**a**-lethal deletion	This paper
DC103	*HML*α *MAT***a** *HMR***a**/*HML*α *MAT*α *HMR***a**	This paper
DC104	*HML*α *MAT***a** *HMR***a**	This paper
DC208	*HML*α *MAT***a** *HMR***a**/α-lethal ring	This paper

Strains DC101, DC102, DC103, DC104, and DC208 are all derived from an *MAT***a** *S. carlsbergensis* yeast (CB11), which does not have the endogenous yeast plasmid *Scp*I (13). Auxotrophic mutations were isolated and used to select the various changes of *MAT* by rare matings (11).

mid subclones made by inserting *Eco*RI/*Hin*dIII fragments into the plasmid vector pBR322. Lengths of heteroduplex regions were determined by comparison with internal standards derived from the vector both by electron microscopic contour measurements (10–15 molecules each) and S1 endonuclease treatment followed by gel electrophoresis (23). Because the techniques gave similar results (±5%), the lengths given below reflect both approaches.

MAT*a × *MAT*α.** Fig. 2*A* shows a heteroduplex between *Eco*RI fragments excised directly from hybrid phage A104.1 and HPM25, containing the *MATa** and *MAT*α alleles, respectively. This combination exhibits a single substitution loop of about 700 bases. A substitution loop of this size is characteristic of all **a** × α heteroduplexes regardless of which genetic locus is involved (*MAT*, *HMR*, or *HML*). This single substitution is the most likely location of the ≈150-bp size difference between **a** and α restriction fragments reported earlier (12). In the diagram of the mating type genes shown in Fig. 1, this substitution is indicated as the Y region and has two forms, Y**a** and Yα. The size of the Y**a** (≈600 bp) and Yα (≈750 bp) regions was determined by subtracting the lengths of the duplexes on each side of the Y**a**/Yα nonhomology from the known lengths of the restriction fragments carrying *MAT***a** and *MAT*α.

Two lines of evidence indicate that the Y**a**/Yα substitution does not represent merely an inversion loop. Heteroduplex molecules between hybrid phage containing *MAT***a** and *MAT*α in opposite orientation showed no duplex regions in the cloned segment, and Southern blot hybridization described in a later section likewise showed no homology.

HML*α × *MAT*a.** The limits of the homology between unlike cassettes at different loci is demonstrated in the heteroduplex between plasmid subclones of *Eco*RI/*Hin*dIII fragments from *HML*α and *MATa** shown in Fig. 2*B*. The plasmids were cleaved at the *Pst* I site in pBR322 before hybridization, leaving a 750-bp duplex segment of vector DNA at the *Eco*RI end of the insert and a 3582-bp segment at the *Hin*dIII end (22). In this case, two regions of homology can be observed flanking the Y**a**/Yα substitution loop. The longer of the two homologous sequences consists of approximately 1450 bp (designated WX in Fig. 1) and the shorter one approximately 300 bp (designated Z in Fig. 1). The endpoints of the homologous regions are defined by substitution loops of unique DNA at each end of the insert, a small one near the *Eco*RI site and a larger one at the *Hin*dIII end. A hybrid between *HML*α and *MAT*α plasmids (also cleaved with *Pst* I; Fig. 2*C*) lacks the Y**a**/Yα substitution loop but exhibits the nonhomologies at each end of the insert. The duplex region between them measures 2550 bp.

HMR*a × *MAT*a and *HML*α.** Heteroduplexes involving *Eco*RI/*Hin*dIII subclones of *HMRa** in pBR322 were prepared by cleaving the molecules with *Sal* I, leaving a 620-bp duplex from pBR322 at the *Hin*dIII end of the insert and a 3709-bp duplex at the *Eco*RI end (22). Comparison of *HMR***a** with *MAT***a** (Fig. 2*D*) and with *HML*α (Fig. 2*E*) shows that the extent of homology is less than that observed between *HML* and *MAT*. The structures are similar to those in Fig. 2 *B* and *C* in that the Y**a**/Yα bubble is the same size. However, the long homology (WX) is not complete. The homology between *HML* and *MAT* extends nearly to the *Eco*RI site at the left of *MAT* (Fig. 2*B*), whereas the *HMR* and *MAT* homology extends only about 700 bp, leaving a large substitution loop (Fig. 2*E*). This difference in structure defines the W and X regions shown in Fig. 1. Region X is found at all three loci, whereas the portion in common between *MAT* and *HML* but not found at *HMR* is designated W. The heteroduplex between genomic *HML*α and *HML***a** restriction fragments shown in Fig. 2*F* indicates that *HML* contains W in both *HML***a** and *HML*α and, thus, the presence or absence of W does not reflect the sex specificity of cassette transfer. Similarly, the alternative *HMR* allele (*HMR*α) is a competent donor of α information although it does not contain the W region. In addition, the S1 endonuclease analysis showed that the short homology between *HML*α and *MAT***a**, Z_L (≈300 bp), is longer than the short homology between *MAT*α (or *HML*α) and *HMR***a**, Z_R (≈250 bp).

Orientation of *HML*, *MAT*, and *HMR* DNAs on chromosome III

The orientation on the chromosome of the restriction maps of *HML*, *MAT*, and *HMR* and the WXYZ regions defined by heteroduplex analysis has been determined by taking advantage of specific chromosome rearrangements that involve these loci. For example, Hawthorne's deletion is a recessive lethal associated with a rare conversion of *MAT*α to *MAT***a** in heterothallic *HML*α *MAT*α *HMR***a** *ho* strains (24). We have proposed that this mutation involves the fusion of *MAT* and *HMR***a** (1) and have provided supportive genetic evidence (11). In an analogous manner, heterothallic cells of genotype *HML*α

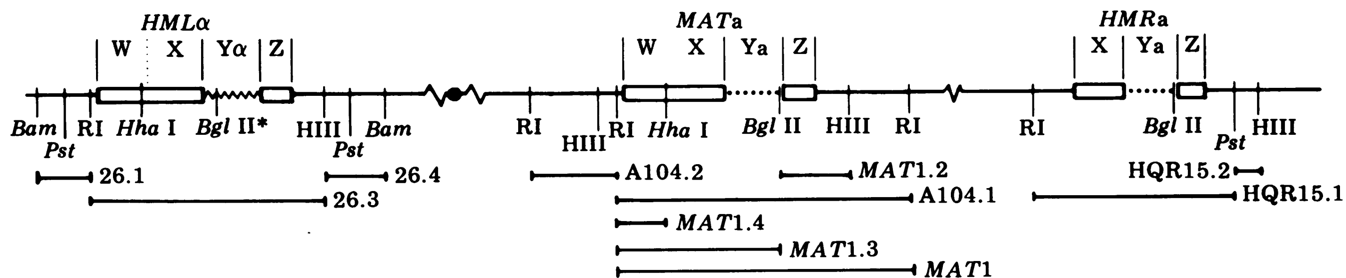

FIG. 1. Schematic representation of yeast chromosome III showing the *HML*, *MAT*, and *HMR* loci and partial restriction maps of each region not drawn to physical or genetic scale. The solid lines below denote specific restriction fragments used as hybridization probes in this work. The orientation of the W, X, Y, and Z regions of each cassette represent conclusions drawn from experiments described in the text. Open bars denote sequences common to two or more cassettes. Zig-zag and dotted lines represent sequences unique to α and **a** sequences, respectively. The heavy dot denotes the centromere. The *Bgl* II site in Yα is found in *S. carlsbergensis* only.

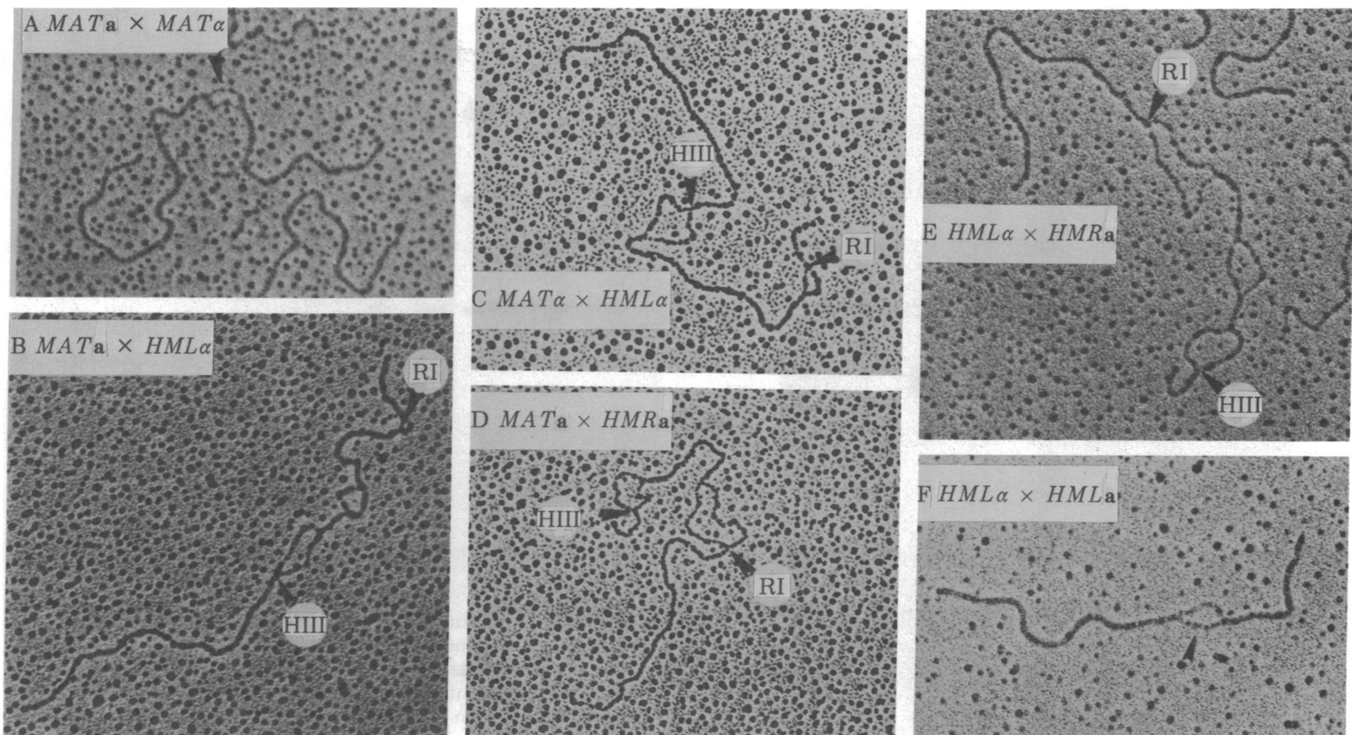

FIG. 2. Heteroduplex structures found between cloned DNA fragments containing individual mating type cassettes: the molecules in *A* and *F* represent chromosomal *Eco*RI fragments excised from the cloning vector. The molecules in *B–E* are made up of chromosomal *Eco*RI/*Hin*dIII fragments cloned into the corresponding sites in pBR322. The hybrid plasmids were cleaved at a single site in the vector DNA as described in the text.

*MAT***a** *HMR***a** *ho* occasionally switch from the **a** mating type to α by an event that generates a recessive lethal chromosome rearrangement. We have proposed that this rearrangement involves the fusion of *MAT* and *HMLα* to form a ring chromosome and have provided both genetic and physical evidence to support this interpretation (11). Figs. 3 and 4 reveal that, as expected, yeast strains carrying these chromosomal rearrangements exhibit new sized restriction endonuclease fragments homologous to *MAT*. These bands (labeled J_D and J_R) are the junction fragments formed by the fusion of *MAT* with *HMR***a** (Hawthorne's deletion, Fig. 4) and *MAT* with *HMLα* (the α-ring chromosome, Fig. 3). We have determined which end of each locus is deleted during the formation of these fusion bands by using as probes to Southern blots of these strains subcloned DNAs unique to the portion of chromosome III on each side of *MAT*, *HML*, and *HMR*. Additional evidence for our interpretation of the junction bands is presented in Fig. 5.

The orientation of *HML* by using the ring chromosome is shown in Fig. 3. Three pairs of DNA tracks digested with *Pst* I and run on the same gel were blotted. In each pair the track on the right represents an α-ring chromosome strain and the left track represents a segregant that has lost the α-ring (11). The locations of hybridization probes 26.1, 26.4, and 26.3 are shown in Fig. 1. All three probes hybridize to the *Pst* I fragment containing *HMLα*, but only 26.4 and 26.3 (Fig. 3, tracks b and c) hybridize to the J_R fragment. Thus, the 26.4 region must be included in the ring chromosome and be in the direction of the centromere (proximal of *HML*), as indicated in Fig. 1. Similarly, 26.1 is indicated as distal because it is not present on the ring chromosome junction fragment. Thus the orientation of *HMLα* on chromosome III is WXYZ, left to right.

The pairs of tracks in Fig. 4 represent *Hin*dIII digests of DNA from a *MATα*/*MAT***a**-lethal (Hawthorne's deletion) strain (DC102) and an isogenic *MATα*/*MAT***a** strain (DC103). Tracks a in Fig. 4 have been hybridized with an *Eco*RI fragment containing *MAT***a** (A104.1). As expected, all fragments containing cassettes are labeled, including the J_D fragment and an additional *Hin*dIII fragment overlapping the probe. Tracks b in Fig. 4 were probed with an adjacent *Eco*RI fragment (A104.2) from the W end of *MAT*. In this case, the only mating type fragments that hybridize are the *MAT* fragment and the J_D fragment. Thus, A104.2 is a unique probe for sequences near *MAT* but not deleted in this chromosomal rearrangement. Therefore, A104.2 is to the left of *MAT* and the orientation of *MAT* is WXYZ, left to right.

Similarly, *HMR***a** was isolated as an *Eco*RI/*Hin*dIII fragment (HQR15, Fig. 1). A *Pst* I site divides that clone into two fragments, one of which carries XYZ sequence (HQR15.1) and one that has no homology with *MAT* (HQR15.2) as shown in Fig. 4, tracks c. However, HQR15.2 does hybridize to the J_D band (Fig. 4, tracks d). Therefore, because the portion of chromosome III between *MAT* and *HMR* is deleted, HQR15.2 must be on the other side (distal) of *HMR*. In addition, HQR15.1 contains a middle repetitive sequence that hybridizes weakly to a number of restriction fragments in addition to mating type sequences. Thus, *HML*, *MAT*, and *HMR* have the same polarity, XYZ, left to right.

By additional Southern blot analysis of *MATα*-lethal deletion and *MAT***a**-lethal ring chromosomes, we have shown that these rearrangements are formed through homologous recombination in the W or X regions. Tracks a–e in Fig. 5 show the pattern of bands homologous to the *HMLα* probe 26.3 in a *Hin*dIII/*Bgl* II double digest of DNA from a series of normal and mutant strains all derived in the same genetic background (see Table 1). *Hin*dIII cleaves outside the mating type cassettes. In these strains *Bgl* II cleaves in both Y**a** and Yα but in different positions (Fig. 1). Thus, such a double digest produces eight fragments homologous to 26.3 in an **a**/α diploid strain (DC103). Each fragment can be identified on the basis of restriction mapping and the heteroduplex analysis previously described. The bands are denoted as proximal (p) or distal (d) relative to the centromere (Fig. 1). Several things about the pattern pro-

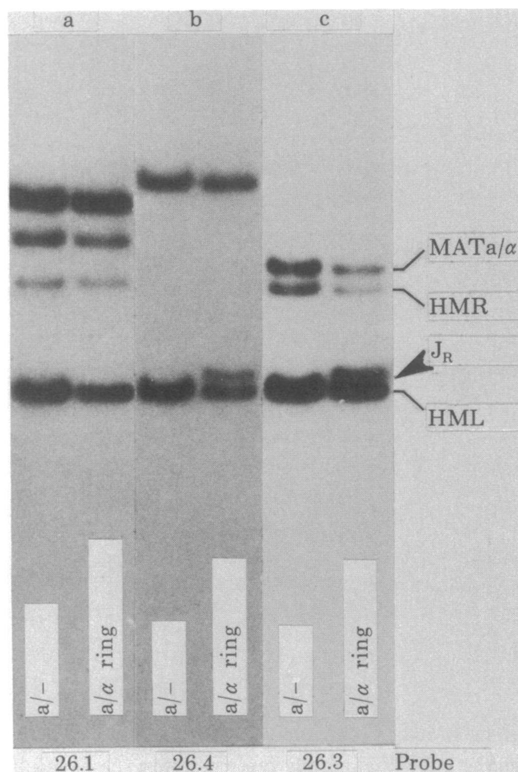

FIG. 3. Southern blots of yeast DNAs digested with *Pst* I and probed with subcloned segments surrounding the *HML* locus (see Fig. 1). Each pair of gel tracks consists of strain XG1 no. 24, carrying the *MAT*α-lethal ring chromosome, and XG1 no. 24C, an aneuploid derivative that has lost the ring. The location of hybridization probes 26.1, 26.4, and 26.3 is shown in Fig. 1. The gel band resulting from the fusion of *HML* and *MAT* in the ring chromosome is denoted J_R. Unlabeled bands in tracks a and b represent *Pst* I restriction fragments flanking *HML*. In addition, 26.1 contains a sequence represented several times elsewhere in the genome.

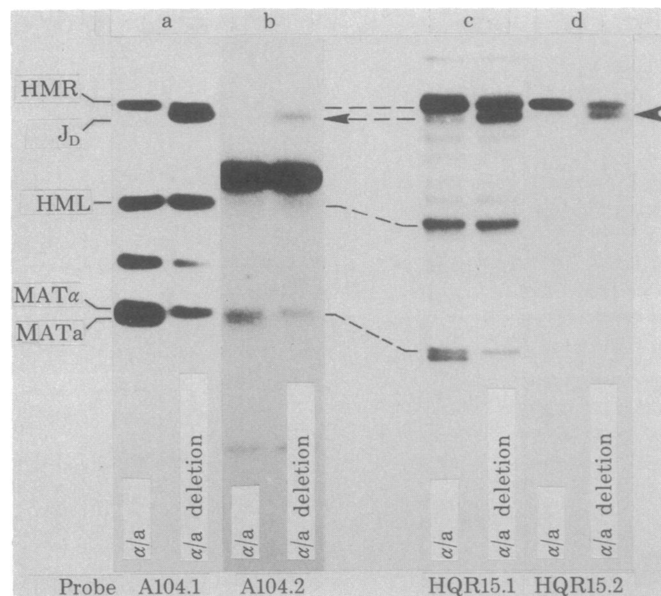

FIG. 4. Southern blots of yeast DNAs digested with *Hin*dIII and probed with subcloned segments adjacent to *MAT* and *HMR*. Each pair of tracks consists of a normal **a**/α diploid (DC103) and an isogenic diploid (DC102) containing the *MAT***a**-lethal deletion. The location of the hybridization probes from *MAT* (tracks a and b) and *HMR* (tracks c and d) are shown in Fig. 1. The band labeled J_D represents the restriction fragment generated by the fusion of *HMR* and *MAT* in the deletion strain. Bands not labeled represent sequences adjacent to *MAT* and *HMR*. Tracks a and b come from a different gel from tracks c and d.

duced by the *MAT*α/*MAT***a**-lethal deletion strain are noteworthy. Because the strain contains one normal α chromosome, both p and d *MAT*α bands, both p and d *HML*α bands, and both p and d *HMR***a** bands are present. However, even though no normal *MAT***a** allele is present (the *MAT***a** phenotype was created by the deletion), the proximal *Hin*dIII/*Bgl* II *MAT***a** band appears. The *Hin*dIII J_D fragment (not shown) is the sum of the proximal *MAT***a** *Hin*dIII/*Bgl* II fragment and the *HMR***a** distal *Hin*dIII/*Bgl* II fragment. Thus, the fusion must have occurred by reciprocal recombination between the *MAT*α X region and the *HMR***a** X region linking the *HMR***a** *Bgl* II site with the proximal *MAT* *Hin*dIII site (see Fig. 6). There is no *MAT***a** distal fragment because that region is deleted. The *HMR***a** proximal band remains because the cell contains one normal chromosome. In like fashion, the *MAT***a**/*MAT*α-lethal ring chromosome strain has lost the *MAT*α distal band by deletion but generated the normal *MAT*α proximal fragment by homologous recombination between *HML*α and *MAT***a** in the WX region (Fig. 6) to form the *Hin*dIII junction band J_R composed of the proximal *MAT*α *Hin*dIII/*Bgl* II fragment and the proximal *HML*α *Hin*dIII/*Bgl* II fragment.

Is there homology between the ends of the cassette?

The *Bgl* II site in the Y**a** region conveniently separates the long and short homologies. We subcloned the original *MAT***a** isolate (A104) and obtained *MAT*1.3 (Fig. 1), which carries only the WX and part of Y**a** regions, and *MAT*1.2, which carries the Z region. When used as a probe for sequences homologous to *Hin*dIII/*Bgl* II double digests of an **a**/α diploid (Fig. 5), *MAT*1.3 hybridizes only to the four bands corresponding to the long homology regions of *HML*α, *MAT***a**, *MAT*α, and *HMR***a**. Similarly, *MAT*1.2 hybridized only to the four bands carrying the short homologies. We therefore conclude that there is little or no homology between the WX and Z regions. Furthermore, because the *MAT*1.3 probe contains nearly the whole Y**a** region attached to WX and because *Bgl* II cuts most of Yα away from WX (see Fig. 1), we can conclude from the lack of hybridization of Y**a** to the YαZ band (*HML*αp in Fig. 5) that Y**a** does not contain Yα sequences in an inverted orientation.

Is the W region present at *HMR* in the inverted orientation?

Substitution loops visualized by heteroduplex analysis are indistinguishable from inversion loops. Therefore, we examined the *HMR* locus for inverted sequences homologous to W. Digestion of *MAT***a** DNA with *Hha* I and *Eco*RI yields a fragment corresponding to the leftmost 850 bp, including 750 bp of the long homology (*MAT*1.4 in Fig. 1). When that fragment is used as a probe to a *Hin*dIII/*Bgl* II digest of an **a**/α diploid (Fig. 5), only three bands are observed, *MAT***a**p (WXY**a**), *MAT*αp (WXYα), and *HML*αd (WXYα). The *HMR***a**p band is not homologous to that *Hha* I fragment. Thus, it is a specific probe for region W, and W is not present at *HMR***a** in either orientation. In addition to the strains derived from *S. carlsbergensis* shown in Fig. 5, we have looked for variation in the position of W sequences in 10 more strains carrying *HML***a**, *HMR*α, *HMR***a**, and *HMR*α alleles. In all cases tested, W was present at *HML* and absent from *HMR*.

CONCLUSION

The results presented here indicate that the *HML*, *HMR*, and *MAT* loci have the same basic structure consisting of two common homologous regions (X and Z) flanking a region containing either a 750-bp sequence unique to the α gene or a 600-bp sequence unique to **a**. One sequence common to *HML* and *MAT* (W) was not found at *HMR* in any of the strains

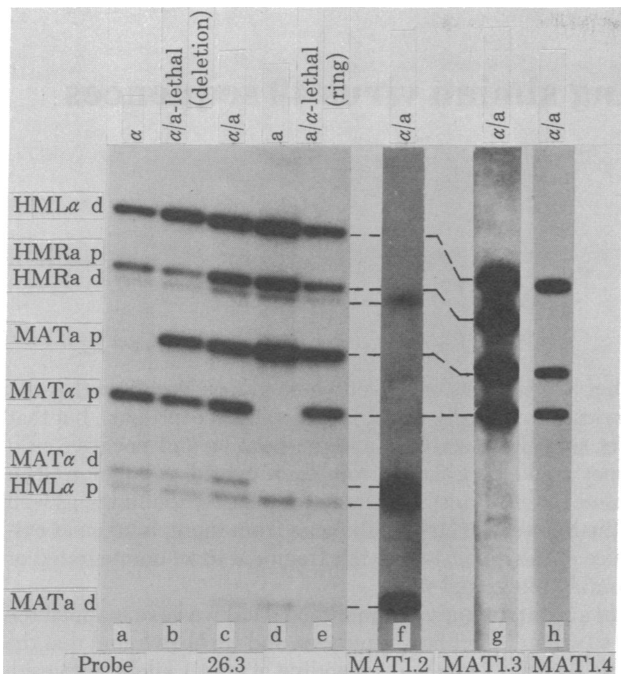

FIG. 5. Southern blots of yeast DNAs digested with *Hin*dIII and *Bgl* II probed with subcloned segments of the *MAT*a locus. The restriction fragments of each locus are denoted as proximal (p) or distal (d) to the centromere (Fig. 1). All strains were derived from the haploid α strain DC101 (see Table 1). Those denoted **a** and α are haploid strains (DC101 and DC104); **a**/α denotes a hybrid (DC102) between the two. The α/**a** deletion (DC102) and **a**/α ring (DC208) strains are described in the text. The location of the probes, 26.3, *MAT*1.2, *MAT*1.3, and *MAT*1.4 are shown in Fig. 1. Tracks g and h were run on a different gel from tracks a–f.

surveyed. Similar results have been observed in a different set of strains by Nasmyth and Tatchell (25). We have confirmed the prediction that the cassettes at *HML*, *MAT*, and *HMR* are aligned with the same polarity (11). In addition, we have shown that the structures visualized by electron microscopy are aligned on the chromosome in the order WXYZ from left to right as chromosome III is traditionally drawn.

It is clear that the portion of the mating type cassettes that is transposed contains the nonhomologous Ya and Yα regions; however, the extent to which the WX or Z regions are transposed is not known. Genetic and physical observations place some restraints on the limits of the substituted region. Although the W region may be involved in cassette transfer from *HML*, no W region sequence required for control of expression or directionality of switching can be transferred from *HML* to *MAT* because it could not be changed back by a cassette from *HMR*. Similar arguments can be applied to the difference between Z_L and Z_R.

The mechanism of cassette transposition is still unknown. We know that DNA sequences from *HMR* and *HML* must be replicated and eventually substituted for the sequences at *MAT*. This could be accomplished by replication of diffusible cassettes from *HML* and *HMR* followed by a substitution reaction at *MAT*. Alternatively, substitution might be accomplished by homologous pairing of *HML* or *HMR* with *MAT* followed by transfer of a strand from the storage loci to *MAT*. The heteroduplex formed at *MAT* would be resolved in the direction of the donated strand and the gap at the donor locus filled in by replication. The regions of homology between *MAT* and *HML* or *HMR* on each side of the transposed region provide an opportunity to initiate and terminate the recombination events required in either mechanism. However, we have not detected sequences homologous to the cassettes that are not associated

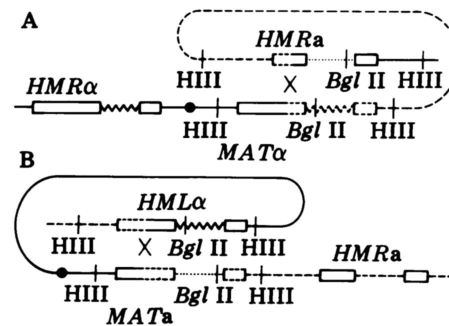

FIG. 6. Diagram of the recombination events involved in the generation of the *MAT*a-lethal deletion (*A*) and the *MAT*α-lethal ring chromosome (*B*). Dashed lines denote regions deleted by the recombination events.

with the chromosomes (12). Further, we have shown that pairing and *reciprocal* recombination occur between these loci to form chromosome rearrangements at low frequency in heterothallic strains. It is therefore reasonable that such pairing followed by nonreciprocal gene conversion could occur at high frequency in the presence of a site-specific recombination enzyme in *HO* strains.

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