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# Directional bias during mating type switching in *Saccharomyces* is independent of chromosomal architecture

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**Haploid *Saccharomyces* cells have the remarkable potential to change mating type as often as every generation, a process accomplished by an intrachromosomal gene conversion between an expressor locus *MAT* and one of two repositories of mating type information, *HML* or *HMR*. The particular locus selected as donor is dictated by the mating type of the cell, a bias that ensures productive mating type interconversion. Here we use green fluorescent protein tagging of the expressor and donor loci on chromosome III to show that this preference for donor locus does not result from a predetermined organization of chromosome III: *HML* and *MAT* as well as *HMR* and *MAT* remain separated in cells of both mating types. In fact, cells in which the inappropriate donor locus is artificially tethered to *MAT* still predominantly select the correct donor. We find, though, that initiation of switching leads to a rapid association of the correct donor locus with *MAT*. Thus, in mating type switching in *Saccharomyces*, donor preference is imposed at commitment to recombination rather than at physical contact of interacting DNA strands.**

*Keywords*: chromosomal dynamics/donor preference/mating type switching/recombination/*Saccharomyces*

## Introduction

Specific interactions between distant chromosomal loci underlie a variety of developmental programs in eukaryotic cells, including globin gene expression and immunoglobulin class switching (Jung *et al*., 1993; Zhang *et al*., 1993). Mating type switching in the yeast *Saccharomyces* provides a particularly tractable system for analyzing such distant interactions during development (Bi and Broach, 1998; Haber, 1998). The mating type of a haploid cell is dictated by the particular allele, **a** or α, present at the mating type locus, *MAT*, located near the center of chromosome III (Herskowitz, 1989; Herskowitz *et al*., 1992). Formation of a double-strand break in the DNA of the *MAT* locus, catalyzed by an endonuclease encoded by the *HO* locus, initiates mating type switching (Strathern *et al*., 1982; Kostriken and Heffron, 1984). Switching then occurs by a gene conversion event that replaces the mating information at the *MAT* locus with that present at either of two repository mating loci, *HML* and *HMR*, located at the opposite ends of chromosome III (180 and 100 kb, respectively, away from *MAT*) (Haber, 1998). This results in replacement of one mating type allele at *MAT* with a copy of the opposite mating type allele taken from either *HML* or *HMR*.

Mating type switching follows a precise developmental program dictated by the intricate transcriptional regulation of the *HO* gene and by a highly regulated interaction between distant regions of chromosome III (Herskowitz *et al*., 1992; Haber, 1998). Namely, mothers, but not daughters, transcribe the *HO* gene and do so only during the $G_1$ phase of the cell cycle. Accordingly, only mothers can switch cell type and the switch occurs prior to DNA replication (Nasmyth, 1993; Long *et al*., 1997; Nasmyth and Jansen, 1997). In addition, cell type dictates which donor locus is selected for participation in the gene conversion event. **a** cells predominantly use *HML* as donor, whereas α cells use *HMR* as donor. Since *HML* normally contains α mating information and *HMR* normally contains **a** mating information, this pattern ensures that most of the switching events result in a change of mating type, rather than a futile replacement of the *MAT* allele with the same sequence (Klar *et al*., 1982).

The fact that cells can promote selective interaction between *MAT* and one of the repository *HM* loci implies that the two loci possess distinguishable features that are recognized in a cell type-specific manner. The discrimination does not derive from the different alleles resident at the donor loci, from the unique sequences flanking either locus, nor from any of the DNA sequences distal to either locus on chromosome III (Weiler and Broach, 1992). Rather, the left arm of chromosome III exhibits a cell type-dependent difference in recombinogenicity such that a large (>40 kb) region of the left arm of chromosome III containing *HML* can undergo intragenic recombination with *MAT* at a frequency at least 20 times higher in **a** cells than in α cells (Wu and Haber, 1995). This **a** cell-specific enhancement of recombination of the left arm of chromosome III depends on a small (<2 kb) segment ~30 kb from the telomere, 16 kb from *HML* (Wu and Haber, 1996), consisting of a pair of binding sites for the transcriptional activator Mcm1 and the mating type-specific transcriptional repressor α2. Deletion of this segment (referred to as RE for recombinational enhancer) causes **a** cells to choose *HMR* (the wrong donor) instead of *HML*, without altering the donor preference in α cells (*HMR* is preferred as usual) (Wu and Haber, 1996; Szeto *et al*., 1997).

These observations provide a working model for donor preference (Figure 1A). In **a** cells, binding of Mcm1 to the RE activates the intrachromosomal recombination potential of the left arm of chromosome III. Accordingly, *HML* becomes the preferred donor. In α cells, α2 represses the activity of Mcm1 bound to the RE and thereby prevents activation of the recombination potential of the left arm.

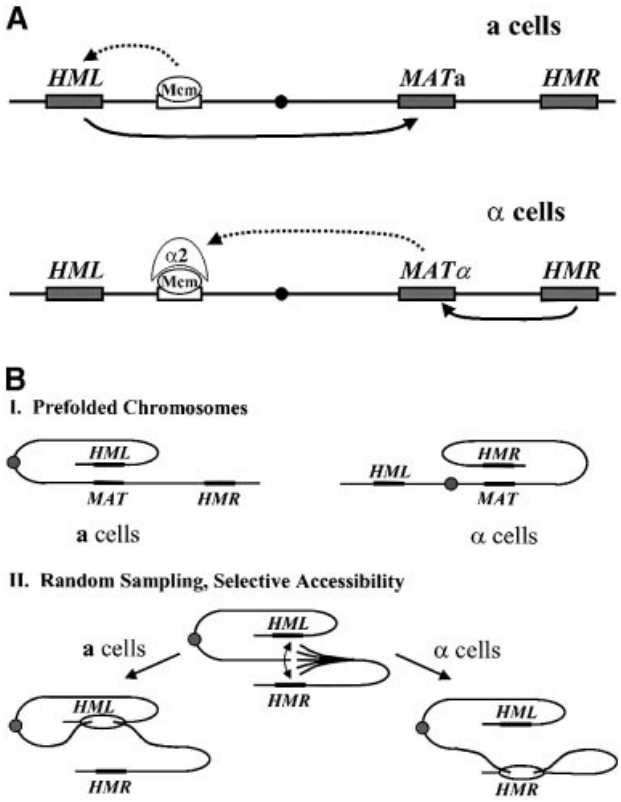



**Fig. 1.** (**A**) Mating type switching in yeast. Schematic of chromosome III, showing the expressor locus, *MAT*, the transcriptionally silent donor loci *HML* and *HMR*, and the recombination enhancer RE. In *MAT***a** cells, Mcm1 binds to the RE and activates *HML* as donor. In *MAT*α cells, α2 represses the activity of Mcm1 bound to the RE, resulting in use of *HMR* through an as yet undefined mechanism. (**B**) Models for the role of chromosome III architecture in mating type switching. Two models could account for donor selection in yeast. Either chromosome III is prefolded in a cell type-specific manner to yield juxtaposition of the preferred donor next to *MAT* or, following double-strand cleavage, the broken end could physically sample both *HML* and *HMR* equally but commit to the preferred donor through, for example, enhanced susceptibility of the preferred donor to strand invasion. The first model would predict that the preferred donor would be located closer to *MAT* than the non-preferred donor, while the second would predict that no cell type-specific organization of the chromosome would be evident. In this latter case, both donor loci would be either equally unassociated with *MAT* or equally associated.

As a result, *HMR* becomes the preferred donor, due to an as yet undefined intrinsic bias for *HMR* as donor (Wu *et al*., 1996). While this model accounts for all current observations, it does not address the fundamental question of the basis of the enhanced recombinogenicity of the left arm of chromosome III in **a** cells. In this report, we probed the mechanistic basis for donor preference by determining the architecture of chromosome III in switching cells. In particular, we determined whether the correct donor locus is located closer to *MAT* prior to initiation of mating type switching. We find that not only do the donor loci fail to show such pre-organization. but also artificially tethering the wrong donor locus to *MAT* fails to redirect donor selection. However, following initiation of switching, *MAT* becomes associated with the proper donor locus in the cell. These results suggest that commitment to recombination, rather than physical proximity, dictates donor preference in yeast.

## Results

Two distinct models could account for the ability of yeast cells to select one donor locus over the other during mating type switching (Figure 1B), each positing a different rate-limiting step in the donor selection process. The first model assumes that finding the appropriate donor would be the rate-limiting step and that the RE leads to a cell type-specific pre-organization of chromosome III to position the correct donor locus closer to *MAT* prior to initiation of switching. That is, *HML* would be nearer *MAT* in **a** cells and *HMR* would be nearer *MAT* in α cells. In this model, donor preference results from the fact that *MAT* would simply be more likely to encounter the preferred donor following initiation of the switching process. Such chromosomal organization probably dictates donor preference during mating type switching in *Schizosaccharomyces pombe* and some recent data suggest that chromosomal architecture could contribute to donor preference in *Saccharomyces* (Klar, 1992; Kostriken and Wedeen, 2001). In addition, the *HML* locus appears to be confined to the nuclear periphery, providing a landmark for potential chromosomal organization (Laroche *et al*., 2000). The second model postulates that no pre-organization exists and that selection of the donor locus occurs at a stage in the recombination process later than physical encounter. The double-strand break would sample both donor loci equally, but commitment to recombination, perhaps at the strand invasion stage or formation of a replication complex, would be regulated by mating type through the RE so that only the correct locus served to complete the recombination event.

### *The relative positions of donor and expressor loci are cell type independent*

In order to distinguish between the two models presented in Figure 1, we created strains in which we could examine the locations of the *MAT* and the donor loci in cells as a function of cell type. To do so, we used the method developed by Belmont and colleagues (Robinett *et al*., 1996; Belmont and Straight, 1998), in which tandem repeats of a repressor binding site are inserted at the chromosomal locus of interest in cells expressing a fusion of green fluorescent protein (GFP) to the binding domain of the repressor that recognizes the binding site. In particular, we inserted an array of lac repressor sites at *MAT* and an array of tet repressor sites at either *HML* or *HMR* in strains expressing both GFP–LacI and GFP–tetR fusions (Robinett *et al*., 1996; Michaelis *et al*., 1997). The different arrays at the two loci preclude adventitious pairing of the tagged loci. In addition, we used a $2n - 1$ chromosome III monosomic strain to increase the size of the cell while maintaining a single chromosome III within the cell. To assure ourselves that the arrays themselves did not interfere with donor preference, we performed donor preference analysis as previously described on strains containing arrays at *MAT* and *HML* or *MAT* and *HMR*. As evident from the data presented in Figure 4, strains containing the arrays at *MAT* and *HMR* exhibited a donor preference pattern essentially identical to that of a wild-type strain. The strains containing arrays at *MAT* and *HML* showed a slightly reduced selection of *HML* in an **a** background, perhaps reflecting the increased distance

between *HML* and the RE as a consequence of the insertion of the *HML* array, but normal selection of *HMR* in an α background. Thus, the tagged strains maintain appropriate donor preference.

We would predict quite distinct results for the location of the GFP spots in our test strains depending on the particular model of donor preference invoked. In a pre-organization model, we would expect to see predominantly one spot in **a** cells tagged at *HML* and *MAT*, and two spots in α cells, while the reciprocal case would obtain in cells tagged at *HMR* and *MAT*. In model 2, we would expect that we would see predominantly two spots in the cell, regardless of cell type or tag locations. We can envisage a variant of either model in which all three loci are tightly associated in the cell under all circumstances, at least to the level of resolution of the light microscope. In this case, we would expect to see a single spot in all strains.

We collected data by fluorescence microscopy for ~200 cells of each tag pair in each mating type background. We examined only unbudded cells, i.e. those in the $G_1$ stage of the cell cycle, and for each cell we collected optical slices at 0.2 μm intervals through the entire vertical dimension of the cell. The image from each slice was enhanced by repeated cycles of computational deconvolution until no significant improvement of the image resulted in a successive cycle (usually 15 cycles). We then reconstructed the three-dimensional image of the cell, determined whether the cell presented one or two spots and, in the latter case, measured the distance between the two spots. The resolution afforded by this deconvolution microscopy allowed us to distinguish spots located at least 0.2 μm apart.

The results of our analysis are shown in Figure 2 and summarized in Table I. Figure 2B shows a cumulative plot of distance between the two spots in each strain, in which the number on the *y*-axis represents the total fraction of cells with spots less than or equal to *x* μm apart. Figure 2C provides a histogram of the fraction of cells with spots at a given distance apart. Table I gives the percentage of cells exhibiting two spots and the average distance between the spots. Consistent with the shorter linear chromosomal distance between *HMR* and *MAT* relative to *HML* and *MAT*, the average three-dimensional distance between *HMR* and *MAT* in cells is less than that of *HML* and *MAT*. However, the three-dimensional distance is not proportional to the linear chromosomal distance. With regard to donor preference, we found that the percentage of cells with two spots and the average distance between those spots for cells tagged at *HML* and *MAT* are the same for both cell types. Similarly, for cells tagged at *HMR* and *MAT*, the percentage of cells with two spots is essentially the same for both cell types, although we have noted a small but consistent reduction in the average distance between *HMR* and *MAT* in α versus **a** cells. This latter observation is discussed below. In general, though, our data demonstate that the preferred donor locus is not preferentially positioned adjacent to *MAT* in the cells prior to switching. Accordingly, cell type does not dictate a specific architecture of chromosome III as a means of promoting donor preference.

We noted that the proportion of cells marked at *MAT* and *HMR* showing single spots was substantially larger than that predicted assuming that the two loci behaved as

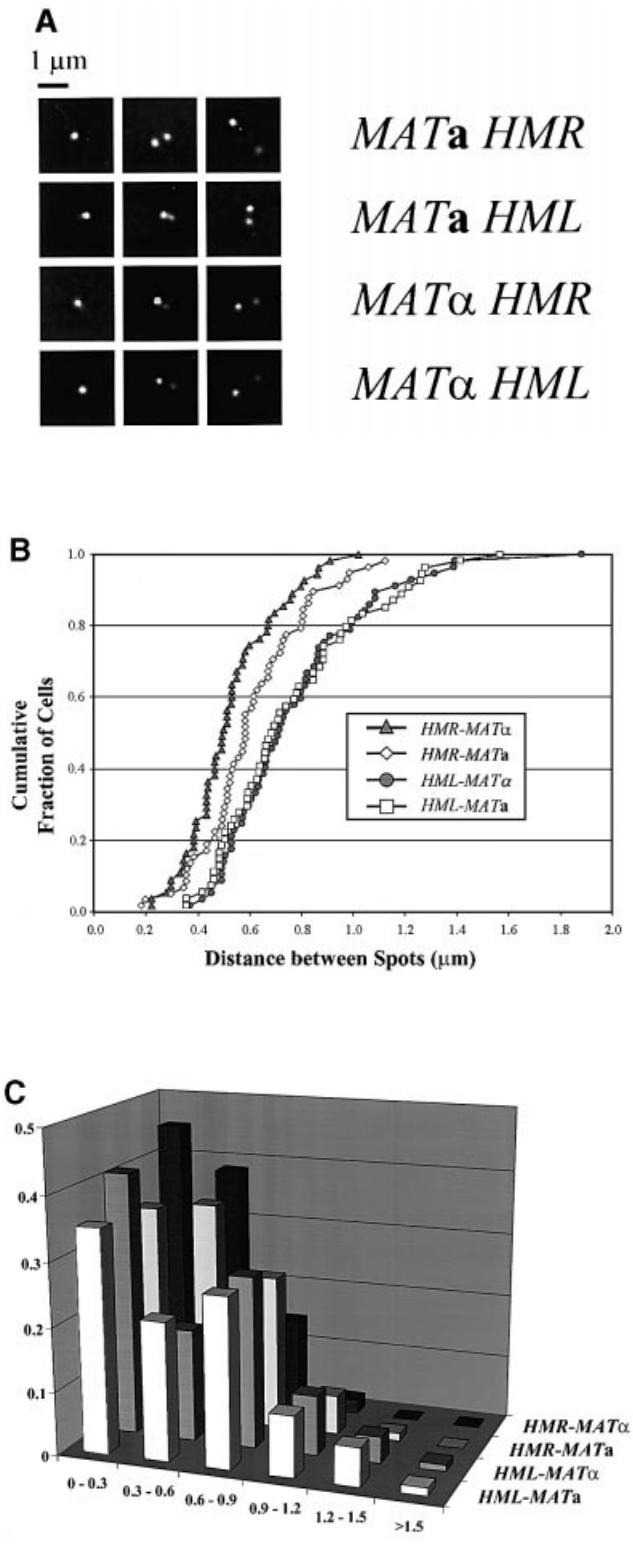



**Fig. 2.** Spatial distribution of donor and expressor loci. (**A**) Deconvoluted fluorescent images of representative cells containing LacO arrays at *MAT* and TetO arrays at *HML* or *HMR* and expressing TetR–GFP and LacI–GFP fusion proteins. Each row is a single strain with the tagged loci indicated to the right of the images. (**B**) Distances between spots in cells tagged at two sites on chromosome III. Plotted is the cumulative fraction of cells with two fluorescent spots separated by a given distance. Data are shown only for those cells exhibiting two spots (cf. Table I). (**C**) Binned data for all cells, indicating the fraction of cells tagged at the indicated loci with spots located within each 0.3 μm distance interval.

**Table I.** Organization of mating loci on chromosome III

| | *RE* | Tagged loci | | | | |
|---|---|---|---|---|---|---|
| | | *MAT***a**-*HML* | *MAT*α-*HML* | *MAT***a**-*HMR* | *MAT*α-*HMR* | ChrIX[a] |
| % with two spots | + | 65 (54/83) | 63 (122/191) | 52 (95/182) | 58 (162/278) | 56 (22/39) |
| Average distance | + | 0.77 ± 0.29 | 0.80 ± 0.30 | 0.65 ± 0.20 | 0.60 ± 0.20 | 0.67 ± 0.33 |
| % with two spots | Δ | 61 (122/191) | 63 (121/192) | 51 (96/186) | 57 (106/187) | NA |
| Average distance | Δ | 0.82 ± 0.31 | 0.85 ± 0.32 | 0.65 ± 0.17 | 0.59 ± 0.17 | NA |

Cells from strains with arrays inserted at the indicated tagged loci and either containing an intact recombination enhancer (*RE*$^+$) or deleted for the recombination enhancer (*RE*Δ) were examined by deconvolution microscopy and the percentage of cells with two spots (absolute numbers are indicated in parentheses) and the average distance (in μm ± SD) between the two spots are presented. Strains used for this study are listed in Table II.
[a]Arrays were inserted at positions 106 and 225 kb from the left end. NA, not applicable.

tethered but freely diffusible sites on a chromosome of average compaction. That is, we predicted ~5–10% of cells with single spots in the *MAT*- and *HMR*-tagged strains, and we observed 40–50% of cells with single spots (see Materials and methods for the calculation and assumptions). Similarly, we would anticipate ~5% of the single spots in the *MAT*- and *HML*-tagged strains, and we observed ~40% of cells with single spots. To test whether this anomolous behavior was a function of either the mating loci or chromosome III, we examined cells in which we inserted the tet and lac arrays 120 kb apart on chromosome IX. The results of the analysis of this strain are also included in Table I. As is evident, the pattern of distances between these two loci was essentially identical to that observed for cells marked at *MAT* and *HMR*. Thus, the unexpected pattern obtained with *MAT* and *HMR* is not specific to the mating loci or chromosome III and, in sum, our results indicate that the architecture of chromosome III and the position of the donor loci relative to *MAT* are not significantly influenced by the mating type of the cell.

Despite the absence of evident differences in distances between the donor and expressor mating type loci as a function of mating type, we examined the effect of deleting the RE on the relative positions of donor and expressor loci. These data were collected as above with isogenic strains from which the 2 kb region spanning the RE had been deleted, and are presented in Figure 3. As is evident, the percentage of cells with two spots and the average distances between those spots are unaffected by deletion of the RE. Thus, the RE does not affect the large-scale architecture of chromosome III.

### *Tethering the non-preferred donor locus to MAT does not invert donor preference*

Although our results indicate that chromosome III is not arranged in the cell so as to juxtapose the preferred donor with *MAT*, we asked whether artificially tethering the inappropriate donor next to *MAT* would reverse the normal donor preference. To test this, we positioned lac arrays at both *HML* and *MAT* or *HMR* and *MAT* and expressed a GFP–LacI fusion protein that retained the LacI tetramerization domains. In this manner, the GFP–LacI proteins bound to one array could bind to the GFP–LacI proteins bound at the second array and bring the two arrays into close physical proximity. Straight *et al*. (1996) showed previously that this technique could maintain juxtaposition of sister yeast chromosomes at mitosis in the absence of the normal chromosomal adhesion complex. To confirm that the GFP–LacI complex could in fact maintain association of the marked donor locus with *MAT*, we examined our test cells by deconvolution microscopy as above. We observed 100% single spots within the cells under conditions of the switching experiment (data not shown), indicating that the LacI fusion protein efficiently tethered the marked donor locus to *MAT*. We then tested these cells for donor selection following mating type switching initiated by a wild-type *HO* gene, using the donor preference assay described previously. The data from these experiments are shown in Figure 4. As can be seen, the efficiency of selecting the proper donor increased slightly in strains in which the proper donor was tethered to *MAT* (>95% selection of *HMR* in α cells with *HMR* tethered to *MAT* versus 92% selection in tagged but untethered α cells, and 75% selection of *HML* in **a** cells with *HML* tethered to *MAT* versus 65% in tagged but untethered **a** cells). In addition, tethering the incorrect donor locus to *MAT* somewhat reduced the selection of the appropriate donor (64% selection of *HMR* in α cells with *HML* tethered to *MAT* versus 92% selection in tagged but untethered α cells, and 68% selection of *HML* in **a** cells with *HMR* tethered to *MAT* versus 94% in tagged but untethered **a** cells). However, in no case did tethering the inappropriate donor to *MAT* reverse or randomize the selection process. Thus, the mechanism that the cell uses to select the appropriate donor operates even when the inappropriate donor is located much closer to *MAT* than the appropriate donor, indicating that the rate-limiting step in donor selection is probably not physical association.

### *Initiation of switching induces convergence of the correct donor locus with MAT*

Since the donor and *MAT* loci must physically interact during the process of switching, we asked whether we could observe the confluence of the two loci in our marked strains following initiation of the switching. Accordingly, we introduced the *HO* gene under the control of the galactose-inducible *GAL1* promoter into our tagged strains. We grew the cells to exponential phase in raffinose (non-inducing) medium and then added galactose to induce expression of *HO* and initiate switching. By examining the *MAT* locus by Southern analysis in this strain and under these conditions, we confirmed that *HO*-mediated cleavage of *MAT* did not occur prior to addition of galactose, but became evident 20 min after induction, reaching a maximum value at 30–40 min (Figure 5). This is consistent with kinetics previously observed for *MAT*

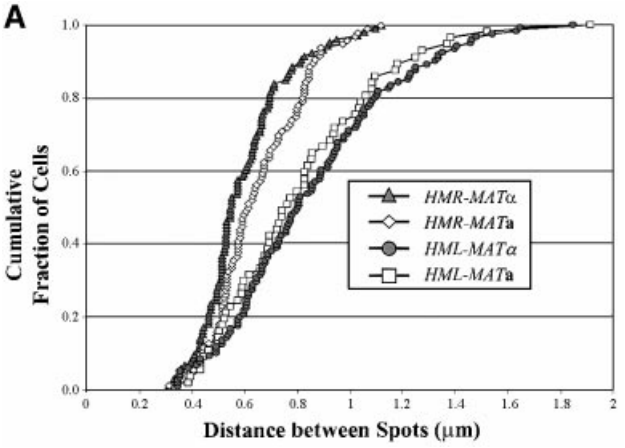
cleavage following induction of a *GAL-HO* construct (Connolly *et al*., 1988). We further examined the kinetics and directionality of switching in this strain by withdrawing samples at 30 min, plating on glucose plates containing 5-fluoroanthranilic acid (to repress expression of *HO* and then select cells lacking the *HO* plasmid during outgrowth) and testing the mating type of the resulting colonies. We found that 65% of α cells and 85% of **a** cells had committed to switching to the opposite mating type by 30 min of induction (data not shown). Thus, these strains exhibit normal donor preference.

To examine localization of the two loci following initiation of switching, we grew cells as above, removed samples at various times after galactose addition, fixed the cells and examined unbudded cells microscopically for the presence of one or two spots. The results of this analysis are presented in Figure 5, in which we show the percentage of cells with two spots as a function of time following addition of galactose. As is evident from these data, in an **a** strain with tags at *HML* and *MAT*, the proportion of cells in the population with two spots decreased markedly following induction of *HO*, reaching a minimum at ~40 min. In contrast, an α strain with the same tags did not show a decrease in the proportion of cells with two spots. Reciprocal results were obtained in strains tagged at *HMR* and *MAT*. In this case, the proportion of cells with single spots increased in α strains, but not in **a** strains, following initiation of switching (data not shown). These results indicate that the *MAT* locus becomes physically associated with the preferred donor locus, but not the non-preferred donor locus, following initiation of switching.

## Discussion

We have explored the role of chromosome III architecture in donor preference during mating type switching and have demonstrated that the preferred donor remains as far from *MAT* prior to initiation of switching as does the non-preferred donor. Furthermore, the cell preferentially selects the preferred donor upon switching even when the non-preferred donor locus is tethered to *MAT*. Once switching is initiated, though, *MAT* becomes associated with the preferred donor locus. These results indicate that the selection process leads to association of *MAT* with the preferred donor, rather than the other way around. Thus, we conclude that factors other than spatial organization underlie donor selection in yeast.

Using a similar approach, Kostriken and Wedeen (2001) concluded that chromosomal architecture did affect donor preference. In their experiments, Kostriken and Wedeen



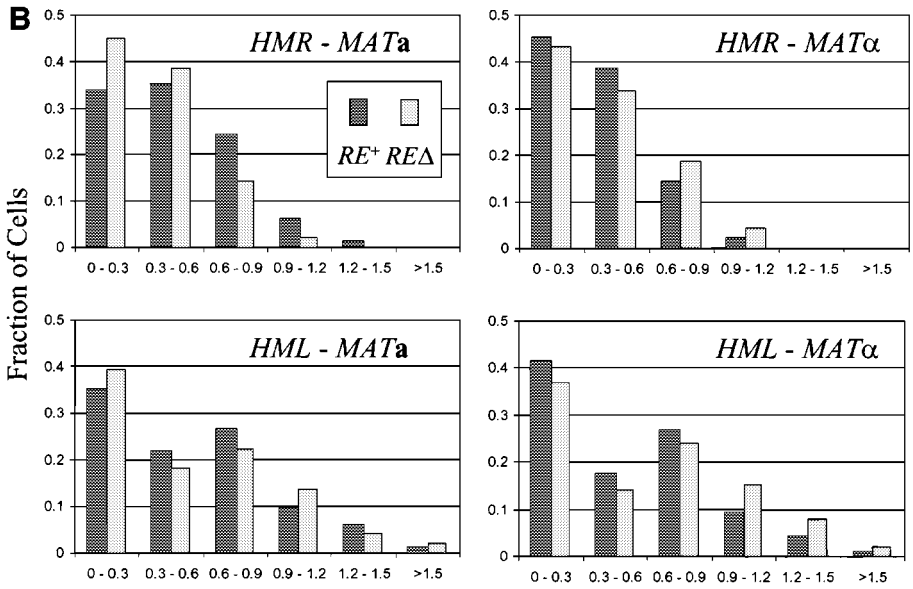




**Fig. 3.** Spatial distribution of donor and expressor loci in strains deleted for the RE. (**A**) Distances between spots in cells tagged at two sites on chromosome III from which the RE has been deleted. Plotted is the cumulative fraction of cells with two fluorescent spots separated by a given distance. Data are shown only for those cells exhibiting two spots. (**B**) Comparison between $RE^+$ and REΔ strains for each combination of cell type and donor locus tag. Data represent separation distances for all cells, indicating the fraction of cells tagged at the indicated loci with spots located within each 0.3 μm distance interval.

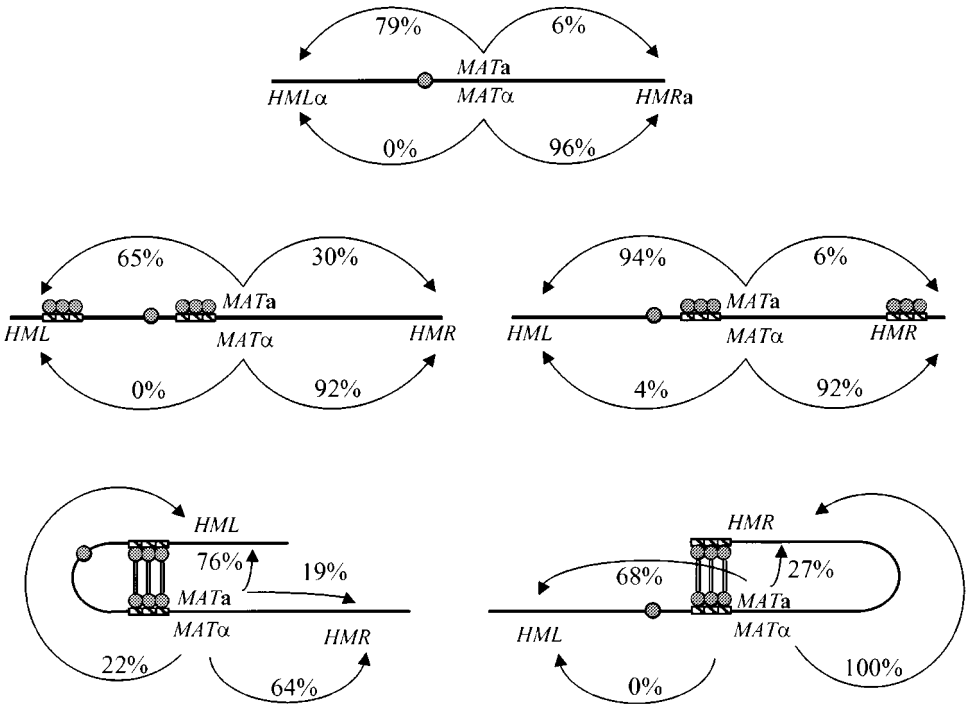



**Fig. 4.** Tethering donor loci to *MAT* does not alter donor preference. Donor preference assays were performed as described in Materials and methods on a wild-type strain (upper figure), strains containing LacO arrays at *MAT* and *HML* or *HMR* and expressing a LacI–GFP construct without a tetramerization domain (middle figures) and the same tagged strains but expressing a LacI–GFP construct with a tetramerization domain (lower figures). The numbers adjacent to the arrows in each diagram indicate the frequency that *MAT***a** cells (above the line representing chromosome III in each figure) or *MAT*α cells (below the line in each figure) select *HML* (arrow to left) or *HMR* (arrow to right). The residual percentage of events represents those colonies that yielded ambiguous donor selection. Approximately 50 colonies were tested for each mating type of each strain.

constructed *HO* strains in which the mating type alleles at *HML* and *HMR* were reversed (**a** information at *HML* and α information at *HMR*). They also rendered the haploid strains non-mating so that cells could continue to switch every generation as the culture grew and thus reach an equilibrium in the culture of the mating type alleles (**a** or α) resident at *MAT*. This equilibrium value provided a measure of the relative selection biases of the cell for *HML* and *HMR*. Wild-type strains showed a steady-state allele occupancy at *MAT* of 20% **a** and 80% α, reflecting the fact that cells are more efficient at correctly selecting *HMR* than *HML* (see Figure 4). Tethering *HML* to *MAT* by a method similar to that described in this report shifted that equilibrium to 45% **a** and 55% α, from which the authors concluded that tethering the donor locus to *MAT* redirects donor preference. We applied a mathematical model of this system to determine the equilibrium allele occupancy at *MAT* as a function of the absolute selection rates of *HML* and *HMR* in **a** and α cells. Using the values presented above in Figure 4 for the rates that we measured experimentally for *HML* versus *HMR* selection in **a** cells and α cells, we obtained a predicted allele occupancy of the *HML*-tagged but untethered strain of 10% **a** and 90% α, and a predicted occupancy in the strain in which *HML* is tethered to *MAT* of 56% **a** and 44% α. This remarkable similarity to the observed values reported by Kostriken and Wedeen suggests that the effects observed by them in their strains are quite similar to those we find in ours. Furthermore, these calculations indicate that only a slight reduction in the efficiency of selection, rather than a reversal in preference, is sufficient to yield the observed shift in the equilibrium values in the experimental design used by Kostriken and Wedeen. Thus, we feel that both sets of observations support the hypothesis that donor preference is not dictated by chromosomal architecture.

While we conclude that chromosomal architecture does not drive donor preference, we acknowledge that location does influence the efficiency of donor locus usage. As noted in our results, tethering a donor locus near *MAT* does increase the likelihood that it will be selected, whether or not it is the preferred donor. In addition, in the absence of a functional RE (in α cells or in **a** cells carrying an RE deletion), *HMR* is the preferred donor through an ill-defined default pathway that is sensitive to position effects. *HMR* is situated closer to *MAT* than is *HML*, not only in the linear distance along the chromosome, but also, as we have found in this study, in the three-dimensional distance within the nucleus. Inserting sequences between *HMR* and *MAT*, thereby increasing its distance from *MAT*, or moving *HMR* from the right arm to the left arm of chromosome III, diminishes its selection under these default conditions, while moving *HMR* closer to *MAT* on the left arm increases its selection (Wu *et al*., 1996; Szeto *et al*., 1997). Thus, proximity plays a role in the default pathway. We have consistently found that *HMR* is somewhat closer to *MAT* in α strains relative to **a** strains, thus placing *HMR* nearer *MAT* when it is the preferred donor. Whether this contributes to the RE-independent α effects on donor preference noted previously remains to be tested (Wu *et al*., 1996). Nonetheless, we previously reported and have recently confirmed that moving *MAT* to a different chromosome does not diminish the cell's ability

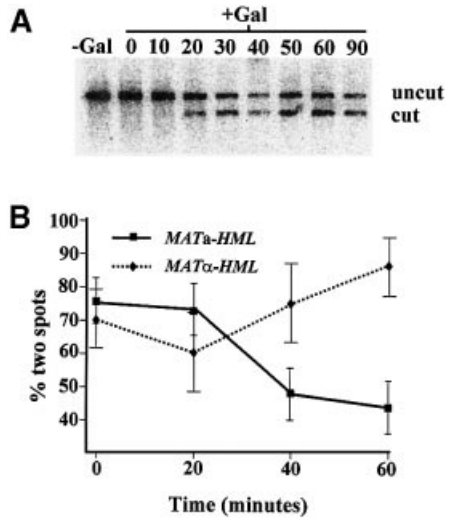



**Fig. 5.** Convergence of *MAT* with the correct donor locus following initiation of switching. (**A**) Strain PJS1392 was transformed with plasmid pPLH222 (*CEN GAL-HO*) and a transformant was grown at 30°C in SC-Leu media to ~$10^7$ cells/ml. Cells were harvested, transferred to SC-Leu media lacking glucose and containing 2% raffinose, and grown for 3 h at 30°C. Galactose was added to 2% and culture samples were removed either prior to galactose addition or at the indicated time following galactose addition. DNA was prepared from the culture samples, digested with *Xba*I, fractionated on a 1% agarose gel, transferred to a Zeta-Probe membrane (Bio-Rad) and probed with labeled DNA specific for *MAT* (the 256 bp *Hin*dIII–*Alu*I fragment immediately centromere distal to *MAT*). The upper band (4178 bp) corresponds to the uncleaved *MAT* locus and the lower band (3033 bp) to HO cut locus. (**B**) Strains PJS1392 (*MAT***a**::*TRP1-LacO*$_{256}$ *HML*::*URA3-TetO*$_{112}$ *HMR*) and PJS1401 (*MAT*α::*TRP1-LacO*$_{256}$ *HML*::*URA3-TetO*$_{112}$ *HMR*) were transformed with plasmid pPLH222 and grown as above. After growth in 2% raffinose for 3 h, cells were transferred to SC+2% raffinose lacking uracil and histidine and supplemented with 7.5 mM 3-aminotriazole, grown for 30 min, transferred to SC+2% raffinose medium for 15 min, fixed for 5 min in 2% paraformaldehyde (final volume) and subjected to microscopy as above. Samples were removed from the culture and made 2% galactose at the indicated time prior to fixation. All the galactose-containing samples were processed in parallel to the sample lacking galactose. Indicated are the percentages of cells with two spots in each sample as a function of the time of growth in the presence of galactose.

to select the appropriate donor preferentially (Weiler and Broach, 1992; P.Simon and J.Broach, unpublished observation). Thus, while position can influence the overall efficiency of the selection process, it does not override the basic selection mechanism.

From these observations, we conclude that at least two steps need to be considered in understanding the process of donor selection. First, the double-strand break needs to come in contact with the appropriate donor locus. Since the relative position of *MAT* to the donor loci does not strongly influence the process of selection, then finding the correct locus is not normally rate limiting. That is, the double-strand break must sample both loci a number of times before committing to recombination. Consistent with this requirement, our observation of the position of the GFP spots in living yeast cells tagged at both *MAT* and *HML* indicated that the chromosomal loci exhibit quite dynamic behavior relative to each other (P.Houston and J.Broach, unpublished observations), consistent with recent reports on the dynamic nature of single loci in yeast nuclei (Heun *et al*., 2001a,b). Thus, it is not unreasonable to assume that the dynamic activity of the loci would allow multiple sampling opportunities in a single switching event. This is also consistent with analysis of recombination in other systems, in which homology search has been shown to be rapid compared with commitment to recombination (Julin *et al*., 1986; Camerini-Otero and Hsieh, 1995; Yancey-Wrona and Camerini-Otero, 1995). As a second step in the process of switching, the broken end from *MAT* must be repaired by replicating the sequence at a donor locus. This involves strand exchange at the donor locus, followed by formation of a replication complex on the newly formed primer template. We would suggest that one of these events is likely to be the rate-limiting step through which donor preference is imposed. For instance, in an **a** strain, the *HML* locus, through the action of the RE, would be more susceptible to invasion than would *HMR*. We would argue that in the absence of the activity of the RE, *HMR* is intrinsically more susceptible to invasion. The mechanistic basis for such susceptibility to invasion remains to be resolved, but a higher density of helicases, such as Chl1, at the preferred locus could play a role (Weiler *et al*., 1995). This model does not eliminate the role of position in donor selection. Increasing the frequency with which *MAT* samples one locus versus the other—by tethering the locus to *MAT* or by removing the competing locus to a more remote site—could increase the use of that locus. However, we would conclude that, under normal circumstances, commitment to recombination, perhaps through relative susceptibility to strand invasion or maturation of strand exchange intermediates, would dictate donor preference.

We observed in these studies that two loci on a single yeast chromosome do not behave as if they were freely diffusible beads tethered to each other by an inelastic string. First, if the two loci diffused freely, constrained only by the limits of the intervening segment of DNA, then the most common position of the two loci in the nucleus would be at their maximum possible separation. In contrast, we find that the median distance between the two loci was substantially shorter than the maximum distance in all the strains tested. This behavior could be accounted for in part by assuming that the DNA possesses a high degree of elasticity *in vivo*, inhibiting the separation of the two points on the DNA to the maximum distance. The nature of this elasticity is unknown. Secondly, we observed an unexpectedly large preponderance of cells in which the two loci were contiguous in space, at least at the resolution of the light microscope. This is not a specific property of the mating loci, since loci on chromosome IX located a similar linear distance apart on the chromosome as *HMR* and *MAT* show the same distribution of distances in the nucleus as do *HMR* and *MAT*. Rather, our data suggest that while segments of chromosomes are quite dynamic in the cell, they display a higher level of organization that renders distant regions of a chromosome adjacent to each other more often than would be expected if they were freely mobile. This is consistent with previous observations indicating that segments of the yeast genome are quite mobile but remain confined to a limited region within the nucleus (Marshall *et al*., 1997). Additional experiments examining the relative position over time in living cells of two loci on the same chromosome should yield more information on the higher order structure of the yeast chromosomes.

**Table II.** Strains used in this study

| Strain | Genotype[a] |
|---|---|
| S150-2B | *MAT***a** *leu2-3,112 his3Δ1 ura3-52 trp1-289 gal2 ho* |
| LS204 | *MAT***a**/*MATα hmlα1α2Δ-inc/hmlα1α2Δ-inc hmr***a***1Δ101-inc/hmr***a***1Δ101-inc HO/HO leu2-3,112/leu2-3,112 his3Δ1/his3Δ 1 ura3-52/ura3-52 trp1-289/trp1-289* |
| PJS1296 | *MAT***a**::*TRP1-LacO*$_{256}$*/MATα hmlα1α2Δ-inc/hmlα1α2Δ-inc hmr***a***1Δ101-inc*::*LEU2-LacO*$_{256}$*/hmr***a***1Δ101-inc leu2-3,112/leu2-3,112 HIS3*::*TetR-GFP,LacI-GFP/his3Δ1 ura3-52/ura3-52 trp1-289/trp1-289 HO/HO* |
| PJS1297 | *MAT***a**/*MATα*::*TRP1-LacO*$_{256}$ *hmlα1α2Δ-inc/hmlα1α2Δ-inc hmr***a***1Δ101-inc*::*LEU2-LacO*$_{256}$*/hmr***a***1Δ101-inc leu2-3, 112/leu2-3,112 HIS3*::*TetR-GFP,LacI-GFP/his3Δ1 ura3-52/ura3-52 trp1-289/trp1-289 HO/HO* |
| PJS1298 | *MAT***a**::*TRP1-LacO*$_{256}$*/MATα hmlα1α2Δ-inc*::*LEU2-LacO*$_{256}$*/hmlα1α2Δ-inc hmr***a***1Δ101-inc/hmr***a***1Δ101-inc leu2-3, 112/leu2-3,112 HIS3*::*TetR-GFP,LacI-GFP/his3Δ1 ura3-52/ura3-52 trp1-289/trp1-289 HO/HO* |
| PJS1299 | *MAT***a**/*MATα*::*TRP1-LacO*$_{256}$ *hmlα1α2Δ-inc*::*LEU2-LacO*$_{256}$*/hmlα1α2Δ-inc hmr***a***1Δ101-inc/hmr***a***1Δ101-inc leu2-3, 112/leu2-3,112 HIS3*::*TetR-GFP,LacI-GFP/his3Δ1 ura3-52/ura3-52 trp1-289/trp1-289 HO/HO* |
| PJS1301[b] | *MAT***a**::*TRP1-LacO*$_{256}$*/MATα hmlα1α2Δ-inc/hmlα1α2Δ-inc hmr***a***1Δ101-inc*::*LEU2-LacO*$_{256}$*/hmr***a***1Δ101-inc leu2-3, 112/leu2-3,112 HIS3*::*TetR-GFP,LacI(4)-GFP/his3Δ1 ura3-52/ura3-52 trp1-289/trp1-289 HO/HO* |
| PJS1302[b] | *MAT***a**/*MATα*::*TRP1-LacO*$_{256}$ *hmlα1α2Δ-inc/hmlα1α2Δ-inc hmr***a***1Δ101-inc*::*LEU2-LacO*$_{256}$*/hmr***a***1Δ101-inc leu2-3, 112/leu2-3,112 HIS3*::*TetR-GFP,LacI(4)-GFP/his3Δ1 ura3-52/ura3-52 trp1-289/trp1-289 HO/HO* |
| PJS1303[b] | *MAT***a**::*TRP1-LacO*$_{256}$*/MATα hmlα1α2Δ-inc*::*LEU2-LacO*$_{256}$*/hmlα1α2Δ-inc hmr***a***1Δ101-inc/hmr***a***1Δ101-inc leu2-3, 112/leu2-3,112 HIS3*::*TetR-GFP,LacI(4)-GFP/his3Δ1 ura3-52/ura3-52 trp1-289/trp1-289 HO/HO* |
| PJS1304[b] | *MAT***a**/*MATα*::*TRP1-LacO*$_{256}$ *hmlα1α2Δ-inc*::*LEU2-LacO*$_{256}$*/hmlα1α2Δ-inc hmr***a***1Δ101-inc/hmr***a***1Δ101-inc* leu2-3,112*/leu2-3,112 HIS3*::*TetR-GFP,LacI(4)-GFP/his3Δ1 ura3-52/ura3-52 trp1-289/trp1-289 HO/HO* |
| PJS1392[c] | *MAT***a**::*TRP1-LacO*$_{256}$ *HML*::*URA3-TetO*$_{112}$ *HMR leu2-3,112 HIS3*::*TetR-GFP,LacI-GFP/his3Δ1 ura3-52/ura3-52 trp1-289/trp1-289 gal2/gal2* |
| PJS1401[c] | *MATα*::*TRP1-LacO*$_{256}$ *HML*::*URA3-TetO*$_{112}$ *HMR leu2-3,112 HIS3*::*TetR-GFP,LacI-GFP/his3Δ1 ura3-52/ura3-52 trp1-289/trp1-289 gal2/gal2* |
| PJS1512[c] | *MAT***a**::*TRP1-LacO*$_{256}$ *HML HMR*::*URA3-TetO*$_{112}$ *leu2-3,112 HIS3*::*TetR-GFP,LacI-GFP/his3Δ1 ura3-52/ura3-52 trp1-289/trp1-289 gal2/gal2 ste5::kan/ste5::kan* |
| PJS1513[c] | *MAT***a**::*TRP1-LacO*$_{256}$ *HML*::*URA3-TetO*$_{112}$ *HMR leu2-3,112 HIS3*::*TetR-GFP,LacI-GFP/his3Δ1 ura3-52/ura3-52 trp1-289/trp1-289 gal2/gal2 ste5::kan/ste5::kan* |
| PJS1532[c] | *MATα*::*TRP1-LacO*$_{256}$ *HML HMR*::*URA3-TetO*$_{112}$ *leu2-3,112 HIS3*::*TetR-GFP,LacI-GFP/his3Δ1 ura3-52/ura3-52 trp1-289/trp1-289 gal2/gal2 ste5::kan/ste5::kan* |
| PJS1516[c] | *MATα*::*TRP1-LacO*$_{256}$ *HML*::*URA3-TetO*$_{112}$ *HMR leu2-3,112 HIS3*::*TetR-GFP,LacI-GFP/his3Δ1 ura3-52/ura3-52 trp1-289/trp1-289 gal2/gal ste5::kan/ste5::kan 2* |
| PJS1521[c] | *MAT***a**::*TRP1-LacO*$_{256}$ *HML*::*URA3-TetO*$_{112}$ *HMR leu2-3,112 REΔ*::*LEU2 HIS3*::*TetR-GFP,LacI-GFP/his3Δ1 ura3-52/ura3-52 trp1-289/trp1-289 gal2/gal2 ste5::kan/ste5::kan* |
| PJS1524[c] | *MATα*::*TRP1-LacO*$_{256}$ *HML*::*URA3-TetO*$_{112}$ *HMR leu2-3,112 REΔ*::*LEU2 HIS3*::*TetR-GFP,LacI-GFP/his3Δ1 ura3-52/ura3-52 trp1-289/trp1-289 gal2/gal ste5::kan/ste5::kan* |
| PJS1520[c] | *MAT***a**::*TRP1-LacO*$_{256}$ *HML HMR*::*URA3-TetO*$_{112}$ *leu2-3,112 REΔ*::*LEU2 HIS3*::*TetR-GFP,LacI-GFP/his3Δ1 ura3-52/ura3-52 trp1-289/trp1-289 gal2/gal2 ste5::kan/ste5::kan* |
| PJS1533[c] | *MATα*::*TRP1-LacO*$_{256}$ *HML HMR*::*URA3-TetO*$_{112}$ *leu2-3,112 HIS3*::*TetR-GFP,LacI-GFP/his3Δ1 ura3-52/ura3-52 trp1-289/trp1-289 gal2/gal2 ste5::kan/ste5::kan* |
| PJS1463[d] | *MAT***a**/*MATα HML/HML HMR/HMR leu2-3,112/leu2-3,112 HIS3*::*TetR-GFP,LacI-GFP/his3Δ1 ura3-52/ura3-52 trp1-289/trp1-289 gal2/gal2* ChrIX(106kb)::*LEU2-LacO*$_{256}$ ChrIX(225kb)::*URA3-TetO*$_{112}$ |

[a]All strains are derived from S288C.
[b]LacI(4) designates the LacI repressor construct containing the tetramerization domain.
[c]Strains are 2*n* – 1 chromosome III monosomes.
[d]Strains are 2*n* – 1 chromosome IX monosomes.

## Materials and methods

### *Strain construction*

Strains used in this study are listed in Table II. The LacO and TetO arrays were targeted to chromosome III by cloning ~2 kb of DNA into plasmids pAFS59 and p306tetO$_{112}$ (Straight *et al*., 1996; Michaelis *et al*., 1997). Sequences spanning *CHA1* (15 511–17 514 bp from the left end of chrIII), *BUD5* (198 185–200 062 bp) and the region telomere proximal to *HMR* (298 947–300 934 bp) were used to target arrays to *HML*, *MAT* and *HMR*, respectively. The *LEU2* marker of the pAFS59 derived plasmids was replaced with *TRP1* by exchanging *Bgl*II fragments with pRS404 to allow two LacO arrays to be integrated using distinct markers. Arrays were targeted to chromosome IX using ~1 kb of DNA for regions around 106 kb (105 491–106 525 bp) and 225 kb (225 185–226 409 bp).

To generate a set of doubly tagged strains, the LacO array was integrated into the *BUD5* locus of **a** and α versions of the S288C-derived haploid strain S150-2B, and the TetO arrays were then integrated near *HML* or *HMR* as described above. In a separate strain construction, the *Eco*RI–*Bam*HI TetR–GFP fusion from YIp128tetR–GFP (Michaelis *et al*., 1997) was inserted into the *Sac*I site of pAFS144 (Straight *et al*., 1996) to yield plasmid pPJS218. Plasmid pPJS218 was integrated into the his3Δ1 locus in **a** and α cells of strain S150-2B. These strains were then transformed with the *Not*I fragment of pCEN03-UG (David Stillman, University of Utah), which consists of CENIII into which has been inserted a GAL1 promoter directed toward the centromeric sequence. This allows galactose-inducible inactivation of chromosome III centromere function. These haploids strains were mated with strains containing pairs of LacO and TetO arrays. After selecting for diploids, strains were grown on galactose and surviving cells were confirmed monosomic for chromosome III by Southern blotting and mating assay. Strains PJS1520, PJS1521, PJS1524 and PJS1533 were derived from strains PJS1512, PJS1513, PJS1516 and PJS1532 by transformation to Leu$^+$ with a PCR fragment obtained from plasmid pPJS254, which consists of the 6.3 kb *Bam*HI chromosomal fragment spanning *RE* from which the *Blp*I–*Pme*I region covering the entire *RE* (*DPS1* and *DPS2*) has been replaced with *LEU2*. Strain PJS1463 is 2*n* – 1 for chromosome IX. For analysis of the position of donor loci relative to *MAT* following activation of *HO*, doubly tagged strains were transformed with plasmid pPY222 (*CEN TRP1 LEU2 GAL-HO*, derived from pJH283 from Dr Fred Heffron), and cultures for analysis were inoculated directly from the transformation plate.

Strains for switching analysis were created using LS204 (Weiler *et al*., 1995) by sequentially integrating the LacI(2)–GFP fusion plasmid pPJS218 at *HIS3* and the LacO arrays at *MAT* and *HML* or *HMR* as above. The tetramerization domain of the Lac repressor was restored

using a PCR primer containing the sequence of the 11-amino-acid domain. This construct (contained on the *URA3* plasmid pPJS231) was used to replace the dimeric Lac repressor in the above strains. All constructions were confirmed by Southern blot analysis.

### Visualization of tagged arrays in cells

Cells from an overnight culture in SC medium (Kaiser *et al*., 1994) were inoculated into fresh SC and grown for 1–2 h at 30°C. Cells were transferred to SC medium lacking uracil and histidine and supplemented with 7.5 mM 3-aminotriazole, and grown for 30 min. Cells were then transferred to SC medium for 15 min and then fixed for 5 min in 2% paraformaldehyde (final volume). Cells were pelleted, resuspended in 1× PBS 20% glycerol, and mounted onto coverslips using 1% Low-Melt agarose in 1× PBS.

Cells were examined using a Nikon Eclipse TE200 microscope with a 100× objective (1.4 aperture) and a planaplochromatic light source. Z-stacks containing 50 slices spaced 0.15 µm apart were deconvolved using SoftWoRx v. 2.5, and GFP spots were localized by finding the pixel(s) with the highest signal intensity.

### Donor preference assays

Switching assays were performed as described (Weiler *et al*., 1995) with minor modifications. Briefly, diploid strains containing the *mat***a***1*Δ-inc allele at *HMR*, the *matα1α2*Δ-inc allele at *HML* and the indicated operator arrays were sporulated and then dissected on –HIS plates to induce the LacI–GFP fusions. After 2 days of growth at 30°C, segregants were replica plated to drop-out plates to determine genotype, which identifies the initial mating type of the germinated spore. Colony PCR to determine the allele at *MAT* was performed using 5′-GACTCT-ACCCAGATTTGTATTAGACG-3′ and 5′-GATAAGAACAAAGAA-TGATGCTAAGAATTGA-3′ as primers. PCR products were run on a 0.7% agarose gel in 1× TAE. Colonies predominantly exhibiting the *mat***a***1* allele were scored as selecting *HMR* as donor, colonies predominantly exhibiting *matα1α2* were scored as selecting *HML* as donor, and colonies that failed to exhibit one predominant allele were scored as ambiguous.

### Calculations

The expected percentage of cells with one spot, assuming a random distribution of the *MAT* and donor loci in the cell, was calculated by positing that the tagged locus could randomly occupy any position within a sphere surrounding *MAT* whose radius equaled the maximum distance ($Dist_{max}$) observed between *MAT* and the donor locus. For simplicity, we assumed that those cells in which the donor locus resided within a vertical column whose *X*–*Y* distance from MAT was less than the minimum optically resolvable distance between two points ($Dist_{min}$) would appear as a single spot. Thus, the expected fraction of cells presenting a single spot would be at most the volume of the column $[2 \times Dist_{max} \times \pi \times (Dist_{min})^2]$ divided by the volume of the sphere $[4 \times \pi \times (Dist_{max})^3/3]$, or $1.5 \times (Dist_{min}/Dist_{max})^2$. $Dist_{min}$ and $Dist_{max}$ were determined experimentally for each strain, with $Dist_{min}$ taken as the smallest measured distance between two resolvable spots.

The predicted allele occupancy at *MAT* for a strain configured as described in Kostriken and Wedeen (2001) was calculated using our experimentally measured probability of an **a** cell selecting *HML* ($P_{\mathbf{a}L}$) or *HMR* ($P_{\mathbf{a}R}$) or of an α cell selecting *HML* ($P\alpha_L$) or *HMR* ($P\alpha_R$). The percent **a** cells in a population at generation *N*, $\mathbf{a}_N$, derives from the percent **a** cells in the population at generation $N-1$, $\mathbf{a}_{N-1}$, as follows: $\mathbf{a}_N = (1 - P_{\mathbf{a}R}) \times \mathbf{a}_{N-1} + P\alpha_L \times (1 - \mathbf{a}_{N-1})$ (recall that in this strain **a** information resides at *HML* and α information at *HMR*). Similarly, the percent α cells in the population at generation *N*, $\alpha_N$, which will be $1 - \mathbf{a}_N$, can be independently calculated by $\alpha_N = (1 - P\alpha_L) \times \alpha_{N-1} + P_{\mathbf{a}R} \times (1 - \alpha_{N-1})$. By iteration, these formulae yield steady-state values (<1% change in each succeeding generation) for the percent **a** or α cells in the population within 10–15 cycles (generations). The steady-state values are completely independent of the value chosen for the percent **a** cells in the population at the first generation.

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