A homolog of male sex-determining factor SRY cooperates with a transposon-derived CENP-B protein to control sex-specific directed recombination

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Schizosaccharomyces pombe cells switch mating type by replacing genetic information at the expressed *mat1* locus with sequences copied from *mat2-P* or *mat3-M* silent donor loci. The choice of donor locus is dictated by cell type, such that *mat2* is the preferred donor in *M* cells and *mat3* is the preferred donor in *P* cells. Donor choice involves a recombination-promoting complex (RPC) containing Swi2 and Swi5. In *P* cells, the RPC localizes to a specific DNA element located adjacent to *mat3*, but in *M* cells it spreads across the silent mating-type region, including *mat2-P*. This differential distribution of the RPC regulates nonrandom choice of donors. However, cell-type-specific differences in RPC localization are not understood. Here we show that the *mat1-M*-encoded factor Mc, which shares structural and functional similarities with the male sex-determining factor SRY, is highly enriched at the *swi2* and *swi5* loci and promotes elevated levels of RPC components. Loss of Mc reduces Swi2 and Swi5 to levels comparable to those in *P* cells and disrupts RPC spreading across the *mat2/3* region. Mc also localizes to loci expressed preferentially in *M* cells and to retrotransposon LTRs. We demonstrate that Mc localization at LTRs and at *swi2* requires Abp1, a homolog of transposon-derived CENP-B protein and that loss of Abp1 impairs Swi2 protein expression and the donor choice mechanism. These results suggest that Mc modulates levels of recombination factors, which is important for mating-type donor selection and for the biased gene conversion observed during meiosis, where *M* cells serve as preferential donors of genetic information.

development | transposition | directionality | yeast

Directed recombination between alternative DNA sequences plays a critical role in cellular differentiation in a variety of systems. Examples of such recombination events include the lineage and developmental stage-specific V(D)J recombination in the immune system of mammals (1) and mating-type switching in the yeasts *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* (2–5). In these distantly related yeast species, cells alternate between two distinct mating-type alleles by copying genetic information from one of the two silent donor loci to the active mating-type locus via highly orchestrated recombination processes (6–8).

The mating-type region of *S. pombe* contains three linked loci—*mat1*, *mat2*, and *mat3*—located on chromosome 2. In wild-type homothallic strains, designated *h*⁹⁰, *mat2* is located ~17 kb centromere-distal to *mat1*, whereas *mat3* is separated from *mat2* by an 11-kb interval (Fig. 1) (8, 9). The mating type of a haploid cell is determined by the presence of *P* or *M* information at *mat1* (8). Each allele consists of two divergently transcribed genes (10). *mat1-P* contains the *Pc* and *Pi* genes, and *mat1-M* contains the *Mc* and *Mi* genes. *mat2* and *mat3* contain the same genetic information as *mat1-P* and *mat1-M*, respectively, but are maintained in a silent state. These silent mating-type cassettes are embedded in a 20-kb heterochromatin domain surrounded by inverted repeat (*IR-R* and *IR-L*) boundary elements (11). Heterochromatin assembly in *S. pombe* involves factors that are conserved in higher eukaryotes. Methylation of histone H3 lysine 9 (H3K9me) by Clr4/Suv39h provides binding sites for chromodomain proteins, including the HP1 proteins Swi6 and Chp2 (12). H3K9me and chromodomain proteins are

enriched throughout the silent *mat2/3* interval and provide a recruiting platform for various effectors, including silencing activities and proteins involved in mating-type switching (12, 13).

Mating-type switching is initiated by double-strand break at *mat1* that is repaired by a gene conversion event involving the copying of information from *mat2* or *mat3* (8, 14, 15). Importantly, the choice of donor is nonrandom (16) and is determined by the location of the donor cassettes on the chromosome rather than by their genetic content (5, 8). *mat1-M* cells favor *mat2-P* as a donor, whereas *mat1-P* cells favor *mat3-M* as a donor locus. Heterochromatin proteins, in particular Swi6/HP1, aid in donor choice (5, 9). Factors that affect Swi6 localization at the *mat* locus impair mating-type switching (17–21). Swi6 facilitates cell-type-specific spreading of a recombination-promoting complex (RPC) (18). In *P* cells, the RPC binds to a Swi2-dependent recombination enhancer element (*SRE*) adjacent to the *mat3-M* locus (18). However, in *M* cells, the RPC spreads across the heterochromatin domain, including the *mat2-P* locus. The components of the RPC include Swi5, a protein involved in recombination/repair (22, 23), and an AT-hook protein Swi2 (18, 22). In addition to loading the RPC at the *SRE*, Swi2 facilitates its spreading through association with Swi6 (18). The differential spreading of the RPC at the *mat* locus suggests a potent regulatory mechanism by which cell type (*M* or *P*) dictates donor selection during mating-type switching.

In this study, we address the mechanism that regulates cell-type-specific spreading of the RPC. We report that *mat1-Mc*, related to the mammalian sex-determining factor SRY (24–26), localizes to the promoter regions of the *swi2* and *swi5* loci. Mc is required for elevated levels of RPC components in *M* cells, compared with those in *P* cells, and its loss results in defective spreading of the RPC from the *SRE* across the *mat2/3* interval. Mc also binds loci implicated in sexual differentiation, as well as retrotransposon LTRs and loci encoding noncoding RNAs. Interestingly, Mc localization at LTRs and selected gene promoter regions requires a CENP-B homolog, Abp1, which also affects Swi2 expression in *M* cells. Our results suggest a mechanism for cell-type control of donor choice during mating-type switching and offer an explanation for the mating-type-related bias of gene conversion observed in other parts of the *S. pombe* genome (27).

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Fig. 1. Schematic representation of the *S. pombe* mating-type locus. The three mating-type cassettes—*mat1*, *mat2*, and *mat3*—are shown. Each allele consists of two divergently transcribed genes. *mat1-P* contains the *Pc* and *Pi* genes and *mat1-M* contains the *Mc* and *Mi* genes. *mat2* and *mat3*, which contain the same information as *mat1-P* and *mat1-M* and serve as donors of genetic information during *mat1* switching, are maintained in a silent state. *IR-L* and *IR-R* indicate boundary elements that surround the heterochromatin domain. *cenH* serves as an RNAi-dependent heterochromatin nucleation center (50). The black rectangle adjacent to *mat3* represents the *SRE*.

Results

Swi5 Affects Directionality of Mating-Type Switching. Swi5 affects mating-type switching (28, 29), but its role in donor choice and its relationship to other factors involved in this process have not been studied. We determined the effects of single and double mutants on mating-type switching in a homothallic (*h⁹⁰*) strain using an iodine-staining assay (Fig. 2*A* and *B*). Cells that switch efficiently to the opposite mating type form colonies with a homogenous distribution of *M* and *P* cells and can thereby readily mate and sporulate, resulting in a dark iodine staining. In contrast, cells that switch their mating type with a lower efficiency form lightly stained colonies due to poor mating and sporulation within the colony. As expected, a *swi5Δ* mutant produced lightly stained colonies with a sectored/mottled staining pattern (Fig. 2*A*) (28, 29). Consistent with Swi2 and Swi5 acting together as components of the RPC, *swi2 swi5* double mutants showed

mating-type switching defects similar to those displayed by the individual single mutants (Fig. 2*B*). To confirm that reduced iodine staining in *swi5Δ* was due to defective utilization of one or both donor loci, we analyzed *mat1* genetic content by multiplex PCR using mating-type allele-specific (i.e., *mat1-P* or *mat1-M*) primers. Wild-type strains efficiently use both donors, as indicated by the almost equal intensity bands representing *mat1-P* and *mat1-M* (Fig. 2*A* and *B*). However, *swi5Δ* or *swi2Δ swi5Δ* cells were predominantly of the *P* mating type (Fig. 2*B*), implying that *mat2-P* is the preferred donor in these mutants.

Loss of Swi6 compromises the spreading of Swi2 from the *SRE* to the surrounding sequences. As a result, *swi6* mutants predominantly use *mat3-M* as a donor, resulting in reduced iodine staining (5, 18, 28) (Fig. 2*A*). However, cells containing a *swi2 swi6* double-mutant combination show elevated heterologous switching and iodine staining (18). This is believed to be caused by the random utilization of donors in cells lacking both RPC and heterochromatin structure at the *mat* locus. *swi5 swi6* double mutants showed a significant increase in switching to the opposite mating type, compared with *swi5* or *swi6* single mutants (Fig. 2*A*). The effects of *swi5Δ* on mating-type switching were confirmed in *h⁹⁰* background cells, in which the contents of the donor cassettes have been swapped (5) (Fig. 2*C*). Because cells select a donor on the basis of the location of the silent cassette, switching in *h⁹⁰* cells culminates in the replacement of *mat1* information with the same allele (5). Deletion of *swi5* alone or in combination with the *swi6* mutant had a predicted effect on donor choice, consistent with the results from experiments using *h⁹⁰* cells (Fig. 2*C*). Thus, Swi5 is a critical factor involved in the nonrandom utilization of donor mating-type loci, irrespective of the mating-type configuration.

Mc Localizes to *M*-Specific Genes, Retrotransposon LTRs, and Non-coding RNA Loci. Because the spreading of Swi2 and Swi5 occurs specifically in *mat1-M* cells, addressing the mechanism by which cell type regulates the spread of the RPC is critical for understanding the directionality of switching. We therefore studied the roles of mating-type genes, in particular *mat1-M*, in the process of mating-type switching. Of the two genes in the *mat1-M* cassette, *Mi* is induced specifically during meiotic induction (10). However, *Mc* is expressed during vegetative growth (10) and might have a role in the directionality of switching. Like its SRY homolog (26), *Mc* belongs to the family of HMG-box proteins (24, 30) that modulate expression of target loci (31). As a first step toward addressing the possible functions of *Mc* in mating-type switching, we constructed a strain expressing a myc epitope-tagged *Mc* (*Mc-myc*). The tagged *Mc* is expressed under the control of native regulatory elements. The stable *mat1-M* (*mat1Msm0*) (32) cells expressing *Mc-myc* mated efficiently with opposite mating-type (*mat1-P*) cells (Fig. S1), suggesting that the tagged protein is functional.

We used the strain expressing *Mc-myc* to perform genome-wide localization of *Mc*. Chromatin immunoprecipitation coupled with DNA microarray (ChIP-chip) analyses showed that *Mc* binds to specific sites on all three chromosomes, although no major peaks mapped to the *mat* locus (Fig. 3*A*). *Mc* binding occurred preferentially in intergenic regions, in particular in between divergently transcribed loci. *Mc* binding was detected upstream of several genes that are expressed in an *M* cell-type-specific manner (Fig. 3 and Fig. S2). These included genes such as *mfm2* and *mam1* that are involved in the production and secretion of *M* factor, respectively (33, 34), and *mam3*, which encodes the *M* cell-type-specific agglutinin that allows specific aggregation between cells of opposite mating type (35). Interestingly, *Mc* was found to be enriched at the promoter region of *scm3* (Fig. 3*B*), which encodes a chaperone involved in loading of the centromere-specific histone H3 variant, CENP-A (36–38). *Mc* likely induces the expression of this locus because a previous study has shown that *scm3* is expressed at higher levels in *M* cells, compared with *P* cells (35). Surprisingly, we also

Fig. 2. Swi5 regulates the directionality of mating-type switching. (A) Effect of *swi5Δ* and *swi6-115* on mating-type switching in the *h⁹⁰* background. (Upper) Diagram of the mating-type region in *h⁹⁰* strains. (Lower Left) Iodine-staining phenotypes of colonies formed by indicated strains. Colonies were replicated onto Edinburgh Minimal Medium and allowed to grow at 25 °C for 3 d before staining with iodine vapors. (Lower Right) Effects of *swi5Δ*, *swi6-115*, or *swi5 swi6* mutations on the predominant mating type adopted by *h⁹⁰* cells, determined by multiplex PCR using primers to amplify *mat1-P* and *mat1-M* sequences simultaneously. The *P/M* mating-type ratio is shown beneath each lane. (B) The same experiments were performed using *swi2Δ*, *swi5Δ*, and *swi2 swi5* double mutants. (C) The same experiments were performed using *swi5Δ*, *swi6-115*, or *swi5 swi6* mutations in the *h⁹⁹* background.

Fig. 3. Genome-wide mapping of Mc. (A) Chromosomal distribution profiles of Mc. Schematic diagrams of *S. pombe* chromosomes are shown above the plots. ChIP-chip relative enrichments of Mc-myc are plotted. Selective peaks of Mc are indicated. LTR, long terminal repeat; wtf, with Tf; ncRNA, noncoding RNA. (B) Mc localization at promoter regions of indicated genes.

found Mc binding upstream of the *map2* and *map4* genes (Fig. S2) that express specifically in *P* cells (35, 39), raising the possibility that Mc regulates expression of these loci.

These analyses also detected prominent Mc peaks near several loci that show no obvious induction in *M* cells (Fig. 3 and Fig. S2). In some of these cases, Mc localization corresponds to sites of noncoding RNA (ncRNA) production or antisense transcripts (Fig. 3A). Several Mc peaks correspond to WTF (With Tf) elements on chromosome 3 and solo LTRs dispersed across the genome (Fig. 3). Mc localization was detected only at a subset of LTRs (Fig. S3), suggesting that specific features of these LTRs or their chromosomal context influence Mc targeting to these loci.

mat1-Mc Is Highly Enriched at *swi2* and *swi5* Promoter Regions. Remarkably, we found that Mc is highly enriched at the promoter regions of *swi2* and *swi5* (Fig. 3A and B). This is significant because differential expression of Swi2 and Swi5 in *M* and *P* cells may help explain differences in the spreading of the RPC at the *mat* locus. To confirm this result, we performed conventional ChIP assays to detect Mc binding at these loci. Mc could be readily detected at both *swi2* and *swi5* loci (Fig. S4). Results of ChIP and ChIP-chip further demonstrated that Mc levels are considerably higher at *swi2* than at *swi5* (Fig. 3 and Fig. S4). These findings suggest a model in which mat1-Mc modulates levels of Swi2 and Swi5 proteins to promote differential spreading of the RPC observed at the *mat2/3* interval of *M* and *P* cells.

Swi2 and Swi5 Are Differentially Expressed in *M* and *P* Cells. We next investigated whether Swi2 and Swi5 are differentially expressed in *M* and *P* mating-type cells. For this purpose we constructed stable *M* (*mat1Msm10*) (32) or stable *P* (*mat1PA17*) (40) strains expressing myc-tagged Swi2 or Swi5. Western blot analyses with anti-myc antibody revealed that Swi2 is expressed at higher levels

in *M* cells, compared with *P* cells (Fig. 4A). In view of a previous report of comparable protein levels (18), the differential expression of Swi2 in *M* and *P* cells was further confirmed by using independent strains. Further analyses showed that Swi5 levels were also slightly higher in *M* cells than in *P* cells, although the difference is smaller compared with Swi2 (Fig. 4B). We next investigated whether Mc is responsible for the differential expression of the RPC components, in particular the pronounced difference in Swi2 levels between *M* and *P* cells. Mutation in *mat1-Mc* caused reduction in Swi2 and Swi5 levels (Fig. 4A and B), although the effect on Swi5 was less robust. This result is consistent with the relatively lower levels of Mc detected at the *swi5* locus, compared with the *swi2* locus (Fig. 3B and Fig. S4). Taken together with results presented above, our analyses demonstrate that Mc regulates the levels of RPC components. Moreover, the failure of the RPC to spread from the high-affinity *SRE* recruitment site near *mat3* to *mat2* is most likely attributable to lower Swi2 and Swi5 levels in *P* cells, compared with higher levels of these proteins in *M* cells.

Fig. 4. mat1-Mc affects Swi2 and Swi5 protein levels. Cell extracts from indicated strains were subjected to Western blot analyses. Swi2-myc (A) and Swi5-myc (B) are detected using a myc (9E10 or A14) antibody. Tat1 is shown as a loading control.

mat1-Mc Affects the Spreading of the RPC Across the Silent Mating-Type Region. We next sought to determine if *mat1-Mc* affects spreading of the RPC. For this purpose, we compared the distribution of the RPC across the silent *mat* region of strains that carry either the stable *mat1-M* wild-type allele or the *mat1-Mc⁻* mutant allele (*mat1Msmt0*). ChIP was performed to map the binding of Swi2-Myc across the entire *mat2/3* interval, as described previously (11, 18). As expected (18), Swi2 was found throughout the 20-kb silent mating-type domain in wild-type *mat1-M* cells, but its localization was attenuated and restricted largely to the region containing the *SRE* in *mat1-Mc⁻* mutant cells (Fig. 5). Defects in Swi2 expression and RPC spreading observed in *mat1-Mc⁻* mutant cells could be reversed by the ectopic expression of Mc from an inducible promoter (Fig. S5). Together, these results suggest that Mc, involved in proper expression of RPC components, affects the spreading of the RPC at the *mat2/3* region. As a control, we also tested the effects of mutation in *mat1-Pc*. *mat1-Pc⁻* mutant cells showed Swi2 distribution patterns similar to those in wild-type *mat1-P* cells (Fig. S6), with restricted RPC localization at the *SRE* (18). Therefore, the differential spreading of the RPC in *P* and *M* cells is not due to *mat1-Pc* inhibiting the spreading of this complex. Rather, *mat1-Mc* appears to actively promote RPC spreading by modulating levels of Swi2 and Swi5 proteins.

CENP-B Homolog Abp1 Facilitates Mc Localization at *swi2* and LTRs. Abp1 regulates donor choice and is required for RPC spreading across the silent *mat* region (29). Because our previous work has shown that Abp1 localizes to gene promoters and LTRs (41), we wondered if loss of Abp1 impacts Mc binding to these loci. ChIP-chip analyses were performed to compare Mc distribution in wild-type and *abp1Δ* cells. Remarkably, *abp1Δ* abolished Mc localization at LTRs and *swi2* (Fig. 6A). Abp1 is localized at the *swi2* promoter (Fig. 6A), in addition to its localization at LTRs (41),

and hence likely directly facilitates Mc localization at these loci. Conventional ChIP confirmed Abp1 involvement in Mc localization at *swi2* (Fig. 6B). Loss of Abp1 had no major impact on Mc distributions at *swi5* (Fig. S7). Similarly, Mc binding at the *map2* locus was not adversely affected, but *abp1Δ* showed considerable reduction of Mc enrichment at the promoter regions of *scm3*, *mam3*, and *mfm2* (Fig. S7). We next asked if Abp1 affects expression of Swi2 protein. Western analysis was performed to assay Swi2 levels in stable *M* (*mat1Msmt0*) cells carrying either wild-type or *abp1Δ* mutant alleles. *abp1Δ* resulted in a severe reduction in Swi2 levels (Fig. 6C). Together with previous data (29), these results suggest that Abp1 affects spreading of the RPC and directionality of mating-type switching at least in part by affecting Mc-mediated increase in Swi2 levels in *M* cells.

Discussion

Cell-type-specific selection of donor mating-type loci during mating-type switching in *S. pombe* offers an excellent system to study directed recombination and programmed interactions between distant chromosomal loci. The selection of donor locus during mating-type switching requires two primary components: heterochromatin assembled across the *mat2/3* region and the RPC (5, 9, 18, 21). In addition to promoting spreading of the RPC, heterochromatin is believed to impose structural organization at the *mat* locus that promotes preferential utilization of *mat2* (5, 9, 18). During the switching process triggered by double-strand break formation at *mat1* (8), heterochromatin-mediated organization guides the homology search process to scan *mat2* for accessibility. In *M* cells, the presence of the RPC at *mat2* aids the repair machinery in selecting this donor for recombination. However, in *P* cells, the absence of the RPC at *mat2* makes this locus less accessible for recombination, and the homology search continues until repair machinery encounters *mat3*, where the RPC bound to

Fig. 5. *mat1-Mc* is required for spreading of the RPC across the *mat2/3* region. ChIP analysis was performed with indicated strains to measure Swi2-Myc levels across the silent *mat* locus. DNA isolated from ChIP (α -myc, antibody 9E10) and whole-cell crude extract (WCE) were subjected to multiplex PCR to amplify DNA fragments from the *mat* locus, indicated by numbered bars, together with an *act1* fragment used as an internal control. The ratios of the intensities of *mat* and *act1* signals in the α -myc and WCE lanes were used to calculate the relative fold enrichment. Quantification of these results is plotted in alignment with the map of the *mat* locus.

Fig. 6. Abp1 affects Mc localization at *swi2* locus and Swi2 protein levels. (A) ChIP-chip was performed to map Mc distribution in *mat1Msmt0* strains carrying either wild type or the *abp1Δ* allele. Mc levels at *swi2* and LTR loci, which also show Abp1 enrichment (41), are plotted. (B) Conventional ChIP with anti-Mc antibody was performed to confirm loss of Mc binding at *swi2* in *abp1Δ* cells. Relative fold enrichments of Mc at *swi2* compared with the *leu1* control are shown. (C) Cell extracts from indicated strains were subjected to Western blot analyses using a myc (A14) antibody. Tat1 is shown as a loading control.

SRE promotes recombination. The results presented in this study provide support for this model and offer insights into the mechanism for differential localization of the RPC in *M* and *P* cells.

We show that Swi5 acts in a manner similar to Swi2 to promote proper utilization of donors during mating-type switching (Fig. 2) (18). Loss of Swi5 impairs recombination at donor loci, allowing heterochromatin-facilitated interactions between *mat1* and *mat2-P* to dominate. As a result, colonies formed by *swi5Δ* predominantly contain *P* cells (Fig. 2). In contrast, *swi6* mutants are defective in both heterochromatin-mediated preferential interaction between *mat1* and *mat2* and in the spreading of the RPC in *M* cells (5, 9, 18). In this situation, where RPC localization is restricted to *SRE* in both *P* and *M* cells, *mat3-M* is the preferred donor (Fig. 2) (5, 9, 18). However, defects in heterochromatin and loss of the RPC in *swi5 swi6* double-mutant cells confer equal probability that *mat2-P* and *mat3-M* serve as donors, regardless of the cell type (Fig. 2), albeit at reduced frequency. These findings are further supported by experiments performed using *h⁹⁹* cells (Fig. 2C). Together, these results support that Swi2 and Swi5 act together to promote nonrandom utilization of donors via a process that also involves heterochromatin formation in the *mat2/3* region.

A key feature of the donor choice mechanism is that the spreading of the RPC from the *SRE* entry site occurs in *M* cells but not in *P* cells (18). We show that *mat1-Mc* is required for the spreading of the RPC. Because HMG-box proteins perform architectural roles (31), *Mc* could potentially affect RPC distribution through structural organization of the *mat* locus. However, *Mc* also localizes to *swi2* and *swi5* loci and is required for elevated levels of RPC components in *M* cells (Figs. 3 and 4). Importantly, the localization of *Mc* at *swi2* requires a CENP-B homolog, Abp1, the loss of which impairs Swi2 protein expression (this study) and RPC spreading (29). These results suggest that, in addition to silencing retrotransposons, CENP-B binding in different chromosomal contexts recruits factors important for other chromatin transactions, such as transcription (41). Abp1 bound to the *swi2* promoter region facilitates preferential targeting of *Mc*, which normally binds an *M*-box sequence motif to activate *M*-specific genes by recruiting Ste11, a key transcription factor in the sexual differentiation pathway (24, 30, 42). Thus, Abp1-dependent or -independent recruitment of *Mc* to *swi2* and *swi5*, respectively, might stimulate Ste11-dependent expression of these loci. Indeed, Ste11 is required for a subtle increase in *swi5* transcript levels in *M* cells (35). However, considering that the HMG box of SRY-related proteins resembles RNA-binding domains, and that these factors have roles in RNA metabolism (43, 44), it is possible that *Mc* acts in a similar manner to modulate Swi2 and Swi5 protein levels. Indeed, promoter-associated transcription factors have been shown to affect mRNA processing (45). In any case, the elevated levels of the RPC ultimately translate into its increased association with the chromatin and spreading, presumably in a manner similar to dosage-dependent spreading of heterochromatin proteins (11).

It is noteworthy that Abp1 cooperates with an SRY homolog, *Mc*, to regulate directed recombination during mating-type switching. In addition to highlighting the crosstalk between the sexual differentiation machinery and transposon-derived CENP-B proteins, these results raise the possibility that mechanisms involved in propagation of transposons have been co-opted for mating-type switching and sexual differentiation. An *Mc*-mediated increase in the levels of RPC subunits has wide-ranging

implications. For example, increased association of Swi5 with chromatin in *M* cells may account for preferential utilization of the chromatids of *mat1-M* parents as donors of genetic information during meiotic gene conversion (27). This mating-type-based bias in selection of donor alleles, which shares parallels with the phenomenon of meiotic drive described in higher eukaryotes, requires Swi5 protein (27). These results suggest that sex-specific differences in a key factor, induced by *mat1-Mc*, form the basis for preferential propagation of alleles and meiotic drive.

Our analyses detected *Mc* enrichment upstream of several loci that are expressed preferentially in *M* cells, including *scm3* gene. *Scm3* is related to mammalian holiday junction-recognizing protein (46) and could potentially act together with Swi2-Swi5 to promote mating-type switching. We also found *Mc* localizing to *map2* and *map4*, which are expressed specifically in *P* cells (35, 39). Both of these genes possess long 5' UTRs that contain several *M* boxes. Whether *Mc* affects expression of these loci remains to be investigated. However, it is possible that *Mc* mediates repression of these *P*-specific loci, in addition to activating *M*-specific loci, to establish mating-type-specific expression profiles. Another intriguing observation is that *Mc* localizes to sites of noncoding RNA production and several LTRs. Moreover, *Mc* binding at LTRs requires Abp1, which is also enriched at these loci (41). Further detailed investigation is needed to explore the significance of *Mc* localization at LTRs and ncRNA loci. However, it is tempting to speculate that *Mc* affects the expression of these loci, which could potentially have regulatory roles in establishing an *M* cell-type-specific gene expression program. In sum, the results presented in this study suggest that *Mc* targets several loci and that it regulates the levels of RPC components critical for proper choice of donor loci during mating-type switching.

Materials and Methods

Yeast Strain. Strains containing *swi5Δ*, *Swi5-myc*, and *Mc-myc* alleles were constructed by using a PCR-based module method (47). The construction of *mat1-Mc⁺* or *mat1-Pc⁺* mutant strains is shown in Fig. S8. *swi2Δ*, *Swi2-myc*, and *abp1Δ* have been described previously (18, 41). Mutant *swi6* strains used in this study contain the missense *swi6-115* (W269R) allele that causes severe reduction in Swi6 protein levels (48).

Mating-Type Switching Assays. Iodine-staining procedure was used to estimate the efficiency of switching as described previously (9, 49). Switching efficiency was also examined by a quantitative PCR assay. In this assay, DNA prepared from wild-type and mutant cells is used to perform multiplex PCR to determine the genetic content at the *mat1* locus, as described previously (18). PCR fragments corresponding to *mat1-P* and *mat1-M* alleles were resolved on polyacrylamide gels and quantified using Phosphorimager.

ChIP and ChIP-chip. ChIP and ChIP-chip using myc antibody (A-14; Santa Cruz) or affinity-purified rabbit *Mc* antibody raised against full-length recombinant protein (this study) were performed as described previously (13). Enrichment values were corrected for nonspecific signals from control samples with the same antibodies used to perform ChIP. ChIP analysis was carried out essentially as previously described (11, 18). Microarray data are available at the National Center for Biotechnology Information's Gene Expression Omnibus repository under accession number GSE32996.

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