Mating-type Gene Switching in Saccharomyces cerevisiae

CHENG-SHENG LEE and JAMES E. HABER
Department of Biology and Rosenstiel Basic Medical Sciences Research Center
Brandeis University, Waltham, MA 02454-9110

ABSTRACT The budding yeast Saccharomyces cerevisiae has two alternative mating types designated MATα and MATα. These are distinguished by about 700 bp of unique sequences, Ya or Yα, including divergent promoter sequences and part of the open reading frames of genes that regulate mating phenotype. Homothallic budding yeast, carrying an active HO endonuclease gene, HO, can switch mating type through a recombination process known as gene conversion, in which a site-specific double-strand break (DSB) created immediately adjacent to the Y region results in replacement of the Y sequences with a copy of the opposite mating type information, which is harbored in one of two heterochromatic donor loci, HMLα or HMRα. HO gene expression is tightly regulated to ensure that only half of the cells in a lineage switch to the opposite MAT allele, thus promoting conjugation and diploid formation. Study of the silencing of these loci has provided a great deal of information about the role of the Sir2 histone deacetylase and its associated Sir3 and Sir4 proteins in creating heterochromatic regions. MAT switching has been examined in great detail to learn about the steps in homologous recombination. MAT switching is remarkably directional, with MATα recombining preferentially with HMLα and MATα using HMRα. Donor preference is controlled by a cis-acting recombination enhancer located near HML. RE is turned off in MATα cells but in MATα binds multiple copies of the Fkh1 transcription factor whose forkhead-associated phosphothreonine binding domain localizes at the DSB, bringing HML into conjunction with MATα.

INTRODUCTION

The budding yeast Saccharomyces cerevisiae propagates vegetatively either as MATα or MATα haploids or as MATα/MATα diploids created by conjugation of the opposite haploid types (Fig. 1). Mating type is determined by two different alleles of the mating-type (MAT) locus.

Like many other fungi, budding yeast has acquired the capacity to convert some cells in a colony from one haploid mating type to the other (Fig. 1) by a process called homothallism. The subsequent mating of cells to the opposite mating type enables these homothallic organisms to self-diploidize. The diploid state provides yeast with a number of evolutionarily advantageous strategies unavailable to haploids, most notably greater resistance to radiation and the ability to undergo meiosis and spore formation under nutritionally limiting conditions. Mating-type gene switching in S. cerevisiae is a highly choreographed process that has taught us much about many aspects of gene regulation, chromosome structure, and homologous recombination.

The MAT locus lies in the middle of the right arm of chromosome 3, ~100 kb from both the centromere and the telomere. The two mating-type alleles, MATα and MATα, differ by ~700 bp of sequences designated Ya and Yα, respectively (Fig. 2A). Ya and Yα contain the promoters and most of the open reading frames for proteins that regulate many aspects of the cell’s sexual activity (for reviews, see 1, 2, 3, 4, 5, 6). The MAT locus is divided into five regions (W, X, Y, Z1, and Z2) on the basis of sequences that are shared between MAT and the two cryptic copies of mating-type sequences located...
at HMLα and HMRα (Fig. 2B). HMLα and HMRα are maintained in an untranscribed, heterochromatic configuration with a highly ordered nucleosome structure (7, 8, 9, 10, 11, 12, 13). HMLα and HMRα serve as donors during the recombinational process that allows a MATα cell to switch to MATα or vice versa.

FUNCTIONS AND REGULATION OF MAT PROTEINS

MATα encodes two proteins, Matα1 and Matα2. Matα1 pairs with a constitutively expressed protein, Mcm1, to activate transcription of a set of α-specific genes (1, 14, 15), including those encoding the mating pheromone, α-factor, and Ste3, a trans-membrane receptor of the opposite mating pheromone, α-factor. The α- and α-factor mating pheromones trigger G1 arrest of the budding yeast expressing the opposite mating type and facilitate conjugation, thus ensuring that the zygote will contain two unreplicated nuclei. MATα2 encodes a homeodomain helix–turn–helix protein that acts with Mcm1 to form a repressor that binds to a 31-bp symmetric site with Mcm1 in the center and Matα2 at the ends (16). Matα2–Mcm1 represses α-specific genes, including those that produce α-factor (MFα1 and MFα2) and Ste2, a trans-membrane receptor of α-factor. Repression also requires the action of Tup1 and Ssn6 proteins (17, 18, 19, 20).
MATα is not required to turn on α-specific genes, which are constitutively expressed in the absence of MATα2. However, Mata1 is required, along with Mata2, to form a very stable corepressor that turns off a set of haploid-specific genes. Because of this transcriptional repression by the a1–α2 repressor, MATα/MATα diploids acquire several important features: First, they are nonmating because the a1–α2 repressor turns off transcription of MATα1, the activator of α-specific genes, while α-specific genes are repressed by MATα2 (5, 21, 22, 23, 24, 25). Secondly, they are able to initiate meiosis and spore formation because RME1, repressor of meiosis 1, is turned off. Thirdly, they have a bipolar budding pattern (26) because of the suppression of Axl1, which is required, in conjunction with a number of other gene products (Bud3, Bud4, Bud5, and Bud10), for axial budding (Fig. 1) (27, 28). Fourthly, nonhomologous end joining (NHEJ) is turned off by a1–α2 repression of the NEJ1 gene and by the partial repression of another NHEJ component, LIF1 (29, 30, 31, 32), so that DNA

**FIGURE 2** (A) Arrangement of MAT, HML, and HMR on chromosome 3. The gene conversion between MATα and MATα is illustrated. Both HML and HMR could be transcribed but are silenced by the creation of short regions of heterochromatin (ordered nucleosomes are presented as blue circles) by the interaction of silencing proteins with flanking cis-acting silencer E and L sequences. The recombination enhancer (RE), located 17 kb centromere-proximal to HML, acts to promote the usage of HML as the donor in MATα cells. (B) Control of mating type-specific genes. Transcription of α- and α-regulatory genes at MAT occurs from a bidirectional promoter. The Mcm1 protein, in combination with Mata1 and Mata2, activates the transcription of α-specific genes or represses α-specific genes, respectively, while a Mata1–Mata2 repressor turns off haploid-specific genes. Mata2 has no known function. doi:10.1128/microbiolspec.MDNA3-0013-2014.f2
double-strand breaks (DSBs) can only be repaired by HR. It is possible that NHEJ is repressed to prevent end joining in meiosis when more than 100 DSBs are created. Last, the expression of the HO endonuclease gene is suppressed by the a1–α2 repressor, once cells of opposite mating type conjugate to form a diploid.

The phenotypic switch from MATa to MATa is quite rapid—withina single cell division. Hence, it is not surprising that Mata1, Mata1, and Mata2 transcription regulators are quite rapidly turned over, degraded by ubiquitin-mediated proteolysis by the proteasome (33, 34). In contrast, the a1–α2 corepressor is much more stable (24).

MAT switching depends on four phenomena: (i) a cell lineage pattern that ensures that only half of the cells in a population switch at any one time, to ensure that there will be cells of both mating types in close proximity; (ii) the presence of two unexpressed (silenced) copies of mating-type sequences that act as donors during MAT switching; (iii) the programmed creation of a site-specific DSB at MAT that results in the replacement of Ya or Yα sequences; and (iv) a remarkable mechanism that regulates the selective use of the two donors (donor preference).

**CELL LINEAGE AND CONTROL OF HO GENE EXPRESSION**

MAT switching provided a powerful early model to study the determination of cell lineage. Only half of the cells in a colony are able to switch mating type in any one cell division (Fig. 1). To be more specific, any haploid cell that has previously divided is capable of switching MAT, while new daughter cells cannot (35). Thus, a germinating MATa spore will produce a MATa daughter, and then in the next generation the original mother cell will switch to MATa along with its second daughter, while the first daughter (and its daughter) remain MATa. The axial budding pattern of haploids places two MATa cells immediately adjacent to two MATa cells and they readily conjugate, forming MATa/MATa diploids in which the HO endonuclease gene is turned off so that further mating-type switching is repressed.

Nasmyth’s laboratory (36, 37, 38) first demonstrated that the control of this lineage pattern depends on the asymmetric expression of the HO endonuclease gene, which is restricted to mother cells that have divided at least once. Control of HO expression depends on the localization of Ash1 mRNA to the daughter cell prior to cell division (39). The Ash1 repressor protein, localized only in the daughter cell, suppresses the expression of Swi5 transcription factor (Fig. 1) (40, 41), which is required for HO expression (36), thus restricting HO expression to the mother cell in the next G1 stage of the cell cycle. Since Ash1 mRNA localization was first discovered, more than 20 other mRNAs have been shown to show similar localization in Saccharomyces (42, 43).

In addition to the spatial control, HO transcription is confined to a narrow window in the cell cycle, after the cell passes “start” in the G1 stage of the cell cycle. The cell division kinase, Cdk1 (Cdc28), is required for HO expression and becomes active only after the start. Cdc28 combines with G1 cyclins to activate the Swi4–Swi6 transcription factor required for transcription of the HO gene to initiate MAT switching. In addition, the HO endonuclease protein is quite unstable, with a half-life of only 10 min, so that mother cells experience a brief pulse of endonuclease activity. The HO endonuclease is rapidly degraded by the ubiquitin-mediated SCF protein degradation complex (44, 45).

HO is a member of the LAGLIDADG family of site-specific endonucleases (reviewed in 46); it recognizes a degenerate 24-bp sequence that spans the MAT-Y/Z border (47, 48). A haploid yeast has three possible targets for HO: the MAT locus, HMLa, and HMRa, but only the MAT locus is accessible under normal conditions. Thus, combining all these controls, there is a single programmed DSB inflicted at the MAT locus only in mother cells and prior to the initiation of DNA replication.

**SILENCING OF HML AND HMR: A MODEL FOR GENE SILENCING BY HETEROCHROMATIN**

The presence of intact but unexpressed copies of mating-type genes at HML and HMR implied that these two loci have to be maintained in an unusual, silent configuration. Both HML and HMR are surrounded by a pair of related but distinct silencer sequences, designated HML-E, HML-I, HMR-E, and HMR-I (Fig. 3). Each of these elements contains several binding domains for different regulators, including the origin recognition complex proteins (ORC), the Rap1 transcription factor, and the transcription factor Abf1 (49). These cis-acting elements interact, directly or indirectly, with several trans-acting factors to repress transcription of these genes. The most critical roles in this process are played by four silent information regulator (Sir) proteins, discussed below. Together, these gene products and cis-acting sequences create short regions (∼3 kb) of heterochromatin, in which the DNA sequences of HML and HMR are
Mating-type Gene Switching in Saccharomyces cerevisiae

found as a highly ordered nucleosome structure (7, 8, 9). These heterochromatic regions are transcriptionally silent for both PolIII- and PolII-transcribed genes (10, 11) and resistant to cleavage by several endogenously expressed endonucleases, including the HO endonuclease (12, 13). Silencing is weakened if the distance between the two silencer sequences is increased. The reader is encouraged to consult more detailed reviews of HML and HMR silencing for more details (13, 50, 51, 52, 53, 54, 55, 56, 57, 58).

In addition to the action of the Sir proteins and cis-acting silencers, the extent of silencing appears to be strengthened by the location of both HMR and HML relatively near chromosome ends (telomeres) that also exhibit gene silencing (59, 60, 61, 62). There are some important differences in the silencing of HML and HMR. HMR appears to be more silenced than HML, because several mutations, including the histone H4-K16N mutation, which strongly affect HML silencing have little effect on HMR silencing (63) (J. E. Haber, unpublished data). Moreover, whereas overexpression of HO endonuclease will create some cleavage at HML, HMR is much more resistant to such cutting (12, 64). Interestingly, if both HML and HMR are inserted ~60 kb from a telomere on chromosome 6, both HML and HMR show equivalent silencing defects with a histone H4-K16N mutation (65), indicating that there are “booster” sequences in the vicinity of HMR that make it much more silent. Furthermore, silencing of HMR requires passage through S phase (but not replication per se), while “substantial silencing of HMLα” could be established without passage through S phase (66). The spread of silencing to adjacent regions is restricted by several boundary elements (67, 68, 69).

The establishment and maintenance of silencing requires four Sir proteins. Mutations in these genes lead to coexpression of both HMLα and HMRα in a haploid cell, thus producing a nonmating phenotype normally seen in MATa/MATα diploids (35, 70, 71, 72). A deletion of any of these—SIR2, SIR3, and SIR4—completely abolishes silencing, while loss of SIR1 had a less extreme phenotype. Sir1 interacts with the ORC complex at silencers to recruit the rest of the Sir complex. In the absence of Sir1, silencing—already established—can be epigenetically maintained for several generations without it (73). The keystone of these silencing proteins is the NAD+-dependent Sir2 histone deacetylase, which is responsible for deacetylating a number of lysines on the N-terminal tails of histones H3 and H4 (74). Sir3 protein directly binds to various domains on nucleosomes, including H4-K16 and H3-K79 (75). Methylation of histone H3-K79 by the Dot1 methyltransferase correlates with the unsilenced chromatin state (76). Sir3 also binds with Sir4 (77), which, in turn, interacts with yKu70 (78), which is important in telomere associations with the nuclear periphery (79). Regulation of acetylation of the N-terminal tails of histones H3 and H4 is directly implicated in silencing, first by mutations that replace the four evolutionarily conserved lysine residues (59, 80, 81, 82, 83). More direct evidence came from the fractionation of chromatin in terms of the state of acetylation of histone H4-K16 (84), showing that HML and HMR are preferentially recovered in the hypoacetylated fraction. Deletion of the first 32 amino acids of the N-terminal tail of histone H3 unsilences HML but not HMR, whereas deletion of the N-terminal 16 amino acids of H4 unsilences both loci (85). A number of other proteins also contribute to silencing, including the acetyltransferase Esa1 and Sas2 protein (86).

It should be noted that silencing also occurs adjacent to yeast telomeres, and many of the genes involved in HML/HMR gene silencing also play a role in telomeric silencing (reviewed in 13, 50, 51, 53, 54, 55, 56, 57, 58, 87). At telomeres, there are no specific silencer sequences, but the telomere-associated Rap1 protein interacts with both Sir3 and Sir4 (88, 89, 90). Moreover, telomere termini are also enriched in yKu70–yKu80, which also recruit Sir4 (91, 92). Telomeric silencing can extend for more than 10 kb (93), but strong silencing is confined to the first 1–2 kb (94). Several mutations that strongly affect telomeric silencing (e.g., yku70Δ) have either no effect on HM loci or have an effect only...
with a partially debilitated HMR-E sequence, suggesting that HM loci are more strongly silenced than telomeres (88, 89, 95).

**FIRST MODELS OF MAT SWITCHING**

Early studies of MAT switching recognized the existence of two additional key loci that were required for the replacement of MAT alleles: HML and HMR (96, 97, 98). A remarkably insightful hypothesis by Oshima and Takano (1971) suggested that these loci were the seat of controlling elements that could transpose to MAT and activate opposite mating-type alleles (99). Coupled with the key experiments of Hawthorne (1963), these ideas led Herskowitz’ laboratory (100, 101) to suggest a specific version of the transposition model known as the “cassette model” in which an unexpressed copy of Ya sequences was located at HML. (HMLa) and unexpressed Ya sequences were found at HMRa. These sequences could be transposed to the MAT locus, where they would be expressed. In these early models, there was no suggestion that MAT switching involved homologous recombination; rather, a site-specific duplicative transposition was imagined. Subsequent studies (102, 103, 104, 105) confirmed that there were indeed two additional copies of mating-type information at HML and HMR. Most laboratory strains carry HMLa and HMRa, but natural variants exist that carry the opposite configuration: HMLa and HMRa (96, 106, 107). One early surprise in the molecular analysis of MAT, HML, and HMR was that the two donor cassettes did not carry simply the Ya and Yα donor sequences that could be “played” in the cassette player of the MAT locus, but that they were in fact intact, complete copies of mating-type genes carrying their own bidirectional promoters (Fig. 2). However, these genes were not transcribed. The two unexpressed cassettes differ in the extent of homology they share with MAT. HMR, HML, and MAT all share two regions flanking the Y sequences, termed X and Z1. HML and MAT share additional sequences, termed W and Z2 (Fig. 2).

We now know that during switching there is no change in either donor sequence; that is, MAT switching does not involve a reciprocal exchange of Ya and Yα sequences but rather a copying of the sequences from either HMLa or HMRa to replace the original MAT allele (108). This asymmetric recombination event is termed gene conversion. The idea that HML and HMR could repeatedly serve as donors during MAT switching provided an explanation for an early observation of Hawthorne (1963) that a mutant MATa cell could be replaced by MATα, which then switched to a wild-type MATα allele. Subsequent “healing” and “wounding” experiments were carried out in which mutations at MAT were corrected by recombination with the donor or in which a mutation at the donor was introduced into the MAT locus (100, 109, 110). In some cases, the replacement of MAT information included not only the Y region but at least part of the flanking X and Z1 regions as well that were shared by MAT and its two donors (110, 111).

In the 37 years since the cassette model was articulated, the MAT switching system and other HO-induced DSBs have been the object of intense study, to learn both about gene silencing and about the multiple mechanisms of DSB repair by homologous recombination, nonhomologous end joining, and new telomere addition (112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125). Some related studies have been done by inserting small HO endonuclease recognition sites at other locations and from the induction of other site-specific endonucleases, most notably I-SceI (119, 123, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137). Additional information about DSB repair has been gleaned from the analysis of DSB-induced recombination in meiotic cells (reviewed in 138, 139, 140, 141). By and large, the results are sequence independent; however, some interesting aspects particular to MAT switching are noted below.

**MAT SWITCHING: A MODEL FOR HOMOLOGOUS RECOMBINATION**

The conversion of one mating type to the other involves the replacement at the MAT locus of Ya or Yα by a gene conversion induced by an HO endonuclease-mediated DSB (142, 143). The process is highly directional, in that the sequences at MAT are replaced by copying new sequences from either HMLa or HMRa, while the two donor loci remain unchanged. Directional gene conversion reflects the fact that the HO endonuclease cannot cleave its recognition sequence at either HML or HMR, as these sites are apparently occluded by nucleosomes in silenced DNA. Thus, the MAT locus is cleaved and becomes the recipient in this gene conversion process. In cells deleted for SIR2, SIR3, or SIR4, where HML or HMR is unsilenced, HO can readily cut these loci and they become recipients (144, 145).

Normally, the HO gene is tightly regulated to be expressed only in haploid mother cells and only at the G1 stage of the cell cycle (36); however, the creation of a galactose-inducible HO gene made it possible to express
HO at all stages of the cell cycle and in all cells (146). This made it possible to deliver a DSB to all cells simultaneously and to follow the appearance of intermediates and final products by physical analysis of DNA extracted at times after HO induction (12, 147, 148). An example of Southern blot analysis of MATα switching to MATα is shown in Fig. 4. Physical monitoring of recombination at MAT has yielded much of what we know about DSB-induced mitotic recombination (reviewed in 3, 113, 115, 149, 150).

The overall process of MATα switching to MATα is illustrated in Fig. 5. Following HO cleavage of MATα, the ends are resected in a 5′-to-3′ direction, creating a 3′-ended single-stranded DNA (ssDNA) tail that assembles a filament of the Rad51 recombinase protein. This protein–DNA complex engages in a search for a homologous sequence (in this case HMLα) with which repair can be effected. The homology search culminates in strand exchange in which the ssDNA base pairs with the complementary sequence in the donor, creating a displacement loop, or D-loop. The 3′ end of the invading strand is then used as a primer to initiate copying of one strand of the donor locus, and the newly copied strand is displaced until it can anneal with homologous sequences on the opposite end of the DSB. The 3′-ended nonhomologous tail of the Y region is clipped off and the new 3′ end is used to prime a second strand of DNA synthesis, completing the replacement of MATα by MATα. This process is known as the synthesis-dependent strand-annealing (SDSA) mechanism, in which all the newly copied DNA is found at the recipient locus while the donor remains unaltered (151). Each of these steps is discussed in more detail below.

**HO cleavage**

The HO endonuclease cleaves a degenerate recognition site of 24 bp in vitro (47), although sites of 117 bp down to 33 bp are generally used when the HO recognition site is inserted at other locations. A site with only 21 bp results in inefficient single-strand nicking that, by replication, can be converted to a DSB (152). Single base-pair mutations in the recognition site, such as MAT-1 (inconvertible) or MAT-stk (stuck), abolish or greatly reduce switching (153, 154). HO cutting generates 4 bp, 3′-overhanging ends (143). In vivo, the DSB is processed by several 5′-to-3′ exonucleases to create long 3′-ended tails (148). As discussed more fully below, the 3′ end is remarkably resistant to exonucleolytic removal. There do not seem to be any 3′-to-5′ exonucleases that act on a 3′-overhanging end, but protection of the ssDNA tail depends on binding of the ssDNA-binding protein complex, RPA (155).

MATα switching, induced by a galactose-regulated HO endonuclease, is a surprisingly slow process, requiring more than 1 h to complete, independent of the time during the cell cycle (12, 147, 148). It is possible that MATα switching may be more rapid when HO is expressed from its normal promoter in G1, only in mother cells, and in a coordinated fashion with other genes (142). However, additional experiments using HO whose expression is restricted to the G1 phase of the cell cycle (36) showed similar slow kinetics, as did expressing a conditional allele of HO under normal cell-cycle control (M. Yamaguchi, M. Gartenberg, and J. E. Haber, unpublished data).

**5′-to-3′ resection of the DSB ends**

5′-to-3′ resection proceeds rather slowly, at a rate of ∼4 kb h−1 (∼1 nucleotide s−1) (156, 157). In a strain where there is no repair of the DSB, resection will continue at roughly this rate for more than 24 h, while if the DSB is being repaired, the slowly proceeding resection proteins most likely are displaced by the repair DNA synthesis machinery.
It is possible that resection is downregulated once recombination intermediates are established. Southern blot analysis of the rate of 5′-to-3′ degradation first implicated the trio of interacting proteins, Mre11, Rad50, and Xrs2 (MRX complex) (158, 159, 160). MRX somehow associates with Sae2, although a direct interaction has not been demonstrated, and together these proteins appear to do the initial 5′-to-3′ resection at HO-induced DSB ends. Mre11 has 3′-to-5′ exonuclease activity, and both Mre11 and Sae2 have endonuclease activity (161, 162, 163); however, mutation of the nuclease motifs of Mre11 has only a modest effect on resection (161, 162, 164). The nuclease activity of Mre11 is required in mitotic cells to cleave hairpin ends (165, 166) and in meiosis to remove the Spo11 protein from DSB ends (but in both of these cases, Sae2 is also necessary). Deleting Sae2 more significantly retards resection (167). However, while MRX–Sae2 appears to get resection started, extensive resection depends on two competing pathways of resection, one comprising the 5′-to-3′ exonuclease Exo1 and the other consisting of the Sgs1–Top3–Rmi1 (STR) helicase complex coupled to the nuclease function of Dna2 (which itself has nuclease activity that is not relevant for this process) (157, 168, 169, 170). The activity of Exo1 is impaired by the binding of yKu70 and yKu80 proteins, which are important in NHEJ (171, 172).

**FIGURE 5** Mechanism of MAT switching. Key steps in the switching of MATα to MATα by a synthesis-dependent strand-annealing (SDSA) mechanism. An HO-induced DSB is resected by 5′-to-3′ exonucleases or helicase endonucleases to produce a 3′-end single-stranded DNA (ssDNA) tail, on which assembles a Rad51 filament (shown only on one side of the DSB). The Rad51::ssDNA complex engages in a search for homology. Strand invasion of MAT-Z into the homologous HML-Z can be detected by anti-Rad51 chromatin immunoprecipitation followed by quantitative PCR using the primer pair P and P. Strand invasion can form an interwound (plectonic) joint molecule (D-loop) that can assemble DNA replication factors to copy the Ya sequences, which can be monitored by a primer extension assay using the primer pair P and P. The D-loop is thought to migrate as DNA synthesis proceeds. Unlike normal replication, the newly copied strand is postulated to dissociate from the template and, when sufficiently extended, anneal with the second end, still blocked from forming a plectonic structure by the long nonhomologous Yα tail. The single-stranded tail is clipped off once strand annealing occurs by the Rad1–Rad10 flap endonuclease, so that the new 3′ end can also be used as a primer to fill in the gap. Consequently, all newly synthesized DNA is found at the MAT locus, while the donor is unaltered. However, a small fraction of DSB repair events apparently proceed by a different repair mechanism involving the formation of a double Holliday junction. doi:10.1128/microbiolspec.MDNA3-0013-2014.f5
When both Sgs1 and Exo1 are deleted, there is very limited resection, which appears to depend on MRX and Sae2. This result suggests that MRX acts first and hands off resection to Exo1 or the STR–Dna2 complex, but this idea is contradicted by the long-observed fact that deletion of MRX proteins only reduces resection by about 50% in cycling cells. However, in G2-arrested cells, deletion of Rad50 eliminates nearly all resection (173). These observations suggest that there must be an alternative resection activity that is absent in G2-arrested cells. Moreover, in G1 cells prior to the activation of Cdk1 at the start point of the cell cycle, there is nearly no resection (174, 175). Inhibition of Cdk1 at other points in the cell cycle also blocks resection (174, 175). This control is exerted through Cdk1’s phosphorylation of both Sae2 and Dna2 (168, 176). Taken together, it seems that the modest inhibition of resection in cycling cells by deleting MRX or Sae2 argues that MRX–Sae2 does not act as the obligate gatekeeper of resection.

Resection of course must plow through chromatin, and it is not yet clear how these complexes accomplish the necessary chromatin remodeling. Deleting the Arp8 subunit of the Ino80 chromatin-remodeling complex has—in some hands—a modest effect on resection. However, a much more profound inhibition of resection is seen when the Swi2/Snf2 homolog, Fun30, is deleted (177, 178, 179). Fun30 is an ATPase that has been shown to displace a positioned nucleosome in vitro (180, 181). Resection of HO-cut ends is also reduced by the phosphorylation of histone H2A (called γ-H2AX) by the Mec1 and Tel1 checkpoint kinases (homologs of mammalian ATR and ATM). Interestingly, Fun30 associates less strongly in vitro with nucleosomes carrying H2A-S129E, mimicking γ-H2AX (179).

Recruitment of Rad51 recombinase and the search for homology

As long 3’ tails are generated, they are bound by the Rad51 recombination protein, which facilitates a search for homologous regions, to initiate recombination (Fig. 5). Chromatin immunoprecipitation (ChIP) experiments have shown that once ssDNA is generated, it is first bound by the ssDNA-binding protein complex, RPA, which is then displaced by Rad51 (182). The loading of Rad51 depends completely on the Rad52 protein (183, 184). In the absence of the Rad55 and Rad57 proteins, which are known as Rad51 paralogs, Rad51 filament assembly is slow and apparently incomplete, and MAT switching fails to occur. In other DSB-mediated repair events where the donor is not silenced, recombination also fails in the absence of Rad55 or Rad57 (183, 184).

ChIP, using an anti-Rad51 antibody, allows one to visualize the kinetics of Rad51 loading onto ssDNA. The same approach permits visualization of the synthesis between the MAT DSB and the donor, as Rad51 will associate with both the invading MAT strand and the HMLα duplex DNA. This step takes ~15 min after appearance of Rad51 assembly at the DSB (149, 183, 184). The time to achieve pairing between MAT and HML has also been seen microscopically by examining green fluorescent protein (GFP)-tagged LacO and TetR arrays situated close to HML and MAT, respectively (185, 186). It should be noted that the time to pair with donor sequences located interchromosomally is significantly longer than what occurs between HML and MAT (187). The relatively rapid encounter between these two loci is undoubtedly aided by the cis-acting recombination enhancer (RE), located ~17 kb centromere-proximal to HML, which will be discussed in detail below. It is striking that the amount of homology shared by MAT and its donors is quite small, especially on the Z side, which seems to initiate copying of the donor. MAT and HMR only share 230 bp, while MAT and HML share 327 bp. In contrast, there is much more extensive homology on the W/X side, but this lies beyond the nonhomologous Y sequences, so this DSB end cannot directly initiate new DNA synthesis. Thus, the efficiency of repair is dictated by the smaller Z side. We will discuss the mechanism of donor preference in detail below, but suffice it to say that one can set up an experiment in which MAT will normally switch with HMR as a partner and HML is the “wrong donor.” By artificially increasing the size of the homology on the Z side of HML from 327 to 650 to 1800 bp, one can significantly increase its use as a donor in competition with HMR (188). It has been estimated that encounters between the Rad51 filament and the preferred donor will happen on average four times before some irreversible step will lead to the completion of recombination (188). The location of a donor in the genome also seems to play an important role in homology searching. Donors located in the regions showing higher contact probability, determined by genome-wide chromosome configuration capture, are used more efficiently to repair a DSB on a different chromosome (C.-S. Lee, R. Wang, and J. E. Haber, unpublished data). Once resection exposes both the X and Z homologous regions flanking the DSB, they can each synapse with their homologous sites in HML (149). However, for the left end of the DSB to act as a primer, the nonhomologous Ya tail has to be removed. The nonhomologous tail
is clipped off by the Rad1–Rad10 endonuclease (189, 190). In other HO-induced events (and probably in MAT switching), removal of the nonhomologous tail also requires a number of other proteins, including Msh2–Msh3, Saw1, and Slx4 (191, 192). This “chippers” acts apparently only after annealing of the resected end with the newly copied complementary strand to create a branched, annealed structure with a 3′-ended tail. This step also requires the action of either of the two DNA damage-responsive protein kinases, Mec1 or Tel1, to phosphorylate Slx4 (193). Although a branched structure with the nonhomologous tail might form when MAT-X synapses with the donor, tail clipping preferentially occurs later, apparently after the annealing of the elongated first strand in the MAT-X region (149).

During strand invasion, any mismatch in the synapse could be corrected by mismatch repair machinery. McGill et al. (111) used artificial restriction sites inserted at different places in the X and Z regions and showed a highly biased transfer of markers from the donor to MAT. In the absence of mismatch repair, a single base-pair mutation only 8 bp from the 3′ end of the HO cut, in the Z region, was most often retained during switching (154). A kinetic analysis further demonstrated that mismatch correction occurred soon after the strand invaded the donor locus (194). That mutant sequence in the invading Z DNA was corrected to the genotype of the donor is the most direct in vivo demonstration of the idea that mismatch repair will preferentially correct a mismatch adjacent to a nick (in this case, the 3′ end of an invading strand) (195, 196).

In addition to anti-Rad51 ChIP, strand invasion has been examined with a polymerase chain reaction (PCR)-based nucleosome protection assay (149). As noted before, HML has very highly positioned nucleosomes, whereas there is little order to nucleosomes at MAT. At the time of synopsis, there is a transient reduction in protection of nucleosomes at the site of strand invasion. By delaying the initiation of new DNA synthesis, it was possible to detect the change more reliably. This study produced two interesting results. First, the region of reduced nucleosome protection extended to several nucleosomes in the nonhomologous Y region prior to the initiation of new DNA synthesis, suggesting that the D-loop is extended beyond the 3′ end of the invading strand, perhaps by helicases. It has not been determined yet whether the extended and apparently open region would bind RPA. Interestingly, a mutation of the largest subunit of RPA, rfa1–t11 (K45E), has normal Rad51 filament formation but fails to engage in synopsis with donors (182). This result could mean that RPA is needed to stabilize the D-loop.

The other important finding was that nucleosome rearrangement at HML did not occur in a rad54Δ mutant. Rad54 is a Swi2/Snf2 homolog that has been shown to engage in chromatin remodeling in vitro (197). Deletion of Rad54 did not abolish strand invasion as measured by the association of Rad51 with the donor (183), but this association apparently is distinct from the full chromatin rearrangement necessary to complete DSB repair. Without Rad54, there is no primer extension and new DNA synthesis. One possible explanation for this result is that the kind of association of MAT and HML strands in the absence of Rad54 is a paranemic joint in which the invading strand does not intertwine with the donor duplex, whereas with Rad54 a plectonemic, interwound structure is formed with the displaced strand in an extended D-loop (Fig. 5).

**Copying the donor sequences**

The initiation of new DNA synthesis can be detected by a PCR-based primer extension assay, using two primers—one complementary to sequences distal to MAT and one in the Yu region of the donor (148). PCR amplification can take place only after the invasion of the 3′-ended single strand into the Z region of the donor locus and the copying of at least 50 nt, thus creating a recombination intermediate that covers both primers. This step occurs ~15–20 min after synopsis between MAT and HML is observed by ChIP and 15–30 min prior to the completion of gene conversion, as monitored by both Southern blotting and a second PCR assay, detecting the time when the donor Y sequences are joined to the proximal side of MAT (148, 149).

Physical analysis has also made it possible to analyze conditional lethal mutants to ask which DNA replication enzymes are required for MAT switching. Unlike break-induced replication, where only one end of a DSB can invade homologous sequences (reviewed in 198), only a fraction of the proteins necessary for origin-dependent DNA replication are also required for MAT switching (136). In this process, which appears to involve the elongation of one strand and then the elongation of the second strand, primase and DNA polymerase α, the lagging strand DNA polymerase, are not required, while both DNA polymerases δ and ε appear to act either sequentially or redundantly (199). The proliferating cell nuclear antigen (PCNA) clamp is required, but the GINS–Cdc45–Mcm helicase complex is dispensable (199). DNA synthesis during MAT switching does not need most of the loading factors required at an origin for normal replication, including the ORC proteins, Cdt1 and Cdc6 (200). However, the Dpb11–Sld2–Sld3...
proteins are required (149). These proteins have been shown to be part of a preloading complex at origins (201), but how they would work when DNA copying is not dependent on an autonomously replicating sequence or on other early-functioning proteins is unknown. Three other mutations that impair break-induced replication do not block MAT switching or other gene conversion events: (i) deletion of the nonessential DNA polymerase δ subunit, Pol32; (ii) inactivation of the Pif1 helicase; and (iii) the dominant pol30-FF248, 249AA mutation of PCNA (119, 136, 202, 203).

The fact that DNA synthesis during gene conversion does not use all the processivity factors employed in normal replication may explain why gene conversion is much more susceptible to mutation of the replicated sequences. Taking advantage of several features of MAT switching, it was possible to select for mutations that arose during gene conversion (204). The majority of mutations were base-pair substitutions, but the rest (∼40%) represented various types of template jumps, as if the DNA polymerase was less processive than would be found during normal replication. Surprisingly, all types of mutation representing template jumps were eliminated in a strain with a proofreading defect in DNA polymerase δ. We surmised, on the basis of some in vitro studies of a similar proofreading mutation (205), that the proofreading-defective mutant enzyme is in fact less prone to dissociate from the template. This result argues strongly that DNA polymerase δ is a major player in MAT switching. However, there was also evidence that DNA polymerase ε was active, since a proofreading-defective mutant of DNA polymerase ε resulted in the appearance of +1 frameshifts. The appearance of these mutations in gene conversion was apparently independent of the mismatch repair system and insensitive to the error-prone DNA polymerase ζ or another translesion DNA polymerase, DNA polymerase η. However, DNA polymerase ζ does seem to be principally responsible for mutations in ssDNA outside the regions of shared homology that must be filled in after the DSB is repaired (206, 207).

Direct evidence that MAT switching proceeds by an SDSA mechanism was obtained by Ira et al. (151), who used “heavy–light” density transfer methods to analyze the location of new DNA synthesis during MAT switching. These experiments confirmed that all the newly copied DNA is in the recipient MAT locus, while the donor remains unaltered.

Completion of switching

One of the other striking aspects of MAT switching is that it is very rarely accompanied by crossing over, which produces lethal outcomes in MAT switching (208, 209). Crossovers are not expected when the SDSA mechanism is used, because there is no stable single or double Holliday junction that would be cleaved to produce crossovers. Moreover, such crossovers would be lethal between MAT and either HML or HMR. On the basis of ectopic recombination studies in which HO induces a gene conversion between MATa on chromosome 5 and an uncuttable MATa-inc allele on chromosome 3 (and where the normal donors are deleted), it seems that crossovers, which are viable in this scenario and can be easily scored, are prevented predominantly by the action of two helicases, Sgs1 (with its partners Top3 and Rmi1) and Mph1 (210, 211). The Sgs1 complex acts as a dis-solvase to remove double Holliday junctions that would possibly become crossovers if removed by resolvases. Mph1 appears to ensure that the SDSA pathway is used rather than the alternative DSB repair mechanism that is much more prevalent in meiosis.

Finally, it is worth noting that MAT switching represents a case of gap repair rather than strictly break repair; that is, the regions of homology located by the two DSB ends are separated on the donor template by ∼700 bp. In a related study, using HO-cleaved LEU2 sequences, Jain et al. (123) found that break repair and gap repair, when the gap was larger than ∼2 kb, are surprisingly different repair processes. Break repair occurs with relatively rapid kinetics, whereas there is a delay of hours in new DNA synthesis when there is a long gap. This delay is quite similar to that seen in break-induced replication, where only one end of the DSB is homologous to a template and repair can only occur by assembling a complete replication fork. Consequently, long gap repair and break-induced replication depend on Pol32 and are impaired by pol30-FF248, 249AA, while break repair (and MAT switching) is unimpaired. Apparently the two ends of the DSB need to be in contact with each other at the time of strand invasion; they must pair close enough to each other, and in the proper orientation, to permit some signal to be propagated. We termed the assessment of the nature of the strand invasion—the difference between break repair and gap repair—as a manifestation of a recombination execution checkpoint. MAT switching appears to have a small enough gap to be treated as break repair.

**DONOR PREFERENCE**

During MAT switching, there is an elaborate mechanism that gives yeast the ability to choose between its two donors. MATa cells use HMLα as the template to repair...
the DSB ~85–90% of the time, while \( \text{MAT}_{\alpha} \) cells preferentially choose \( \text{HMR}_{\alpha} \). This preference makes sense in that it leads to a productive switch to the opposite mating type. However, donor selection is not dictated by the \( \text{Ya} \) or \( \text{Yu} \) content of the donors: a strain with reversed silent information (\( \text{HML}_{\alpha} \text{MAT}_{\alpha} \text{HMR}_{\alpha} \)) still chooses \( \text{HML} \) ~85–90% of the time (212, 213). Weiler and Broach (213) showed that replacing the entire \( \text{HML} \) region including its silencers with a cloned \( \text{HMR} \) locus did not change donor preference, suggesting that the location of the donor, not the sequence differences between \( \text{HML} \) and \( \text{HMR} \), directs donor selection. There must therefore be one or more \( \text{cis} \)-acting sequences, outside of the donors themselves, that activate or repress one or both donors, depending on mating type.

\( \text{MAT}_{\alpha} \)'s choice of \( \text{HMR} \) over \( \text{HML} \) occurs independently of the \( \text{MAT}_{\alpha}1 \) gene but is strongly dependent on \( \text{MAT}_{\alpha}2 \), the gene that acts as a repressor of \( \text{a} \)-specific genes (100, 214, 215, 216, 217, 218). \( \text{MAT}_{\alpha} \) donor preference does not depend on a functional \( \text{MAT}_{\alpha}1 \) gene (219). \( \text{HMR} \) appears to be used as the default locus and all the active regulation is in making \( \text{HML} \) more accessible (Fig. 6). Thus, a \( \text{MAT}_{\alpha} \) cell deleted for its preferred donor, \( \text{HML} \), can easily use \( \text{HMR} \), but some of \( \text{MAT}_{\alpha} \) cells die when their only choice of a donor is \( \text{HML} \) (216, 220, 221). Cells experiencing an unrepaired DSB become arrested at a G2/M checkpoint (222), which should theoretically have allowed cells time to locate a donor and repair the DSB by gene conversion, but as DSB ends are resected, other recombination events—for example, between several Ty retrotransposons located 30 kb proximal to \( \text{MAT} \) and Tys on other chromosomes—may lead to the death of cells (223).

Identification of a recombination enhancer

Wu and Haber (220) identified the key \( \text{cis} \)-acting element responsible for donor preference (now called the recombination enhancer or RE) by creating a series of truncations and internal deletions. A 2.5-kb deletion located 17 kb proximal to \( \text{HML} \) completely reversed donor preference, so that a \( \text{MAT}_{\alpha} \) cells now used \( \text{HML} \) only 10% of the time instead of 90% (Fig. 6). Deletion of this sequence also abolished \( \text{MAT}_{\alpha} \) donor preference for donors located 41, 62, and 92 kb from the left end. Deletion of this region had no effect on \( \text{MAT}_{\alpha} \) cells, which continued to use \( \text{HMR} \) most of the time.

Further mapping of RE was accomplished by inserting subfragments of the smallest deletion back into the chromosome. This led initially to the identification of a 700-bp RE that restored \( \text{MAT}_{\alpha} \) donor preference almost to wild-type levels. Subsequent analysis has narrowed down the most important sequences to ~250 bp, although full activity resides in a region of ~400 bp. This further refinement was accomplished by showing that a syntenic region in \( \text{Saccharomyces carlsbergensis} \) and in \( \text{Saccharomyces bayanus} \) (but not in more distant species such as \( \text{Saccharomyces servazzii} \)) contained an active RE that would substitute for the \( \text{S. cerevisiae} \) RE (224, 225). By comparing the divergent sequences of these REs, Wu et al. (224) defined five well-conserved subdomains, named A–E (Fig. 6). Deletion analysis indicated that subdomains A, C, D, and E are all important for donor preference, while subdomain B is not. Further analysis revealed that each of the subdomains A, D, and E contains one or more binding sites for the Fkh1 transcription regulator (225). As we will see below, this protein plays a central role in the activity of RE. A key finding was that one could replace the entire RE with multimers of only subdomain A, D, or E to mimic RE activity. Most strikingly, four copies of the 21-bp subdomain A was sufficient to raise \( \text{HML} \) usage from ~10% in the absence of any RE to 65%. The activity of the 4×A construct depends on Fkh1 (225).

**FIGURE 6** Protein binding to consensus elements in the RE. In \( \text{MAT}_{\alpha} \) cells, Mcm1 binding facilitates the binding of Swi4–Swi6 and multiple copies of Fkh1. These proteins are important for the ability of RE in promoting \( \text{HML} \) usage in \( \text{MAT}_{\alpha} \) cells. In \( \text{MAT}_{\alpha} \) cells, binding of the \( \text{Mata}2–\text{Mcm1} \) repressor to a 31-bp conserved operator, shared by an-specific genes, leads to the formation of highly positioned nucleosomes between the two flanking genes and excludes binding of Fkh1 or Swi4–Swi6. doi:10.1128/microbiolspec.MDNA3-0013-2014.f6
Role of a Mata2–Mcm1 operator in turning RE off in MATα cells
RE lies in perhaps the largest “empty” region of the yeast genome, ∼2.5 kb with no open reading frames or regulatory sequences associated with the flanking genes, KAR4 and SPB1. In MATa, the region is “open” and binds a number of proteins, whereas in MATα cells, highly positioned nucleosomes cover the entire 2.5-kb intergenic region harboring RE (224). The 90-bp subdomain C, which has no Fkh1-binding site but is important for donor preference, harbors a conserved 31-bp consensus Mata2–Mcm1 binding site. In MATα cells, a2–Mcm1 repressor binding, in conjunction with the corepressor Tup1, alters chromatin structure and thus turns off RE (217). Mutation of the Mata2-binding sites is sufficient to alter donor preference in MATα, increasing HML usage from ∼10 to ∼50% (217, 224). RE contains no open reading frame, but indeed there are two “sterile” transcripts of the RE region that are transcribed in MATα but not in MATα (218). However, these two noncoding RNA transcripts are not important for RE activity (85, 220, 224). It is interesting to note that the 2.5-kb region between KAR4 and SPB1 contains a second a2–Mcm1 binding site centromere-proximal to the defined RE element. This second repressor site may have a role in repressing HML usage in MATα cells.

Activation of RE in MATα cells depends on the Mcm1 protein
A 2-bp mutation that eliminates Mcm1 binding in the Mata2–Mcm1 operator sequence completely inactivates RE and reduces HML usage from ∼90 to 10–20% in MATα cells (224). Similarly, a single amino acid substitution mutation in MCM1 (mcm1-R89A), which reduces Mcm1 binding, also showed a reduction in HML usage (224). Surprisingly, in MATa cells, where there is no Mata2 protein, the mutant RE with the 2-bp operator mutation that prevents Mcm1 binding has an array of positioned nucleosomes that is similar to what is seen in normal MATα cells (224). Apparently, other sequences within RE can, at least in MATα cells, organize a phased nucleosome structure in the absence of Mata2–Mcm1 binding.

Clearly, Mcm1 binding is critical in the activation of the normal RE sequence, but it is also evident that, since multimers of domain A, D, or E (which lack Mcm1-binding sites) are sufficient to promote preferential use of HML, the importance of Mcm1 is in regulating other chromatin features of the normal RE.

RE binds Fkh1 and Swi4–Swi6
Binding of Fkh1 to several domains of RE was confirmed by ChIP against a functional epitope-tagged Fkh1–haemagglutinin construct. Fkh1 bound only in MATα, and preferentially in the G2/M stages of the cell cycle (227). Deletion of Fkh1 markedly reduced HML usage in MATα from ∼85 to ∼35%, which has more HML usage than when RE was fully deleted. This result suggests that there must be other proteins involved in the action of the complete RE (227). In contrast, when a 4×A construct was used in place of the whole RE, deleting Fkh1 dropped HML usage to the same level as deleting RE (227). Further inspection revealed that subdomain C contains a Swi4–Swi6-regulated cell cycle box (SCB) that binds the G1-to-S-phase cell-cycle regulators Swi4–Swi6. When the SCB was mutated or if Swi6 was deleted, HML usage dropped, again to ∼35% (227). Swi4–Swi6 and Fkh1 are not in the same pathway in the regulation of RE activity because a deletion of SCB, coupled with fkh1Δ, further reduced HML usage to ∼15%.

Other donor preference mutations
Two other trans-acting factors have been shown to play less decisive roles in donor preference. Screening directly for donor preference mutations has not been very productive, largely because mutations that affect HO expression and cis-acting mutations that reduce HO cleavage tend to interfere with the evaluation of donor choice scored at the colony level. Only chl1Δ has emerged in this way. Deletion of the CHL1 gene reduces HML usage in MATα cells from 80 to 60% but has no effect on MATα (215). CHL1 encodes a nuclear protein with putative helicase activity that is implicated in the establishment and maintenance of sister-chromatid cohesion (228). Deletion of CHL1 causes a large increase in both the loss and gain of chromosomes by mitotic chromosome nondisjunction (229, 230). The other factors implicated in donor preference are the yKu70 and yKu80 proteins. The Ku complex plays many roles in chromosome architecture and in DSB repair. These proteins are required for the predominant mechanism of nonhomologous end joining that can rejoin the 4-bp 3′-overhanging DSB ends created by HO, but they are also critical in associating telomeres with the nuclear periphery and in ensuring the full activity of telomerase. When Ku proteins are deleted, telomeric regions are delocalized from the periphery, but, paradoxically, it seems that HML is more frequently associated with the nuclear periphery in a yku70A mutant (231). This might explain why, when either Ku protein is deleted, there...
is an ~10% reduction in HML usage in MATa but no
discernible effect on MATα. It seems that Chl1 and
yKu70–yKu80 may all act in the Swi4–Swi6 pathway, as
double mutants SCBΔ cbl1Δ and SCBΔ yku80Δ con-
tinue to use HML ~30% of the time, whereas fkb1Δ cbl1Δ and fkb1Δ yku80Δ both resemble fkb1Δ SCBΔ
(227). Telomeres are also tethered by the interaction of
the Sir4 silencing protein with Esc1 (232, 233); how-
ever, deletion of ESC1 had no important detect in donor
preference, and a yku70Δ esc1Δ double mutant also did
not exhibit a significant perturbation of donor prefer-
ence (E. Coïc, unpublished data). Hence, it is not clear
that anchoring RE or HML to the nuclear periphery
plays any meaningful role in this regulation.

**RE acts over a long distance and is portable**

When HML was deleted and either HML or HMR was
inserted at other chromosome 3 locations, the donor
could be activated at several sites along the entire left
arm in MATα cells, although the efficiency decreased as
the donor was moved further from RE (219, 220). Con-
versely, RE itself can be moved to sites along the left arm
and still stimulate the use of HML (234). RE can also
stimulate HML usage when MAT is moved to a different
chromosome (221). Moreover, RE also works with non-
MAT sequences. In a competition assay, an HO-induced
DSB in a leu2 sequence on chromosome 5 can be repaired
with a LEU2 gene placed near RE or a second, competing
donor located ~100-kb centromere-proximal on chro-
mosome 5. The presence of RE increased the use of the
adjacent interchromosomal LEU2 donor from ~15 to
~50% (85). The increased usage of the RE-adjacent
donor was reflected in a more rapid appearance of the
association of the Rad51 recombination protein with the
donor sequences, as measured by ChIP (85), suggesting
that RE accelerates the search for homology and/or the
conversion of an initial encounter between the DSB end
and the donor into a stable recombination intermediate.

Further evidence that RE is portable has come from
two additional experiments. First, insertion of a second
copy of RE near HMR in MATα cells increased the usage
of HMR from ~10 to ~50% (234). Secondly, when
MATα, HML, HMR, and RE were moved from chro-
mosome 3 to the larger chromosome 5 in roughly the
same configuration, the use of HML increased from ~40
to ~90% with the help of RE (234).

**RE affects other recombination events**

The effect of RE is not restricted to recombination in-
duced by HO endonuclease. If HML is replaced with
an allele of the leu2 gene and a second leu2 allele was
placed elsewhere on chromosome 3—or even on another
chromosome—the rate of Leu* spontaneous recom-
bination was ~10 times higher in MATα cells than in
MATα in an RE-dependent manner (216). There was
no significant mating-type-dependent difference when a
similar experiment was done with one leu2 allele in place
of HMR, thus supporting the conclusion that donor
preference was effected through changes in the left arm
of the chromosome, with HMR being a more passive,
although efficient, participant.

**How does RE work?**

There is no obvious difference in the positioning of nu-
cleosomes over HML in MATα versus MATα strains
(8), suggesting that RE does not make HML more ac-
cessible by altering the silencing of HML. Furthermore,
the silencing of HML does not seem to interfere with the
recombination with MAT, and donor preference is un-
changed if the donor regions are unsilenced but contain
alterations so that they cannot be cleaved by HO (188).
Although HML and HMR have been shown to reside
preferentially near the nuclear periphery, it appears that
this tethering does not prevent the donors from engaging
MAT in the center of the nucleus (231).

One attractive idea to explain the role of RE is that it
changes the localization or the higher-order folding of
the entire left arm of chromosome 3 to make HML more
flexible and available in locating and pairing with the
recipient site in MATα cells. Several approaches suggest
that there are differences in chromosome arrangement in
the two mating types, but these changes, in the absence
of a DSB, do not seem to explain donor preference. First,
chromosome segments near MAT, HML, and HMR can
be fluorescently tagged by binding LacI–GFP or TetR–
GFP (or some other color) to LacO or TetO arrays and
thus the distance between these elements can be esti-

dated. In one such study of fixed cells, however, HML
was not closer to MATα than HMR (185). More detailed
studies using fluorescent techniques (I. Lassadi and K.
Bystricky, personal communication) or by sequencing-
based chromosome conformation capture (termed 5C)
(J. Dekker, personal communication) suggest that in
MATα cells the left arm of the chromosome is more ex-
tended and perhaps more associated with the nuclear
periphery. If RE is deleted in MATα cells the arm is more
“crumpled” and more like the configuration seen in MATα
cells. These data suggest that the inactive RE, bound by
the Matu2–Mcm1 repressor, actually impairs left arm usage
in MATα cells, so that RE would positively affect usage
in MATα but might also negatively affect HML usage in
MATα.
FIGURE 7 Role of the recombination enhancer in MATα donor preference. (A) Arrangement of HMLα, MATα, and HMRα–BamHI (HMRα-B). When RE is replaced by four LexA-binding sites (LexABS), HML usage is strongly impaired. Expression of LexA–FHA (the phosphothreonine-binding domain of Fkh1) fusion protein completely rescues HML usage, while expression of the mutant LexA–FHA^{R80A}, which has lost phosphothreonine-binding activity, fails to rescue it. (B) Southern blot data after induction of switching showing the proportion of BamHI-digested MATα or MATα-B DNA in the strains depicted above. (Figure modified from 85.)

A simple model of the mechanism underlying the Fkh1-regulated donor preference

When RE was replaced with four copies of the LexA operator, HML usage was very low, but the use of the left donor could be markedly increased by expressing a LexA–Fkh1 fusion protein (85). LexA–Fkh1 was then truncated so that it contained only the first 230 amino acids, comprising the forkhead-associated (FHA) domain. This construct was shown to completely restore HML usage (Fig. 7), whereas other fusions carrying the transcription regulatory domain near the C terminus had no activity. In addition, mutation of a conserved arginine residue, which is required for the phosphothreonine-binding activity of FHA domains, abolished LexA–FHA activity (Fig. 7). These results suggested that Fkh1 binds to RE in MATa cells and that its FHA domain physically interacts with proteins containing a phosphothreonine residue, around the DSB, and thus tethers HML close to MAT. Consistent with this model, ChIP experiments showed the association of LexA–FHA with MAT only after a DSB was induced, even in strains where HML itself was deleted (85). Whereas phosphorylation of histone H2a (γ-H2AX) spreads more than 50 kb on either side of the DSB, FHA binding is confined to only a few kilobases around the break. However, the binding partner of the FHA domain remains to be determined. Neither of the two DSB-dependent phosphorylations of histones, H2a-S129 or H4-S1, which spread around the MAT locus, is responsible for donor preference. Moreover, donor preference is not influenced in the mutants deleted for N-terminal tails of histones H3 and H4 or proteins known to associate with the DSB ends, including Mre11 and Sae2. As for the identity of the damage-dependent protein kinase that phosphorylates the threonine residue, casein kinase II but not the ATR and ATM homologs, Mec1 and Tel1, is involved in the donor preference regulation (85).

A simple model to explain donor preference is presented in Fig. 8. The cluster of Fkh1 proteins bound at RE in MATa (but not MATα) comes into contact with DSB-induced, casein kinase II-dependent phosphorylated threoninines in proteins that bind near the DSB ends. This association effectively tethers the nearby HML locus close to MAT and facilitates its use in MAT switching. This tethering brings HML from its location 200 kb from MAT to within about 20 kb of the DSB, while HMR remains 100-kb distant, and can account for the strong preferential use of this donor. It is still not clear whether there are any other constraints preventing HML use in MATα cells or whether there are any facilitating sequences that aid HMR usage. Of course, this is hardly the entire story since RE also binds Swi4–Swi6 in domain C, in a cell-cycle-dependent fashion. The activity of the entire RE is likely to be substantially more elaborate.

UNANSWERED QUESTIONS

Although MAT switching is probably the best-understood example of repair of a DSB by homologous recombination, there are still many aspects of the process that are not well understood. Recent studies have shown that a broken chromosome explores a larger fraction of the nuclear volume (233, 236), but how this affects repair is still not clear. The manner in which the Rad51 filament locates its partner is also a matter of active investigation (237, 238). The roles of several chromatin remodelers in gaining access to heterochromatic donor sequences also remain to be elucidated, and the overlapping roles of several DNA polymerases still need to be untangled. Indeed, recent evidence has shown that the repair process itself is surprisingly mutagenic (204), suggesting that the repair DNA synthesis process is quite different from the normally processive and accurate chromosomal replication. Finally, although it is evident that RE brings HML into proximity of the DSB, the relevant target sites (phosphorylated residues on proteins near the DSB) have yet to be identified. As some of these questions are answered, there will be new issues raised by the continuing advances in our ability to study molecular events in vivo in ever greater resolution.
REFERENCES


Mating-type Gene Switching in *Saccharomyces cerevisiae*


Lee and Haber


Mating-type Gene Switching in *Saccharomyces cerevisiae*


