Donor Locus Selection During *Saccharomyces cerevisiae* Mating Type Interconversion Responds to Distant Regulatory Signals

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ABSTRACT

Mating type interconversion in homothallic strains of the yeast *Saccharomyces cerevisiae* results from directed transposition of a mating type allele from one of the two silent donor loci, HML and HMR, to the expressing locus, MAT. Cell type regulates the selection of the particular donor locus to be utilized during mating type interconversion: MATa cells preferentially select HMLa and MATα cells preferentially select HMRa. Such preferential selection indicates that the cell is able to distinguish between HML and HMR during mating type interconversion. Accordingly, we designed experiments to identify those features perceived by the cell to discriminate HML and HMR. We demonstrate that discrimination does not derive from the different structures of the HML and HMR loci, from the unique sequences flanking each donor locus nor from any of the DNA distal to the HM loci on chromosome III. Moreover, we find that the sequences flanking the MAT locus do not function in the preferential selection of one donor locus over the other. We propose that the positions of the donor loci on the left and right arms of chromosome III is the characteristic utilized by the cell to distinguish HML and HMR. This positional information is not generated by either CEN3 or the MAT locus, but probably derives from differences in the chromatin structure, chromosome folding or intranuclear localization of the two ends of chromosome III.

Many cellular processes require productive interaction of two chromosomal regions located tens of kilobases apart. For instance, the β-globin locus control region (LCR) is located 10–20 kb upstream of the gene cluster it activates. Developmental regulation is imposed on this long range interaction to effect sequential expression of the globin gene variants (Evans, Felsenfeld and Reitman 1990). A different cellular process, immunoglobulin heavy chain class switching, requires that sequences (switch regions) separated by 50–100 kb be brought into close apposition. Isotype switching occurs by a programmed genome rearrangement during which the DNA separating one of six constant region gene segments and the variable region gene segment is deleted (Jack et al. 1988). The particular antigen encountered by B cells frequently determines which isotype will be produced, indicating that the genome rearrangement event is regulated by external cues. Although compaction of the DNA between two distant chromosomal regions can suffice to bring them into proximity, the regulation of this event by internal or external signals implies a genetic program.

Mating type interconversion in the yeast *Saccharomyces cerevisiae* provides an experimentally tractable example of a genetically regulated interaction between distant chromosomal regions. The mating type of a haploid yeast cell is determined by which of two distinct alleles is present at the MAT locus, resident on chromosome III: those carrying the MATa allele are a cells and those carrying the MATα allele are α cells. MATa and MATα encode distinct transcription factors that regulate the battery of α-specific and α-specific genes responsible for the mating phenotype of the cell (reviewed in Herskowitz 1989). Single copies of the α and α mating type genes are also present at two other loci, HMR and HML, located near the right and left ends of chromosome III, respectively (Harashima, Nogi and Oshima 1974; Strathern et al. 1980; Nasmyth and Tatchell 1980). The mating type genes resident at HMR and HML are transcriptionally silent and do not contribute to the phenotype of the cell (Klar et al. 1981; Nasmyth et al. 1981). Rather, these loci serve as templates for gene conversion of the MAT locus during a mating type switching event, described below (Oshima and Takano 1971; Hicks, Strathern and Herskowitz 1977; reviewed in Herskowitz and Oshima 1981). In order for interconversion, or switching, to occur, one of the two telomeric regions harboring the donor template must interact with the centrally located MAT locus. This long distance interaction is genetically regulated, since cell type governs selection of the locus that will productively interact with MAT (Strathern and Herskowitz 1979).

Mating type interconversion occurs in homothallic
strains of *S. cerevisiae* (those carrying the dominant *HO* gene) as often as every generation (Hicks and Herskowitz 1976; Strathern and Herskowitz 1979). Switching is initiated by a double strand DNA cleavage within the *MAT* locus, catalyzed by a site specific endonuclease encoded by *HO* (Strathern et al. 1982; Kostriken et al. 1983; Kostriken and Heffron 1984). Repair of the double strand break at *MAT* by gene conversion, using either *HMLa* or *HMRa* as the template, yields unidirectional transposition of the mating type information resident at the donor locus to *MAT*. If the mating type allele transposed from the donor cassette is different from the one previously at *MAT*, then the cell switches mating type.

By examining pedigrees of standard homothallic strains of yeast, Strathern, Hicks and Herskowitz deduced several rules that define the pattern of switching (Hicks and Herskowitz 1976; Strathern and Herskowitz 1979). First, only those cells that have previously budded are capable of switching. Second, cells switch in pairs; i.e., if a cell undergoes mating type interconversion, then both mother and daughter produced from that cell are switched. Third, a cell capable of switching will switch to the opposite mating type around 85% of the time. The first and second rules can be explained on the basis of restricted expression of *HO*. *HO* endonuclease is present only during G1 and only in those cells that have budded (Nasmyth 1983; reviewed in Breeden and Nasmyth 1985). The third rule indicates that the selection of a donor locus for transposition is not random. If it were, then productive switching, leading to a change in mating type, would occur no more than 50% of the time. That switching usually leads to a change in mating type indicates that the cell is able to distinguish *HMLa* from *HMRa* and that it has the capability of selecting one over the other.

We have attempted to define those features of *HML* and *HMR* that are used by the cell to distinguish between them during donor selection in the course of mating type interconversion. Previous work from other laboratories has shown that the alleles resident at the donor loci do not influence donor locus selection. *MATα* strains carrying the *α* allele at both donor loci or carrying the *α* allele at *HML* and the *α* allele at *HMR* still preferentially select *HML* as donor during interconversion (Rine et al. 1981; Klar, Hicks and Strathern 1982). These observations demonstrated that donor preference is independent not only of the allele resident at the donor loci but also of the mating type of the strain subsequently to the switch. That is, the cell does not continue switching until a change of mating type occurs. Rather, the cell selects one donor, on the basis of some feature of *HML* or *HMR* other than the resident allele, and uses that donor in a single mating type conversion event.

We have examined a variety of features other than the resident allele in an effort to define how the cell distinguishes between *HML* and *HMR*. The results of these analyses, presented in this report, indicate that the cell utilizes cues well removed from the donor loci themselves. Moreover, we found that the directional signals do not reside at or near the *MAT* locus. We conclude that the left and right arms of chromosome III possess distinct characteristics, and that it is this difference, and not sequences near the ends of the chromosome, that enables the cell to distinguish between the donor loci.

### MATERIALS AND METHODS

**Media and culture conditions**: Solid and liquid medium were prepared as described by Sherman, Fink and Hicks (1979). Yeast strains were usually grown on YEPD. For transformations and prototrophic selection during matings, the cells were grown on synthetic minimal medium or synthetic medium supplemented with uracil and all amino acids but those omitted for selection. *α*-Aminoacidic acid plates were used when transforming yeast cells with a plasmid that disrupted the *Lys2* gene, to select against the *Lys2* population of untransformed cells (Chattoo et al. 1979). For 5-fluoro-orotic acid (5FOA) selection, cells were grown on synthetic medium plates supplemented with 0.1% 5FOA.

**Genetic techniques**: Yeast cells were transformed by the LiAc method of Ito et al. (1983) using the one step transformation method of Rothstein (1983) or the two step replacement method of Scherer and Davis (1979) as modified by Boeke et al. (1987). The mating phenotypes of strains were assayed by printing patches of the strains to be tested onto a lawn of either DC14 (*MATα his3*1) or DC17 (*MATα his3*2) on plates that select for diploid growth. For spore to cell matings, individual cells of the haploid strain were microinjected adjacent to spores dissected from the sporulated diploid. Each potential mating pair was examined microscopically over several hours for the formation of a zygote, indicating successful diploid formation.

**Plasmid constructions**: The plasmids utilized in this study are diagrammed in Figure 1, and their construction is described in the following.

Plasmid pKSWh2 was constructed in two steps. First, the 8.8-kb EcoR1 to HindIII *MATα*×*183* fragment from the plasmid α×183 (provided by K. Tatchell) was cloned into *YEp5* to yield plasmid pKSWh1. Plasmid α×183 consists of YEp7 carrying the 4.3-kb *MATα* HindIII fragment into which has been inserted a 9-bp *XhoI* linker insertion mutation that does not affect *MATα* function (Tatchell et al. 1981). Plasmid pKSWh2 was then made by filling in the *XhoI* site in the plasmid pKSWh1 to create a 13-bp insertion, which causes a frame-shift within the *mato2* coding region. A *PacI* site was created at the insertion site by this procedure, which was used to follow the *α*2 allele during subsequent yeast strain manipulations.

Plasmid pKSWh14 consists of a *URA3*-marked deletion derivative of *HMLα* in *pUC19*. The plasmid contains the 6.6-kb BamH1 fragment spanning *HML*, from which the internal 4-kb *ClaI* to EcoRV region has been removed. In addition, the 1.1-kb *URA3* gene is inserted at a *BglII* site that marks a small deletion of the *HML E* site (from plasmid pDM41; Mahoney and Broach 1989).
Plasmid pKSW20 consists of pUC19, with the 6.6-kb BamHI fragment spanning HMLα inserted at the BamHI site and the 2.2-kb LEU2 XhoI-SaiI fragment inserted at the SmaI site. Plasmid pKSW25 is identical to pKSW20 except for the deletion of regions W and Z2. The deletions were made by oligonucleotide mutagenesis using the Bio-Rad Muta-GENE in vitro mutagenesis system. The HML fragment was cloned into M13, each deletion was made individually and the two deletions were combined upon recloning the fragment into pUC19. The oligonucleotide used to delete the W domain of HML was 5'-CCTTCTTGAACGAGTTTGAATGACGATT-3' and that used to delete the Z2 domain was 5'-AAGAGCAGTGAAAGATTTCTATATGAGTGTATAAACAAAC-3'.

Plasmids pKSW6LEU, pKSW8LEU and pKSW9LEU are similar plasmids constructed from plasmid pBR322. Each has the 2.2-kb LEU2 SaiI-XhoI sequence inserted as a HindIII fragment into the HindIII site of pBR322. Plasmid pKSW6LEU was constructed by inserting the 5.2-kb BamHI to HindIII fragment spanning HMLα into the PvuII site of the vector, a process that reformed the BamHI site. The HML fragment is oriented such that W is nearer the β-lactamase gene. Plasmids pKSW8LEU and pKSW9LEU were constructed by inserting the 4.1-kb EcoRI to HindIII fragment spanning HMRα, derived from plasmid pJA82.6~268 (ABRAM et al. 1984), at the PvuII site of pBR322, a process that reformed the EcoRI site. The HMR fragment in pKSW8LEU is oriented such that the X region is nearer the β-lactamase gene; pKSW9LEU has HMR in the reverse orientation.

Plasmid pKSW45 was constructed by first inserting the 3.2-kb mata1α65 EcoRI to Sso3A1 fragment from the plasmid α65 (provided by K. TATCHELL) into the EcoRI to BamHI sites of the pUC18 polylinker. Plasmid α65 consists of YEp7 carrying the 4.3-kb mata1α65 HindIII fragment (TATCHELL et al. 1981). The mata1α65 mutation is a 213-bp duplication marked with an XhoI linker insertion in the α1 coding region. The 1.1-kb URA3 HindIII fragment was then inserted into the HindIII site of the pUC18 polylinker.

Plasmid pKSW32 is derived from plasmid pKSW27, which consists of the 3.8-kb EcoRI to HindIII fragment spanning mata2 inserted into pUC18. The 0.8-kb NdeI fragment extending from within the Ya region to the Z1/ Z2 boundary was removed from plasmid pKSW27, and replaced with the 2.2-kb LEU2 SaiI-XhoI sequence.

Plasmid pKSW34 contains the 3.8-kb EcoRI to HindIII fragment carrying a MATαmα allele in pUC18. The MATαmα allele was constructed by replacing the 1.2-kb ScaI fragment carrying the Ya region of MAT from plasmid pKSW27 with the ScaI HMLαmα fragment from the plasmid pAK10 (kindly provided by A. Klar). Plasmid pAK10 contains the HMLαmα BamHI fragment in YEp13, created by retrieval of the inc mutation by in vivo gap repair using the HMLαmα strain K673. Plasmid pKSW34 also contains a 4.5-kb lys2 inverted cassette, inserted at the NarI site of pUC18, for directing recombination of the plasmid into the LYS2 coding region (VOLKERT and BROACH 1986). This cassette consists of the lys2 3' region from the internal XhoI site to the BglII site followed directly by the lys2 5' region from the BglII site to the internal XhoI site. The linear fragment produced by cleavage of the plasmid with BglII is essentially the lys2 gene interrupted by pUC and MATαmα.

Plasmid pKSW37 contains a URA3-marked deletion derivative of MAT in pUC18. The plasmid consists of the 4.3-
## Table 1

### Experimental yeast strains

<table>
<thead>
<tr>
<th>Strain</th>
<th>Chromosome III</th>
<th>Genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>KSW4</td>
<td>MATα HMLα HMRα</td>
<td>HO ura3-52 leu2-3,112 his3Δ1 trp1-289</td>
</tr>
<tr>
<td>KSW7</td>
<td>MATα HMLαΔΔΖΖΖ HMRα</td>
<td>HO ura3-52 leu2-3,112 his3Δ1 trp1-289</td>
</tr>
<tr>
<td>KSW10</td>
<td>MATα (hmlα-ter)Δ:HMRLα (hmrα-ter)Δ:HMRA-stk</td>
<td>As KSW4; mfa1-lacZ</td>
</tr>
<tr>
<td>KSW17</td>
<td>MATα (hmlα-ter)Δ:HMRA-stk (hmrα-ter)Δ:HMRLα</td>
<td>As KSW4; mfa1-lacZ</td>
</tr>
<tr>
<td>KSW17-Z11T</td>
<td>MATα (hmlα-ter)Δ:HMRA-stk (hmrα-ter)Δ:HMRLα</td>
<td>As KSW4; mfa1-lacZ</td>
</tr>
<tr>
<td>KSW17α1</td>
<td>mata1 (hmlα-ter)Δ:HMRA-stk (hmrα-ter)Δ:HMRLα</td>
<td>As KSW4; mfa1-lacZ</td>
</tr>
<tr>
<td>KSW19</td>
<td>mataΔ:URA3 HMLα HMRα</td>
<td>As KSW4; lys2Δ:MATα,LEU2</td>
</tr>
<tr>
<td>KSW24</td>
<td>MATα HMLα HMRα cens3Δ: CEN3,URA3</td>
<td>As KSW4;</td>
</tr>
<tr>
<td>KSW25</td>
<td>mataΔ:LEU2 HMLα HMRα</td>
<td>As KSW4; lys2Δ:MATα ·</td>
</tr>
</tbody>
</table>

### Description

- **kb HindIII** fragment spanning **MAT**, from which the internal 3.3-kb **BglII** fragment completely encompassing **MAT** has been removed, with a 1.1-kb **URA3** **BglII** fragment replacing the deleted region.
- Plasmid pKSW55 was constructed by first inserting the 2.2-kb **LEU2** **SalI**-**Xhol** sequence into the **SalI** site of pUC18. The 4.5-kb **lys2** inverted cassette described for pKSW34 was also inserted into the **SalI** site. A 2.5-kb **BglII** fragment that precisely spans **MATα** was then inserted into the **BamHI** site, deleting a small fragment of **lys2**' to the **Xhol** site in the process. The **BglII** sites precisely bracketing **MATα** were created by oligonucleotide mutagenesis of the cloned **MATα** gene, using the Altered Sites mutagenesis kit from Promega.

### Yeast strains and strain constructions

The strains used in this study were all constructed in this laboratory and are listed in Table 1. They have identical genetic backgrounds and derive from a common **HO** haploid precursor, strain KSW3-20C, which was the parent strain for our genetic analyses of mating type interconversion in this and other studies. To construct strain KSW3-20C, strain S150-2B (MATα HMLα HMRα leu2-3,112 his3Δ1 ura3-52 trp1-289 gal2 ho) was made hmlα2 by a two-step gene transplacement using the **URA3** mata2 plasmid, pKSW2. Plasmid pKSW2 was digested with **Hpal**, which cuts the DNA in the **W** region, and transformed into strain S150-2B. The transformants were screened by Southern analysis to identify the desired strain with an integration at **HML**. Isolates that had excised **URA3** and either **HMLα** or **hmlα2** were selected by growth on 5FOA, and **hmlα2** strains were identified by Southern analysis. The **HO** gene was introduced into this strain by crossing it with strain SYF46 (**ura3** **HO**), sporulating the diploid, and identifying a spore clone having the desired genotype. Strain KSW3-20C was haploid, since the **α2** mutation at **HML**, when transposed to **MAT**, resulted in a sterile phenotype. Consequently, strain KSW3-20C was composed of a mixture of haploid **MATα** and **mata2** cells (TANAKA et al. 1984).

Strains KSW4 and KSW7 were made by first replacing the **hmlα2** locus of strain KSW3-20C with **URA3**, by transformation with the **BamHI** **hmlα::URA3** fragment from plasmid pKSW14, to yield strain KSW3-20C-hmlΔ. The **hmlα::URA3** allele was replaced by either **HMLα** or **HMLαAWAZ2**, using plasmids pKSW20 or pKSW23, respectively, in two steps. Strain KSW3-20C-hmlΔ was transformed to Leu+ with pKSW20 or pKSW23 DNA, linearized at the unique **Stul** site in the **HML** flanking sequence to direct its integration next to **URA3**. The wild type **α** allele at **HML**, introduced into the genome by plasmids pKSW20 and pKSW23, and pKSW2, as well as similar plasmids employed subsequently for strain constructions, served as a template for gene conversion of the **mata2** locus, during a normal switching event. Consequently, the **α2** allele was permanently lost in these strains. With this exception, strain KSW3-20C is isogenic to strain KSW4. One transformant of each plasmid was retained and isolates of the transformants in which **URA3**, **LEU2** and vector sequences were excised by recombination, were identified by growth on 5FOA, and confirmed by Southern analysis.

Strain KSW10 was constructed through the sequential deletion and replacement of each end of chromosome III with the **HM** locus of interest. The success of each step of the construction was confirmed by Southern analysis. The left arm of chromosome III was truncated by transforming strain KSW3-20C with the fragmentation plasmid pAR65 (ROSE 1990; derived from the plasmid YCF3 described in VOLLRATH et al. 1988), linearized with EcoRI. This telomeric plasmid contains a DNA fragment centromere-proximal to **HML**, ~0.9 kb away from the **2Z** region, for targeting the recombination event, and the **URA3** gene for selection. The resulting strain, pAR65-KSW3-20C, was transformed...
to Leu+ with SalI-digested pKSW6LEU DNA (HMLa LEU2), which integrated at the new left terminus next to the URA3 marker, directed by homology to pBR322 sequences. The transformant was able to diploidize, through HO-mediated mating type interconversion, using HMLa as a donor locus. In order to select for isolates in which URA3, LEU2 and vector sequence, but not HMLa, had excised by intramolecular recombination, the diploid was sporulated and spores were plated on 5FOA. One such isolate was retained as strain 6LEU6, which, due to HO-mediated mating type interconversion, persisted as a homozygous diploid. The right arm of one chromosome III homolog in strain 6LEU6 was then truncated by transforming the strain to Ura+ with plasmid YIp5-MATa, linearized at the donor locus. In order to select for isolates in which the URA3 allele was identified by Southern analysis. Strain KSW24 was a diploid, homozygous for the MATa locus carried on plasmid pKSW35 DNA. A transfection mating type interconversion, the diploid was sporulated and retained one isolate bearing a T at position Z11 as strain KSW17-Z11T. Strain KSW17-Z11T is isogenic with strain KSW17 except for the single base substitution at position Z11 of MATa.

Strain KSW17a1 was produced from strain KSW17 by introducing the matalx65 mutation at MAT. Strain KSW17 was transformed to Ura+ with plasmid pKSW45 DNA linearized at the unique EagI site in the W region of MAT. A transformant in which the plasmid had integrated at the MATa locus was identified by Southern analysis. Isolates of this transformant that had excised URA3 and either MATa or matal by intrachromosomal recombination, were selected by growth on 5FOA. One isolate that retained the matal allele was identified by Southern analysis and saved as strain KSW17a1.

Strain KSW19 was a derivative of strain KSW3-20C by way of intermediate strains constructed for other purposes, and consequently had a circuitous history. Transformation of strain KSW3-20C with the matΔ::LEU2 HindIII fragment from pKSW32 yielded strain KSW3-20C-matΔ::LEU2, in which part of the matal locus was lost by the 2a-2 promoter and the HO endonuclease cleavage site was replaced with LEU2. Strain KSW3-20C-matΔ::LEU2 was then transformed to α-adaptive resistance with BglII-digested pKSW34 DNA to generate strain KSW12, which had MATaom (and vector sequence) inserted into LYS2, rendering the strain Lys-. We then mated strain KSW12 to an a spore from strain KSW4, by spore to cell mating. The KSW4/ KSW12 diploid was sporulated and Leu+ Lys- progeny (i.e., matΔ::LEU2 LYS2) were screened by Southern analysis for those harboring the HMLa locus. One such strain, KSW4/ KSW12 -10c, was retained. The deletion of MAT in strain KSW4/KSW12 -10C was then replaced with a larger deletion by transforming it to Ura+ with the HindIII fragment encompassing matΔ::URA3 from plasmid pKSW37. We obtained Ura+ Leu- transformants and these were shown to have the correct structure by Southern analysis. One such transformant was retained as strain KSW14. Since this deletion removes the C terminal end of the essential TSM1 gene (as determined by Ray, White and Haber 1991a) we also transformed diploid cells with the same DNA fragment from plasmid pKSW37. Two diploid transformants were sporulated and tetrads from both produced four viable spores that segregated 2:2 for Ura+. We conclude that either the C terminus of the TSM1 protein is not essential for its function or the gene is not essential in our strain background. The final step in the construction of strain KSW19 was the insertion of MATa at LYS2. Plasmid pKSW55 contained, in place of part of the coding region of LYS2, the LEU2 gene and a fragment encompassing MATa, whose endpoints were internal to the deletion present in strain KSW14. Strain KSW14 was transformed to Leu+ using BglII-digested pKSW55 DNA and one Leu+ Lys- transformant was retained as strain KSW19.

Strain KSW24 was created by transforming strain KSW4 to Ura+ using the EcoRI fragment from plasmid pJCS3-6, which carried the inverted CEN3 region, its flanking sequence and a URA3 marker. One transformant was sporulated and a Ura+ clone was retained as strain KSW24. The inversion of CEN3 in strain KSW24 was confirmed by Southern analysis. Strain KSW24 was a diploid, homozygous for the CEN3 inversion, as a result of HO-mediated mating type interconversion.

Strain KSW25 was derived from the diploid, KSW4/
KSW12. It was identified as a Leu+ Lys+ clone (matΔ::LEU2 lys2::MATαωo) that harbored the HMLα allele, as shown by Southern analysis.

Pedigree analysis: Pedigree analysis was performed on α spores in order to determine the frequency of MATα to MATα switches as follows. Diploid cells were sporulated and the ascii were placed on a YE PD plate that had been preincubated overnight with a thick bisecting line of the α tester strain, DC17. Individual tetrads were micromanipulated to within a few hundred micrometers of the mass of α cells and dissected. The spores were placed at 1-mm intervals along the α cell line with 2 mm separating the four spores of each tetrad, and the positions of each spore on the plate were noted. The cells were examined microscopically at frequent intervals after about 4 hr of incubation at 30°. MATα spores germinated but arrested and changed morphology in response to α pheromone (α-factor) produced by the DC17 α cells; MATα spores germinated and divided. The α spore progeny were then continually monitored through the first two cell divisions, and the daughter cells were micromanipulated apart as early as possible. The first switching event occurred in the mother cell and was scoreable at the four cell stage. If the mother cell switched mating type, then two α cells were produced along with the two α cells from the daughter line. Therefore, a successful switch was evident by the production of two α-factor sensitive cells. If the mother cell did not switch mating type, then all four cells remained as α cells. Therefore, four α-factor resistant cells indicated that a switch had not occurred or that the MATα allele was replaced with a new α allele.

Pedigree analysis was performed on α spores in order to determine the frequency of MATα to MATα switches by a similar procedure. Diploid cells were sporulated and the ascii were placed on a YE PD plate outside of a region on which α cell pheromone (α-factor) had been applied. The α-factor was obtained from the liquid media of a culture of α cells and was prepared according to the method of Michaelis and Herskowitz (1988). We applied 120 μl of a 90-fold concentrate to an area of ca. 4 cm². Individual tetrads were micromanipulated into the α-factor region and dissected. The spacing between spores was as described above except they were placed in rows spaced 2 mm apart in the α-factor region. The position of each spore was noted. DC17 α cells were applied in a thick line bisecting the plate, as before. The plate was incubated at 30°, and microscopic examination was initiated after about 4 hr. The MATα spores were identified by their sensitivity to the α-factor: they germinated but arrested and changed morphology. The MATα spores were resistant to the α-factor. The progeny of the α spores were removed from the α-factor region as soon as the mother cell began cell division and were placed adjacent to the DC17 α cells by micromanipulation. The four progeny cells were then examined for sensitivity to α-factor, as described above. A change in cell type was again evident as two α-factor resistant cells (mother line) that divided and two sensitive cells (daughter line) that arrested. However, if the cells did not switch but remained MATα, all of the cells were α-factor sensitive. When determining the frequencies of switching, only those spores that derived from tetrads with four viable spores that segregated 2:2 MATα MATα were counted.

Pedigree analysis of mataI spores was initiated in the same manner as that for α spores, since, like α spores, mataI spores are resistant to α-factor. However, the spore progeny were not moved away from the α-factor-treated area but rather were examined for the acquisition of α-factor sensitivity, which indicated a switch to MATα. We found that the morphological change exhibited by MATα cells responding to α-factor was not as distinctive or persistent as that exhibited by MATα cells in response to α, and was often difficult to score. Therefore, KSW4 α spores were simultaneously assayed as a positive control for switching from MATα (α-factor resistant) to MATα (α-factor sensitive). Nevertheless, a small degree of uncertainty existed in the switching values obtained. A few tetrads that gave rise to two α-factor sensitive spores were presumed to have resulted from meiotic reversion of the α1 mutation, and were not analyzed.

Mother cell viability: Tetrad from strain KSW19 yielding four viable spores were examined for the viability of the mother cell produced by the first cell division. The two progeny of the first mother cell were micromanipulated away from the daughter cell line and placed in a known position on the plate. After two days and then again at about a week, the cell pairs were scored for colony formation. Those that did not form colonies consisted of a cluster of cells that did not appear to change after the second day.

Cell rescue experiment: The rescue of cells that appeared to have switched from α to a was initiated in the same manner as a standard MATα to MATα pedigree analysis. The progeny from α spores that exhibited α-factor sensitivity were identified and scored as a. These cells were micromanipulated to another section of the plate and placed adjacent to strain KSW25 cells, for cell-to-cell matings. The mating pairs were microscopically examined over the next few hours to determine if a mating had taken place. If the cells had mated, the zygote was isolated and moved again to its final position, which was noted. In this manner, seven cells were successfully rescued as diploids. For subsequent experiments, strain KSW25 was transformed with the vector YEp57 in order to make it His+. This enabled us to set up matings between each a cell to be rescued and several a cells of strain KSW25 and then later isolate the diploid cells that resulted from each successful mating by selection for His" Ura+ clones. We observed His" Ura+ colonies only at the positions on the plate that corresponded to the arranged matings. We rescued 13 cells that appeared to have switched from MATα to MATα by this procedure. All 20 diploids were then tested for their mating phenotypes.

RESULTS

In the process of mating type interconversion in the yeast S. cerevisiae, one of the two donor loci residing near the left and right telomeres of chromosome III must be brought into spatial proximity of the mating type locus, MAT, as shown in Figure 2. A unidirectional gene conversion of the MATα allele to the allele residing at the donor locus follows. While an interaction between MAT and the donor locus on either chromosome arm is possible, cell type regulates the relative probabilities that each one will be utilized for interconversion. In an a cell, HML is nearly always selected and in an α cell, HMR, is nearly always selected. Implicit in the deliberate selection of HML or HMR as the preferred donor locus during a switching event is the requirement that the cell be able to distinguish these two loci.

As a means of identifying the feature(s) that distinguish the two loci, we deleted or otherwise manipulated sequences at the donor loci or at other specific sites within chromosome III to reveal any recognition
Donor Locus Recognition Signals

The double deletion derivative, HMLΔWΔZ2, to create strain KSW7, whose structure is depicted in Figure 3A. In order to determine if this alteration of HML affected the cell's ability to distinguish it from HMR, we measured the frequencies at which a and A cells were able to change mating type. This was done by pedigree analysis, as described in detail in MATERIALS AND METHODS. Basically, we first identified a and A spores by their sensitivity to the pheromone produced by the opposite cell type. By analyzing the spore progeny for pheromone sensitivity following two rounds of cell division, we assessed whether a mating type interconversion event, indicative of proper donor locus selection, had occurred.

Results of pedigree analysis from the parent HMLA strain, KSW4, are presented in Figure 2B and those from the HMLΔWΔZ2 strain, KSW7, are presented in Figure 3B. We found that the frequencies of mating type interconversion for strain KSW7 were equivalent to those obtained for strain KSW4. These results unequivocally demonstrate that W and Z are not required at HML to distinguish it from HMR during donor locus selection. Thus, the difference in donor locus structure and accordingly, the extent of homology between MAT and each donor locus, is not important for the distinction of HML and HMR.

The chromosome III telomeres and telomere proximal sequences do not provide signals for differentiating HML and HMR: As a next step toward localizing the sequence(s) recognized by the cell during donor locus selection, we asked if chromosomal sequences distal to the HM loci were required for discrimination. HML is located approximately 10 kb from the left telomere of chromosome III and HMR is located approximately 25 kb from the right telomere (Yoshikawa and Isono 1990). No genes essential for vegetative growth reside in either distal region. The two chromosome III telomeres have the same basic structure: embedded in C1-αA repeats are X regions but no Y' repeats. We deleted both ends of chromosome III, leaving only 1.8 kb distal to W at HML, and 0.8 kb distal to Z1 at HMR (see Figure 4A). Telomeric sequences from Tetrahymena substituted for the natural telomeres of chromosome III. The frequencies with which this strain, KSW10, was able to switch mating type were determined by pedigree analysis. The results from these analyses, presented in

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The result of interlocus transposition was altered. The result of the experiment described below, in which the extra regions of homology between HML and MAT were precisely excised.

Two possible outcomes of this experiment were anticipated. If the donor locus recognition information were not included on the interchanged fragments but remained at the normal location, then we would have expected that mating type switching would occur at wild-type efficiency in strain KSW17. On the other hand, if the donor locus recognition information were not included on the interchanged fragments but remained at the normal location, then we would have expected that the frequency of mating type interconversion would be quite low. In this case an a cell would most often select the donor locus on the left arm and an a cell would most often select the donor locus on the right arm, leading only infrequently to an observable mating type switch.

The frequencies of mating type interconversion obtained for strain KSW17, as explained in the legend to Figure 2.

Figure 4.—Sequences distal to the donor loci are not required for donor preference. (A) Upper line: diagram of chromosome III for strain KSW4, under which are indicated the approximate distances in kilobases between the various landmarks (Yoshikawa and Isuno 1990). Lower line: diagram of chromosome III for strain KSW10, in which sequences 1.8 kb distal to HML and 0.8 kb distal to HMR have been truncated and replaced with telomeric sequence from Tetrahymena (arrows). Hatched regions indicate positions of pBR322 sequences. (B) The frequencies of mating type interconversion obtained for strain KSW10, as explained in the legend to Figure 2.

Figure 3.—Donor preference does not depend on differences in locus structure at the two donor loci. (A) Upper line: diagram of chromosome III for strain KSW4. Lower line: diagram of chromosome III for strain KSW7. HMLΔAZ2 is a deletion allele of HMLa in which the extra regions of homology between HML and MAT are precisely excised. (B) The frequencies of mating type interconversion obtained for strain KSW7, as explained in the legend to Figure 2.

Unique sequences flanking HML and HMR do not function to discriminate the donor loci: Since neither the difference in locus structure between HML and HMR nor the sequences distal to the donor loci provide cues for distinguishing between them, we tested the possibility that the unique sequences flanking the loci contained the critical distinctive features. If this were the case, then the transposition of each donor locus and its flanking sequences to another location on the chromosome would not affect the donor specificity of mating type interconversion. In other words, the loci would be identifiable despite their new positions.

To make the least drastic chromosome rearrangement possible, we simply interchanged the donor loci, along with a substantial amount of flanking sequence from each. This reconstruction retained the donor loci on chromosome III and maintained their telomere proximal positions; only the chromosome arms upon which they reside was altered. The resulting strain, KSW17, was similar in structure to strain KSW10, used in the previous experiment, differing only in the relative placements of HML and HMR (Figure 5A).

Two possible outcomes of this experiment were anticipated. If the donor locus recognition information were relocated along with the donor locus itself, then we would have expected that mating type switching would occur at wild-type efficiency in strain KSW17. On the other hand, if the donor locus recognition information were not included on the interchanged fragments but remained at the normal location, then we would have expected that the frequency of mating type interconversion would be quite low. In this case an a cell would most often select the donor locus on the left arm and an a cell would most often select the donor locus on the right arm, leading only infrequently to an observable mating type switch.

The frequencies of interconversion that we obtained for strain KSW17 by pedigree analysis are presented in Figure 5B. Both a to α and α to a switches were observed only infrequently. Thus, either mating type interconversion was rarely initiated in this strain or the primary result of each switching event was homologous allele replacement. Since the chromosome III end truncations and concomitant manipulations did not adversely affect the efficiency of mating type switching in strain KSW10, we favored the latter interpretation. This conclusion was strengthened by the results of the experiment described below, in
which we observed efficient allele replacement in an isogenic strain, KSW17a1.

In order to substantiate that strain KSW17 undergoes efficient but homologous mating type interconversion, we genetically marked the \( \alpha \) allele at \( \text{MAT} \) and showed that the marked allele was replaced with the homologous unmarked allele from \( \text{HML} \). This was accomplished by introducing a mutation in the \( \alpha_1 \) gene at the \( \text{MAT} \alpha \) locus of strain KSW17, to yield the isogenic \( \text{mata1} \) strain, KSW17a1 (Figure 5C). \( \text{mata1} \) cells are phenotypically distinguishable from \( \text{MAT} \alpha \) cells and from \( \text{MAT} \alpha \) cells, but are equivalent to \( \text{MAT} \alpha \) cells with respect to switching efficiency and donor preference (Rine et al. 1981). The results from pedigree analysis of \( \text{mata1} \) spores from strain KSW17a1 are presented in Figure 5D. The data document that mating type interconversion to \( \text{MAT} \alpha \) is efficient in strain KSW17a1, providing confirmation that strain KSW17 undergoes efficient but homologous switching. Thus, we conclude that \( \text{MAT} \alpha \) cells select whichever donor locus resides on the left arm of chromosome III and that \( \text{MAT} \alpha \) cells select whichever donor locus resides on the right arm of chromosome III.

**CEN3 does not specify which donor locus is chosen:** Since the cues used by the cell to select differentially one donor locus over the other do not derive from features near the loci themselves, we pursued the idea that a mechanism operating at the level of chromosome structure could be responsible. One obvious candidate for a directional signal on any chromosome is the centromere. The nucleotide sequence of \( \text{CEN3} \) has been determined and the asymmetry of the centromere DNA elements give it a defined polarity (Fitzgerald-Hayes, Clarke and Carbon 1982). If the orientation of \( \text{CEN3} \) is the characteristic sensed by the cell to distinguish the left and right chromosome arms and, therefore, \( \text{HML} \) and \( \text{HMR} \), then inversion of the centromeric sequence should reverse donor locus selection. Homologous allele replacement should result, as \( \text{a} \) cells preferentially select \( \text{HML} \alpha \) on the left arm as a donor locus, and \( \text{a} \) cells preferentially select \( \text{HML} \alpha \) on the right arm. On the other hand, if the centromere orientation does not provide any directional information, then mating type interconversion would be unaffected by its inversion.

We inverted the 627-bp \( \text{CEN3} \)-containing fragment on chromosome III and determined the effect of this alteration on donor locus selection by pedigree analysis (Figure 6, A and B). The resulting strain, KSW24, changed cell type at frequencies similar to those of its parent strain, KSW4. Therefore, the orientation of the centromere is not responsible for generating the left-right positional information to which donor locus selection responds.

**MAT need not reside on chromosome III for appropriate donor locus selection:** One possible landmark that could distinguish \( \text{HML} \) from \( \text{HMR} \), or the left arm of chromosome III from the right arm of the chromosome, is the \( \text{MAT} \) locus itself. Any one of a number of characteristics of the \( \text{MAT} \) locus could be
appropriated by the cell for the purpose of discriminating between the donor loci: the orientation of the MAT locus on the chromosome, the relative distances between MAT and the two donor loci, or the position of MAT on the right arm of the chromosome. We addressed all of these possibilities by removing MAT from chromosome III and placing it on a different chromosome. Although a defect in donor preference resulting from such a transplacement would not define the mechanism involved, it would pinpoint MAT as a critical component. However, if the transplacement of MAT did not affect donor preference, then we could conclude that MAT does not serve as a landmark for discriminating between HML and HMR.

We deleted MAT and its flanking sequences from chromosome III, and inserted MAT at the LYS2 locus on chromosome II to create strain KSW19 (Figure 7A). Pedigree analysis of strain KSW19 yielded the data shown in Figure 7B. These data appeared to show that a cells selected HMRa as a donor locus efficiently, but that donor locus selection in a cells was random. This interpretation, though, was confounded by a switching event that was not normally observed in the parent strain. This event was the cell’s inability to repair the HO endonuclease-induced double strand DNA break at MAT, most likely because the MAT locus on chromosome II did not efficiently locate a donor locus. Since cleavage of the MAT locus precluded expression of the resident mating type allele, cells bearing a cleaved MAT locus exhibited the default a mating phenotype. Accordingly, an unrepairable HO endonuclease cleavage was perceived as a conversion of a to a in MATa cells, or as a homologous replacement of the a allele in MATa cells. Therefore, the true rate of a to a conversion was actually much less than the perceived switching frequency. The evidence that the HO endonuclease dsDNA break was not repaired in strain KSW19 is presented below.

In order to ask if the cell can still distinguish HML from HMR when MAT resides on chromosome II, we must discount unfruitful switches, and consider only those events in which gene conversion from a donor locus takes place. From the data provided below, we determined that the HO endonuclease cleavage at MAT on chromosome II was repaired through gene conversion from one of the HM loci in approximately half of the switching events. We therefore concluded from the data in Figure 7B that strain KSW19 exhibited donor specificity during productive mating type interconversion at a level approximately equivalent to that of a wild-type strain. That is, after discounting the nonproductive switching events, MATa cells preferentially selected HMLa over HMRa as a donor locus (~40% vs. <15%) and MATa cells preferentially selected HMRa over HMLa as a donor locus (also ~40% vs. <15%) (Figure 7C). Since only about half of the initiated switches were completed, the interconversion frequencies were approximately 40% for strain KSW19, about half that observed for strain KSW4.

The evidence that the HO endonuclease dsDNA cut was frequently not repaired in strain KSW19 is as follows. If HO cleaves the DNA at MAT on chromosome II and mating type interconversion is not successful, then the unrepaired dsDNA break will eventually lead to cell death (Klar, Strathern and Abraham 1984; M. Resnick, personal communication). This lethality should be observable by following the lineages of mother cells, since these cells initiate interconversion events. As an initial test of this prediction, we separated mother cells from daughter cells after the first division of spores derived from strain KSW19, and examined them for colony forming ability. We found that 62% (13/21) of these mother cells never gave rise to visible colonies. This frequency of inviability was consistent with and best explained by a HO endonuclease-induced lesion at MAT on chromosome II.

A further experiment confirmed both the existence of the unrepaired HO endonuclease dsDNA cut at MAT and the resulting a phenotype of these ill-fated cells. We set up a genetic test for the presence or absence of a MATa locus in those cells observed to switch from a to a. The progeny of MATa mother cells that exhibited an a phenotype were rescued by cell-to-cell matings using the a tester strain, KSW25, as depicted in Figure 8A. If a mother cell had undergone a productive switch from MATa to MATa, then the diploid resulting from rescue at the four cell stage would have been MATa/MATa and, accordingly, non-mating. However, if the HO endonuclease-induced cleavage in the mother cell were not repaired then the resulting diploid would have been matΔMATa and, accordingly, a. By utilizing a MATΔstrain to rescue the cells in question, so that the HO endonuclease cut site was not cleavable (inconvertible), we ensured that each diploid would give rise to a colony that had not undergone subsequent HO-mediated in-
terconversion. The protocol for this experiment, as well as the results we obtained from rescuing 20 cells that appeared to switch from \( \alpha \) to \( \alpha \), is shown in Figure 8B. About half of the diploids recovered by this procedure gave rise to \( \alpha \) mating colonies, indicating that the original mother cell had failed to complete interconversion. The mother cells that subsequently yielded the remaining half of the diploids had successfully switched from \( \textit{MAT} \alpha \) to \( \textit{MAT} \alpha \). These results confirmed that the switching process, but not discrimination of \( \textit{HML} \) and \( \textit{HMR} \), was perturbed in strain KSW19. Furthermore, the relative frequencies with which \( \textit{MAT} \alpha \) cells either switched to \( \textit{MAT} \alpha \) (9/20) or failed to repair the \( \textit{HO} \) endonuclease-induced dsDNA break (11/20) supported our interpretation of the pedigree analysis data from strain KSW19 as presented in Figure 7C (~40% vs. ~45%, respectively). Once we considered only those cells that had successfully completed a switching event, it was clear that \( \textit{MAT} \alpha \) cells preferentially selected \( \textit{HML} \alpha \) as a donor locus and \( \textit{MAT} \alpha \) cells preferentially selected \( \textit{HMR} \alpha \) as a donor locus.

DISCUSSION

Donor locus recognition signals do not reside near \( \textit{HML}, \textit{HMR} \) or \( \textit{MAT} \): We have attempted to define the location and nature of the recognition information that enables a homothallic cell to select preferentially either \( \textit{HML} \alpha \) or \( \textit{HMR} \alpha \) as a donor locus during mating type interconversion. RINE et al. (1981) and KLAR, HICKS and STRATHERN (1982) previously showed that this recognition information does not reside in the allele sequences at the \( \textit{HM} \) loci: the cell does not merely select the donor locus harboring the allele opposite to that residing at \( \textit{MAT} \). Rather, \( \textit{MAT} \alpha \) cells choose \( \textit{HML} \) and \( \textit{MAT} \alpha \) cells choose \( \textit{HMR} \), independent of the allele present at either locus. We have extended these studies to demonstrate that those features perceived by the cell to distinguish between \( \textit{HML} \) and \( \textit{HMR} \) do not derive from the different structures of the \( \textit{HML} \) and \( \textit{HMR} \) loci, from the unique sequences flanking either donor locus nor from any sequence distal to the \( \textit{HM} \) loci on chromosome \( \textit{II} \).

The results presented in this report place certain limits on the nature of the donor locus recognition process. KLAR, HICKS and STRATHERN (1982) previously proposed the existence of a "synaptase," which would facilitate association between \( \textit{MAT} \) and the appropriate donor locus. Such a function is not readily compatible with the evidence that these sites, if they actually exist, must reside at significant distances from the donor loci. In addition, any model invoking a synaptase to provide a cell type-specific link between \( \textit{MAT} \) and the appropriate donor locus would also require a site near \( \textit{MAT} \) through which such a synaptase could act. However, our results suggest that such a site is not present at \( \textit{MAT} \). We found that donor preference was maintained even when we moved the \( \textit{MAT} \) locus, with none of its flanking sequences, to a new position on chromosome \( \textit{II} \). Thus, appropriate donor locus selection does not require any of the sequences flanking \( \textit{MAT} \). The only sequence differ-
ence between \textit{MAT} \( \alpha \) and \textit{MAT} \( \alpha \) that might have generated specificity in this experiment would have been the mating type allele itself. However, several studies have shown that cell type, but not the particular allele-specific sequence at \textit{MAT}, specifies donor selection (\textit{Klar}, \textit{Fogel} and \textit{Radin} 1979; \textit{Tanaka et al.} 1984; our unpublished results). Therefore, the role of \textit{MAT} is primarily a passive one: that of receiving a new mating type allele from the \textit{HM} locus selected through a mechanism independent of the sequences around either the donor or recipient loci.

\textbf{Potential positional signals on chromosome III:} If the donor locus recognition information does not reside at or near the donor loci, or distal to them on the chromosome, from where might the signals derive? We have not yet eliminated the possibility that a specific sequence lying even further centromere-proximal with respect to one or both loci serves to set \textit{HML} and \textit{HMR} apart. We are in the process of creating more extensive transpositions between the arms of chromosome III in an attempt to test this possibility. Meanwhile, we have tested whether other well-defined landmarks of chromosome III might provide positional information. We found that inversion of \textit{CEN3} did not affect donor preference. Similarly, donor preference was not abolished by removing \textit{MAT} from chromosome III. Thus, no positional cues are provided by the orientation of \textit{CEN3} or by the orientation or position of the \textit{MAT} locus relative to the two donor loci. Therefore, none of the obvious loci on the chromosome serve as landmarks for directing the cell to the appropriate donor locus.

In the absence of cues from specific loci, other features of chromosome III have to be invoked to explain how the cell distinguishes \textit{HML} from \textit{HMR}. We can consider, for example, that the cell actually recognizes a difference between the right and left arms of chromosome III, and then uses that information to direct selection of the appropriate \textit{HM} locus. This recognition process could rely on multiple sites dispersed throughout the chromosome. Alternatively, the recognition process could rely on differences in the chromatin structure of the two arms, either generally or across the \textit{HM} loci themselves. The proposed difference in chromatin structure could be regulated by cell type, or, more likely, could simply be recognized by a cell type specific component of the interconversion apparatus.

Finally, the spatial organization of the chromosome within the yeast nucleus might provide information regarding donor selection. Spatial cues could be provided either by the absolute position of the donor loci in the three dimensional organization of the nucleus or by the relative positions of donor and recipient loci. In the former case, one would envision that the ends of chromosome III are restricted to specific fixed locales within the nucleus, so that the cell could be directed to the appropriate arm during interconversion via its nuclear position. In the latter case, yeast chromosome III would be folded such that \textit{MAT} was in proximity to the end of the right arm (\textit{HMR}a) in \( \alpha \) cells but nearer to the left arm (\textit{HML}a) in \( \alpha \) cells. Precedent for a conserved and precise spatial organization of chromosomes within a cell nucleus is provided by Drosophila salivary gland nuclei (\textit{Mathog et al.} 1984). Salivary gland chromosomes have not only a specific orientation within the nucleus but also a characteristic folding pattern.

\textbf{Donor preference versus switching efficiency:} It is important in these and other studies to distinguish between alterations that affect donor preference and those that affect the efficiency of interconversion. For instance, we observed that translocation of \textit{MAT} affected the efficiency of mating type interconversion without affecting donor preference. However, since the system of donor selection is quite plastic--in the

\textbf{FIGURE 8.}—Switching events in a strain with \textit{MAT} on a different chromosome often lead to unrepaird lesions at \textit{MAT}. (A) The diagram illustrates the pedigree of an \( \alpha \) spore of strain KSW19 that appeared to switch to \( \alpha \). The switched cell was isolated and mated to an \( \alpha \) cell of strain KSW25 to form a viable diploid. (B) The nature of the \( \alpha \) to \( \alpha \) switch was revealed genetically as shown. The phenotypically \( \alpha \) cell that was rescued either had an \( \alpha \) allele at the \textit{MAT} locus on chromosome II (\textit{lys2::MAT} \( \alpha \)) or it had an unrepaird lesion in the DNA (\textit{lys2::MAT} \( \alpha \)). Consequently, the genotypes of the diploids formed by mating to the KSW25 \( \alpha \) cell differed as illustrated. This difference was manifest phenotypically in the diploid mating phenotype. \( \alpha/\alpha \) or \( \alpha \). The fraction of diploids formed displaying each phenotype is listed below. Homozygosis of the \textit{lys2::MAT} \( \alpha \) allele could have occurred if the lesion at the \textit{lys2::MAT} locus were repaired (following diploid formation) using chromosome II of strains KSW25 as a template. The mating phenotype of the diploid would still have been \( \alpha \).
absence of the preferred donor locus, the non-preferred locus can be efficiently used as donor (KLAR, HICKS and STRATHERN 1982)—manipulations of one donor locus that rendered its participation in the switching process less efficient 

per se would resemble a defect in donor preference. For instance, HABER, ROWE and ROGERS (1981) analyzed mating type interconversion in mutant strains that contained both a translocated copy of the HML locus and the normal HML still resident on chromosome III. They found that the translocated HML locus was utilized as a donor for switching less efficiently than was the untranslocated locus. We have isolated several mutants having translocations of the only copy of HML to different chromosomes (our unpublished results), and find that its utilization as a donor locus is severely inhibited. Thus, while these results could be interpreted to indicate that the donor selection information was not present on the translocated segment of DNA, a more plausible interpretation is that the efficiency with which the translocated locus can participate in switching is affected.

The diminished efficiency in mating type interconversion resulting from translocating MAT to a different chromosome—and perhaps of moving HML to a different chromosome, as proposed above—suggests that physical linkage of the three mating type loci is critical to the process of mating type switching. However, physical studies on large DNA segments in solution suggest that sites as far apart as are MAT and the two donor loci, even though physically linked, interact with kinetics of independent, unlinked molecules. Thus, the effect of physical linkage on the efficiency of mating type interconversion must result from some higher order organization of the chromosome. This highlights the possibility that either chromosome packaging or the spatial organization of the chromosome underlies efficient interconversion. We imagine that this structural organization is required to facilitate the long distance interaction between MAT and either donor locus during mating type switching. Moreover, our results suggest that efficient interconversion and accurate donor selection, while distinct processes, may rely on the same organizational features and thus have similar mechanistic underpinnings. A similar view emerges from studies of mating type interconversion in Schizosaccharomyces pombe, in which mutations that render DNA across the mating type complex more recombogenic—implying a more open chromatin configuration—also diminish donor preference in mating type selection (KLAR and BONADUCE 1991; A. KLAR, personal communication).

As noted in the Introduction, several developmental processes involve programmed interaction of regions of the genome separated by considerable distances. While cues for the specificity of these interac-

tions might a priori be expected to reside at or near the interacting domains, the results in this report raise the possibility that such specificity may derive from distant sequences or from higher order chromosome structure. Further approaches to define the mechanistic basis for interaction of the mating type loci in yeast should provide a framework for approaching analysis of these more complex systems.

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