Going in the Right Direction: Mating-Type Switching of *Schizosaccharomyces pombe* Is Controlled by Judicious Expression of Two Different *swi2* Transcripts

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ABSTRACT *Schizosaccharomyces pombe*, the fission yeast, cells alternate between P- and M-mating type, controlled by the alternate alleles of the *ma*ting-*t*ype locus (*mat1*). The *mat1* switching occurs by replacing *mat1* with a copy derived from a silenced "donor locus," *mat2P* or *mat3M*. The mechanism of donor choice ensuring that switching occurs primarily and productively to the opposite type, called directionality, is largely unknown. Here we identified the *mat1-Mc* gene, a mammalian sex-determination gene (*SRY*) homolog, as the primary gene that dictates directionality in M cells. A previously unrecognized, shorter *swi2* mRNA, a truncated form of the *swi2*, was identified, and its expression requires the *mat1-Mc* function. We also found that the *abp1* gene (human CENPB homolog) controls directionality through *swi2* regulation. In addition, we implicated a *cis*-acting DNA sequence in *mat2* utilization. Overall, we showed that switching directionality is controlled by judicious expression of two *swi2* transcripts through a cell-type-regulated dual promoter. In this respect, this regulation mechanism resembles that of the *Drosophila* sex-determination *Slx* gene.

FISSION yeast is primarily a haploid single-celled organism whose cells exist as one of two cell/mating types, called P (plus) or M (minus). Under nitrogen-starvation growth conditions, haploid cells of opposite type mate and the resulting diploid zygotic cell undergoes meiosis to produce two *mat1P* and two *mat1M* haploid spore segregants. This 2:2 Mendelian segregation pattern observed in meiosis shows that cell type is controlled by two alleles of a single *mat1* locus. However, the culture starting from a single cell of either type contains a roughly equal proportion of cells of both mating types. This cell-type change is due to the efficient mating-type switching phenomenon, called homothallism, which operates during mitotic growth of the culture (Arcangioli and Thon 2003; Egel 2005; Klar 2007). The mating-type switching process replaces the existing *mat1* locus through the gene conversion process with a copy derived from one of the two epigenetically silenced "donor loci," *mat2P* and *mat3M* (Figure 1).

The switching process is tied to the DNA replication cycle so that only one in four "grandchildren" of a nonswitchable cell switches in ~90% of cell pedigrees (Miyata and Miyata 1981; Egel and Eie 1987; Klar 1990a). The switching process is initiated by a DNA strand-specific epigenetic entity, called an imprint (Klar 1987; Klar 1990b), found at the junction of the homology-box H1 and the *mat1* allele-specific sequence (Figure 1). The imprint is either a strand- and sequence-specific nick and/or two ribonucleotides incorporated in DNA (Beach and Klar 1984; Egel *et al.* 1984; Nielsen and Egel 1989; Kaykov and Arcangioli 2004; Vengrova and Dalgaard 2004). Three genes (*swi1*, *swi3*, and *swi7*) are required for synthesis of the imprint (Egel *et al.* 1984; Singh and Klar 1993; Dalgaard and Klar 2000). The *swi7*, encoding DNA polymerase α, is an essential gene for cellular viability (Singh and Klar 1993). The *swi1* and *swi3* encode replication fork pause factors (Dalgaard and Klar 2000). DNA replication of

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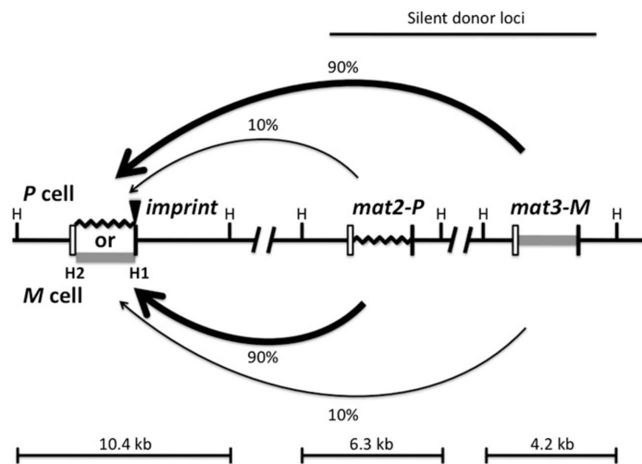

Figure 1 The directionality phenomenon of mating-type switching in fission yeast. The diagram shows *mat1*, *mat2P*, and *mat3M* genes located from left to right in chromosome 2. The *mat1* locus is expressed and it confers cell type, while *mat2P* and *mat3M*, located in the silenced region, act only as donors for providing copies of genetic information for *mat1* switching. The *mat1* can be either *P* (black zigzag line, representing 1104-bp-long DNA sequence) or *M* (shaded bar, 1128 bp). Each *mat* cassette is flanked by the homology regions, boxes H2 (left open box, 135 bp) and H1 (right solid box, 59 bp), which are used for switching-promoted recombination. The imprint site (solid triangle) is located at the junction of the *mat1* allele-specific and the H1 box sequences. The donor preference is determined by the *mat1* cell type; *mat1-P* cells choose *mat3* and *mat1-M* cells choose *mat2*, both with ~90% preference (thick arrow) over the other, less-preferred (~10%, thin arrow) donor locus. The *Hin*dIII restriction enzyme sites (H) flanking each cassette are shown. Digestion of yeast genomic DNA with the restriction enzyme generates three fragments of indicated sizes, each containing a specific *mat* locus. The figure is not drawn to scale.

the imprinted strand at *mat1* is thought to create a transient double-strand break (DSB) in the resulting chromatid. The DSB is repaired by recombination by copying P or M information from one of the two donor loci through the synthesis-dependent strand-annealing (SDSA) mechanism (Arcangioli and De Lahondes 2000; Yamada-Inagawa *et al.* 2007). This strand-specific imprinting/segregation mechanism (Klar 1987; Klar 1990b) explains the generation of one-in-four-granddaughters switching pattern observed in cell pedigrees (Miyata and Miyata 1981; Egel and Eie 1987; Klar 1990a). Interestingly, a similar mechanism of asymmetric cell division, through epigenetic differentiation plus selective segregation of sister chromatids in mitosis, has been recently suggested for generating neuronal bilateral asymmetry in *C. elegans* (Nakano *et al.* 2011).

Interestingly, the donor locus selection is not random; *mat1P* prefers *mat3M*, and *mat1M* prefers the nearby *mat2P* in ~90% of switches (Figure 1). Consequently, switches to the opposite type occur predominantly in standard *mat2P* and *mat3M*-containing strains, called h^{90} strains (for homothallic, ~90% sporulation). This donor preference, called directionality of switching, is not because cells prefer the heterologous information-containing donor locus for switching, but it is because P cells prefer *mat3* and M cells prefer *mat2*, regardless of the donors' genetic content. The directionality control was demonstrated by swapping the donor loci genetic content to *mat2M* and *mat3P* (called h^{09} genotype). Notably, h^{09} cells switched preferentially by futile homologous information replacement (Thon and Klar 1993). Thus, the directionality control dictates *mat1P* to switch preferentially by choosing *mat3*, and *mat1M* by choosing *mat2*, regardless of the donors' genetic content.

Several genes have been shown to effect directionality. Switching-recombination proteins Swi2 and Swi5 physically interact with each other and directly bind to the *mat2/3* region to mediate switching (Akamatsu *et al.* 2003; Jia *et al.* 2004). The distribution pattern of the Swi2/Swi5 complex is cell-type regulated: in P cells, Swi2/Swi5 localizes only to the *mat3* locus; in contrast, in M cells, it spreads to the *mat2* locus (Jia *et al.* 2004). The precise mechanism of the cell-type control on the Swi2/Swi5 spread in the *mat2/3* region is unknown, but the chromatin structure is thought to regulate the Swi2/Swi5 complex spreading because heterochromatin factors, such as Swi6, Clr4, Rik1, Sir2, Clr7, and Clr8, affect the mating-type switching efficiency (Ivanova *et al.* 1998; Shankaranarayana *et al.* 2003; Jia *et al.* 2004; Tuzon *et al.* 2004; Thon *et al.* 2005; Aguilar-Arnal *et al.* 2008). The fission yeast Abp1 (encoding autonomously replicating sequence-binding protein 1), one of the human centromere protein B (CENPB) homologs, has been reported to regulate the activation of DNA replication, to maintain genome integrity by forming heterochromatin at retrotransposons, and to protect replication forks during pausing (Murakami *et al.* 1996; Halverson *et al.* 1997; Okada *et al.* 2007; Cam *et al.* 2008; Zaratiegui *et al.* 2011). Interestingly, the Abp1 protein also controls switching directionality by regulating the Swi2/Swi5 spread on the *mat2/3* region. The mechanism by which Abp1 controls Swi2/Swi5 spread at donor loci is also unknown. However, because Abp1 binds within the donor region, it has been postulated that such binding might regulate directionality (Aguilar-Arnal *et al.* 2008; Cam *et al.* 2008).

In this study, we discovered that *mat1-Mc* and *abp1* genes are required for switching directionality. We showed that the *swi2* gene produces two independent but overlapping transcripts by engaging two different promoters in M cells, but only the longer form is produced in P cells. The previously unrecognized short *swi2* form (*i.e.*, *swi2S*) is the one that controls directionality by promoting the *mat2* donor locus selection in M cells. To some extent, such a two-promoter regulation mechanism resembles that of the sex-determination gene (*Sxl*) of *Drosophila*. Furthermore, we identified a *mat2P* *cis*-acting site (named Swi2-dependent recombinational enhancer 2, SRE2), which is critical for donor locus utilization for switching.

Materials and Methods

Strains, plasmids, and media

The *S. pombe* strains used in this study and their genotypes are listed in Table 1. The *mat1* locus-specific primers

Table 1 Fission yeast stains

Stain	Genotype	Reference
CY146	h^{90}, *leu1-32*, *ura4-D18*, *ade6-210*, *Δste11*::*KAN*	This study
CY148	h^{09}, *leu1-32 ura4-Dl8*, *ade6-210 Δste11*::*KAN*	This study
CY195	h^{90}, *mat3Mi*$^{+}$*mcop7*, *leu1-32*, *ura4-D18*, *ade6-216*, *his2*	This study
CY196	h^{09}, *mat2Mi*$^{+}$*mcop7*, *ade6-210*	This study
CY199	h^{09}, *mat3pcop5Pi*$^{+}$, *ade6-216*	This study
CY200	h^{90}, *mat3mifs15Mc*$^{+}$, *leu1-32*, *ura4-D18*, *ade-216*, *his2*	This study
CY202	h^{09}, *mat2mifs15Mc*$^{+}$, *ade6-210*	This study
CY204	h^{90}, *mat2Pc*$^{+}$*piop7*, *leu1-32*, *ura4-D18*, *his2*, *ade6-210*	This study
CY207	h^{09}, *mat3Pc*$^{+}$*piop7*, *leu1-32*, *ade6-216*	This study
CY212	h^{90}, *ΔSRE2*, *leu1-32*, *ura4-D18*, *ade6-210*, *his2*	This study
CY294	h^{90} *ΔSRE1*, *leu1-32*, *ura4-D18*, *ade6-210*, *his2*	This study
CY301	h^{90}, *mat2P*::*ura4*::*Δ121*, *leu1-32*, *ura4-D18*, *ade6-216*, *his2*	This study
CY303	h^{90}, *mat2P*::*ura4*::*Δ109*, *leu1-32*, *ura4-D18*, *ade6-210*, *his2*	This study
CY308	h^{90}, *mat2P*::*ura4*, *leu1-32*, *ura4-D18*, *ade6-216*, *his2*	This study
CY327	h^{90}, *leu1-32*, *ura4-D18*, *ade6.210*, *Δabp1*::*KAN*	This study
CY345	h^{90}, *leu1-32*, *ura4-D18*, *ade6.210*, *swi2L*::*ura4*	This study
CY347	*mat1M*, *Δmat2/3*::*LEU2*, *leu1-32*, *ura4-D18*, *ade6-216*, *swi2HA*	This study
CY348	*mat1P*, *Δmat2/3*::*LEU2*, *leu1-32*, *swi2HA*, *ura4-D18*, *ade6-216*	This study
PG19	h^{09}, *leu1-32*, *ura4-D18*, *ade6-210*	Thon and Klar (1993)
SP713	*mat1P*, *Δmat2/3*::*LEU2*, *leul-32*, *ura4*$^{-}$, *ade6-216*	Kelly *et al.* (1988)
SP714	*matlM*, *Δmat2/3*::*LEU2*, *leul-32*, *ura4*$^{-}$, *ade6-216*	Kelly *et al.* (1988)
SP715	*mat1Mi*$^{+}$*mcop7*, *Δmat2/3*::*LEU2*, *leul-32*, *ura4*$^{-}$, *ade6-216*	Kelly *et al.* (1988)
SP716	*mat1mifsl5Mc*$^{+}$ *Δmat2/3*::*LEU2*, *leul-32*, *ura4*$^{-}$, *ade6-216*	Kelly *et al.* (1988)
SP717	*mat1Pc*$^{+}$*piop7*, *Δmat2/3*::*LEU2*, *leul-32*, *ura4*$^{-}$, *ade6-216*	Kelly *et al.* (1988)
SP718	*mat1pcop5Pi*$^{+}$ *Δmat2/3*::*LEU2*, *leul-32*, *ura4*$^{-}$, *ade6-216*	Kelly *et al.* (1988)
SP976	h^{90}, *leu1-32*, *ura4-D18*, *ade6-210*	Thon and Klar (1993)
SPA160	h^{90}, *leu1-32*, *ura4-D18*, *Δhis3*, *Δabp1*::*KAN*	Aguilar-Arnal *et al.* (2008)
SP2534	h^{90}, *mat1-Mc-fg2534*, *mat2P*::*ura4*, *ura4-D18*, *leu1-32*, *ade6-210*, *hsp1*	This study
SP2535	h^{90}, *mat1-Mc-fg2535*, *mat2P*::*ura4*, *ura4-D18*, *leu1-32*, *ade6-210*, *hsp1*	This study
SP2536	h^{90}, *mat1-Mc-fg2536*, *mat2P*::*ura4*, *ura4-D18*, *leu1-32*, *ade6-210*, *hsp1*	This study
SP2553	h^{90}, *mat1-Mc-fg2553*, *mat2P*::*ura4*, *ura4-D18*, *leu1-32*, *ade6-210*, *hsp1*	This study
SP2830	h^{90}, *mat1-Mc-fg2830*, *mat2P*::*ura4*, *ura4-D18*, *leu1-32*, *ade6-210*, *hsp1*	This study
SP2832	h^{90}, *mat1-Mc-fg2832*, *mat2P*::*ura4*, *ura4-D18*, *leu1-32*, *ade6-210*, *hsp1*	This study
SP2833	h^{90}, *mat1-Mc-fg2833*, *mat2P*::*ura4*, *ura4-D18*, *leu1-32*, *ade6-210*, *hsp1*	This study
SP2834	h^{90}, *mat1-Mc-fg2834*, *mat2P*::*ura4*, *ura4-D18*, *leu1-32*, *ade6-210*, *hsp1*	This study
YA664	h^{90}, *leu1-32*, *ura4-D18*, *his3-D1*, *arg3-D1*, *swi2HA*	Akamatsu *et al.* (2003)

(TAAGTGGGATGAGTGCTTGCTTTG and AGTGAGTATAT TATGGTAGGGAGTGCGTAGCG) were used to amplify the *mat1-mc*$^{-}$ mutants. The mating-type donor region is silenced and prohibited from homologous recombination by an epigenetic mechanism (Egel 1984). To introduce mutations in donor loci by DNA-mediated transformation, we used a *clr1-5* genotype that permits homologous recombination in the *mat2/3* region (Thon and Klar 1992). All subsequent studies were conducted with strains from which the *clr1-5* mutation was removed by genetic crosses. All mutations were generated by standard polymerase chain reaction (PCR) methods and confirmed by PCR or sequence analysis.

To construct plasmids *pREP3-swi2L*-HA, *pREP3-swi2S*-HA, and *pREP3-swi2S*, PCR fragments amplified from genomic DNA of YA664 (Akamatsu *et al.* 2003) or from a wild-type strain were inserted into the *Bal*I–*Bam*HI sites of the *pREP3* vector (Maundrell 1993).

Strains were cultured in yeast extract with supplementary adenine-containing medium (YEA). For switching, sporulation, Northern blot, and Western blot experiments, Edinburgh minimal medium, supplemented with auxotrophic requirements of each strain (PMA), was used. Media and other standard conditions for growth and genetic analysis were employed as described previously (Moreno *et al.* 1991).

Genomic DNA preparation and Southern blot analysis

Genomic DNA preparation and Southern blot analyses with *Hin*dIII digested yeast genomic DNA were carried out as previously described (Moreno *et al.* 1991). The 10.6-kb *mat1P*-containing *Hin*dIII fragment was used as the probe.

Total RNA preparation and Northern blot analysis

Total RNA was extracted and purified using the TRIzol Plus RNA purification system (Invitrogen, no. 12183-555). Each lane was loaded with 20 μg total RNA of each sample for Northern blot analysis using the kit provided by Ambion (no. AM1940). The *Swi2*P probe consisted of the PCR fragment copied from genomic DNA with primers (ATGCCCATTGTG ATGACCACCCA and AGGTAGAAAGACAGACATTAGAATA GTTTGG). The *Swi2* 5′P probe consisted of the PCR product copied from the yeast cDNA library with primers (TGTATTT

Table 2 ***mat1-mc⁻*** **mutations**

Mutation name	Mutation in *Mc* RNA	Change in Mc protein
2534, 2832	T to C at 20	ATG to ACG in initiation codon
2535	G to A at 21	ATG to ATA in initiation codon
2536, 2553	G to A at 84	Trp to TGA stop codon in codon 22
2833	C insertion at 230	Frameshift in codon 71
2834	C deletion at 165	Frameshift in codon 49
2830	T to C at 443	Trp to Arg in codon 141

CACAAAAGAGGGAGATTCAGT and cDNA 5′-end adaptor primer AAGCAGTGGTATCAACGCAGAGT).

RACE analysis

The mRNA was enriched from total yeast RNA with the Qiagen mRNA kit (Qiagen, no. 72022). A total of 200 ng mRNA was used for each RACE experiment. RACE analysis was performed according to instructions in the RACE cDNA amplification kit (Clonetaq, no. 634923). The Swi2-specific primers used for 5′ and 3′ RACE comprised TTACACTCCC CCTAAGTCTGCTACCTC and GGAGGTAGCAGACTTAGGGG GAGTGTAA, respectively.

Analysis of mat1 locus M/P ratio

To determine the genetic content at the *mat1* locus, a quantitative multiplex PCR procedure was adapted from previous publications (Jia *et al.* 2004; Aguilar-Arnal *et al.* 2008). PCR-amplified products were run on a 1.5% agarose-TBE gel stained with 0.5 g/ml ethidium bromide and captured using a Typhoon scanning machine. Imagequant software was used for quantitative analysis.

Protein extraction and immunoblotting

Strain SP976 cells harboring pREP3-*swi2L*-HA, pREP3-*swi2S*-HA, or pREP3 plasmid was grown in PMA media lacking a leucine supplement for 1 day at 30°. The exponentially growing cells were collected by centrifugation, suspended in 5% trichloroacetic acid buffer, and disrupted by vortexing with glass beads. The extracted protein was subjected to immunoblotting. Mouse anti-HA antibody (Sigma, no. H9658) was used as the first antibody, and peroxidase-conjugated anti-mouse IgG antibody was used as the second antibody.

Results

Selection of mat1-mc⁻ mutations

Ascospores synthesize starch during meiosis and sporulation, while nonsporulating cells do not. Therefore, efficiently switching colonies growing on sporulation medium (pombe minimal adenine, PMA, medium) stain black when exposed to iodine vapors because they contain ascospores, but colonies defective in switching do not stain (Leupold 1955). Using the iodine-staining procedure to screen for mating-type switching-defective mutants from a strain containing the *hypersporulation 1* (*hsp1)* mutation (Michael *et al.* 1995), we found eight independent mutants with a reduced rate of switching. They were sterile for mating and also exhibited an unusual phenotype of repairing their mutations spontaneously. Both phenotypes might be explained should the inefficient *mat1* switching process operate in them to repair/heal their mutations. We postulated that the mutations were located at the *mat1* locus in regions that are replaced with wild-type donor information during the switching process. We also observed that in the *hsp1* genetic background the switched and healed cells mate readily on PMA medium because of their hypersporulation property, and the resulting zygotic asci do not grow further. As a consequence, only the *mat1* mutant cells multiply in PMA medium, making our mutation analyses possible despite the fact that mutations were subject to removal by the switching process (presented below). The DNA of the *mat1* locus of the mutants was amplified with *mat1*-specific primers and sequenced. The eight mutations represented six different sites in the *mat1-mc* gene (Table 2) (Kelly *et al.* 1988). Because the *Mc* gene is required for both mating and sporulation (Kelly *et al.* 1988), both the sterility and mutations repair by switching are explained by their location within the *mat1* cassette.

The mat1 genes are not required for mat1 imprinting

Although our procedure favored selection of mutant cells during growth on PMA solid medium, the persistence of mutations during growth of the culture suggested that the cells were defective in some aspect of the switching pathway. Specifically, we wanted to understand why the switching process had not readily repaired the *mat1-mc⁻* mutations. Recombination required for *mat1* switching is initiated with the synthesis of the *mat1* imprint (Figure 1). The first possibility we considered was that the *mat1-mc⁻* mutations might reduce the imprint level. That is, the Mc transcription factor might be required for the imprinting process. It is not known whether *mat1* genes influence the imprint level. The imprint forms a fragile DNA break site, resulting in a double-strand break (DSB) during the normal DNA extraction process, so the imprint level is usually evaluated by Southern blot analysis (Beach 1983; Beach and Klar 1984). Since our mutations were subject to repair during mitotic growth, for this analysis we employed an *mc⁻* mutant strain with deleted donor loci (Δ*mat2/mat3*) (Klar and Miglio 1986). Because of their donor loci deletion, such strains do not switch at all, and we could thus directly assess the role of Mc in imprinting without the complication of mutations disappearing due to switching-promoted repair

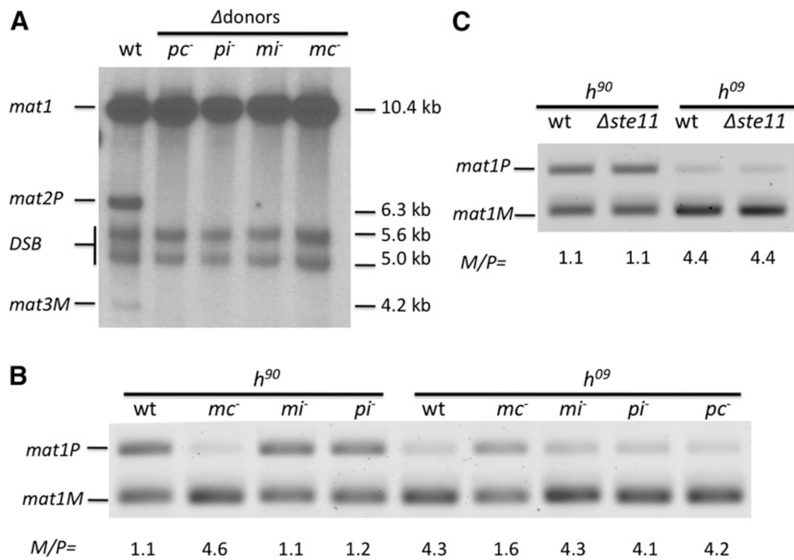

Figure 2 Effect of *mat1* mutations on *mat1* imprint and on directionality of switching. (A) Imprint level determined by Southern blot analysis. The wild-type (wt, SP976; see Table 2 for complete genotype) was the homothallic h^{90} strain, which contains all three cassettes diagrammed in Figure 1. The stocks with mating-type gene mutations in pc^- (SP718), pi^- (SP717), mi^- (SP716), mc^- (SP715) had been deleted for both donor loci; thus bands reflecting *mat2P* and *mat3M* genes were lacking in them. Such strains lacking donor loci cannot switch, and therefore stably maintain their *mat1* mutations. Genomic DNA was digested with *Hin*dIII and the blot was probed with *mat1P* gene-containing 10.6 kb long probe (Figure 1). Locations of the *Hin*dIII sites flanking each cassette are shown in Figure 1. The 5.6-kb and 5.0-kb bands result from the double-strand break (DSB) generated by shearing imprinted DNA during its preparation. The DSB reflects the level of *mat1* imprint (Beach and Klar 1984). (B) Effect of *mat1* genes mutations on directionality of h^{90} (wt, SP976; mc^-, CY195; mi^-, CY200; pi^-, CY204) and h^{09} (wt, PG19; mc^-, CY196; mi^-, CY202; pi^-, CY207; pc^-, CY199) strains. Quantitative multiplex PCR analysis of cultures showed M/P ratio of the *mat1* locus of strains in which mutations introduced in donor loci have been transposed to *mat1* through switching. The PCR reaction included one primer from outside the *mat1* allele-specific sequences and another one from the *mat1-P* or *mat1-M* allele-specific sequences (see *Materials and Methods*). The *mat1* allele-specific PCR product bands are indicated. (C) Effect of Δ*ste11* (h^{90}, CY146; h^{09}, CY148) on directionality. The *mat1* M/P ratio of cultures was determined as described above.

during our analysis. The imprint level observed in the mc^- mutant was similar to that of the wild-type control (Figure 2A). The *mat1P* encodes *mat1-Pc* and *mat1-Pi* genes, and *mat1-M* encodes *mat1-Mc* and *mat1-Mi* genes (Kelly *et al.* 1988). The other three mating-type gene (pc^-, pi^-, mi^-) mutants were also not affected in the imprinting process. These results suggest that none of the four mating-type genes is required for the imprinting step of the switching pathway (Figure 2A).

mat1-Mc gene controls the directionality of switching

We next entertained an alternative model for explaining the persistence of our mutations in *mat1-mc* mutant stocks; the *mat1-Mc* gene might dictate *mat2P* donor preference for switching the *mat1*-M allele. In this hypothesis, our *mat1-mc*$^-$ mutants might switch by a default mode, preferring the *mat3M* as a donor instead of *mat2P*; accordingly, homologous information would be transferred to the *mat1* locus. We previously showed that only a portion of the cassette information can be replaced by switching during the homologous cassette replacement, rather than always replacing the entire cassette (Yamada-Inagawa *et al.* 2007). Accordingly, each switch event might not have repaired *mat1-mc*$^-$ mutations. To determine whether the *Mc* gene dictates *mat2* utilization, we engineered a mc^- mutation in the *mat3M* donor locus. Cells of this culture should alternate between *mat1-P* and *mat1-mc*$^-$ alleles. The wild-type culture contains an equal proportion of *mat1P* and *mat1M* cells due to the efficient switching of both *mat1* alleles, either one to the opposite *mat1* allele. In contrast, according to our hypothesis, the mutant strain should predominate with *mat1-mc*$^-$ cells. To test this possibility, we analyzed the *mat1* locus M/P ratio by PCR as described previously (Jia *et al.* 2004; Aguilar-Arnal *et al.* 2008). For wild-type h^{90} cells, the *mat1P* amount was observed to be nearly equal to the amount of *mat1M*, as expected (Figure 2B). By comparison, the mc^- mutant exhibited much more *mat1M* content. This result suggested that *mat1-Mc* controls directionality by promoting *mat2* utilization in M cells.

We previously showed that donor preference depends on specific chromosomal location and not on the genetic content of donor loci (Thon and Klar 1993). To explore the directionality issue further, we checked the effect of a mc^- mutation on the *mat1* content of an h^{09} strain (*mat2-mc*$^-$ and *mat3P*) whose genetic contents have been swapped with respect to those of standard h^{90} cells. Here, the *mat1P* allele was greatly enriched in the *mat3-mc*$^-$ mutant in comparison to the wild-type cells (Figure 2B). This result confirmed that the *mat2* donor locus is used inefficiently in *mat1-mc*$^-$ h^{09} cells, a result similar to that presented above with h^{90} cells.

Similarly, we also determined whether the other three mating-type genes are involved in switching directionality. Our results showed that none of the three genes we tested (*pc*, *pi*, and *mi*) was involved in the directionality of h^{90} and h^{09} cells (Figure 2B). We did not check the *mat2-pc*$^-$ mutant in an h^{90} cell background because a previous report indicated that the *mat2-pc*$^-$ mutant does not affect the *mat1* locus M/P ratio (Ruusala 1991). Thus, the *mat1-Mc* gene is the only *mat1* gene that controls specific donor-locus choice in both h^{90} and h^{09} genetic backgrounds.

Mc is a known transcriptional factor regulating many M cell-type-specific genes in combination with another DNA-binding protein partner named Ste11 (Kjaerulff *et al.* 1997). Is Ste11 also required for switching directionality? We found that the Δ*ste11* mutation did not affect the *mat1* M/P ratio in both h^{90} and h^{09} backgrounds (Figure 2C). This result is

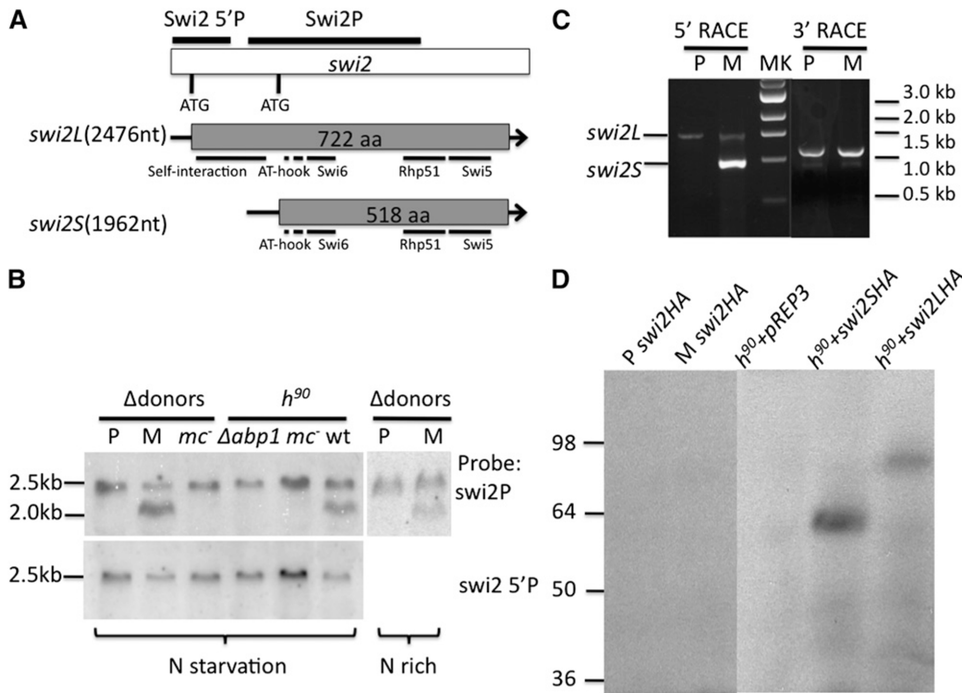

Figure 3 Characterization of the *swi2L* and *swi2S* products. (A) The *swi2* gene, mRNAs, and predicted proteins. Upper drawing shows the *swi2* gene's DNA structure indicated along with its two translation initiation ATG codons. The Northern blot probes, Swi2 5′P, Swi2P, we used are also indicated. The two lower drawings indicate mRNA and ORF length of *swi2L* and *swi2S* forms of the *swi2* gene. Both *swi2L* and *swi2S* predicted proteins share the same open reading frame (ORF) in regions where the genes overlap. The Swi2 protein domains, such as for other proteins interaction, self-interaction, and two AT-hook motifs, are marked (Akamatsu *et al.* 2003). The Swi2S lacks the self-interaction domain. The GenBank accession nos. for *swi2L* and *swi2S* are JQ308182 and JQ308183. (B) Northern blot of *swi2* transcripts. The cultures with indicated genotype were grown in PMA media (nitrogen-starvation condition) and YEA media (nitrogen-rich condition). Strains: P, SP713; M, SP714; wt, SP976; mc^- in h^{90}, CY195; mc^- in Δdonors, SP715; Δ*abp1*, spA160. (C) Rapid amplification of *swi2* cDNA ends (RACE). Results show agarose gel electrophoresis of RACE products obtained by amplifying 5′- and 3′-end products of *swi2* cDNA. (D) Western blot analysis of the Swi2L and Swi2S proteins. Analysis of the *swi2HA* tagged gene from the endogenous locus in donors-deleted P (CY347) and M (CY348) cells is presented in the left two lanes. The remaining lanes reflect analysis of *swi2L-HA* and *swi2S-HA* tagged genes that were expressed from a plasmid vector in h^{90} cells (SP976). The anti-HA antibody was used for Swi2 detection. The h^{90} wild-type culture harboring empty pREP3 vector was used as a negative signal detection control.

consistent with the notion that Mc uses a Ste11-independent pathway to control directionality. However, because the efficiency of switching cannot be determined directly in *ste11* mutants owing to their sterile phenotype, in this case only knowing their *mat1* M/P ratio is not sufficient to assess whether Ste11 controls directionality (see below).

A previously unknown, shorter swi2 (swi2S) transcript requires Mc and Abp1 factors for expression

Next, we addressed how Mc might control the switching direction. As described in the Introduction, assembly of the Swi2/Swi5 complex spreads to both donor loci in M cells, while it is localized at the *mat3* locus in P cells. The precise mechanism of this cell-type-specific spread of the Swi2/Swi5 complex is not known. Because mc^- mutants are defective in *mat2* donor choice, we considered the possibility that such mutants may be defective in the spreading of the Swi2/Swi5 complex at the *mat2/3* region. We therefore examined the *swi2* and *swi5* expression using Northern blot analysis of mRNA isolated from P and M cells, which lacked both donor loci and thus stably maintained their cell type (Klar and Miglio 1986). Analysis of *swi5* did not produce any clear signal, possibly due to its weak expression. *swi2* showed one transcript that was 2.5 kb long (*swi2L*, L for longer) in P cultures, plus another, shorter one that was 2.0 kb long (*swi2S*, S for shorter) in M cultures (Figure 3, A and B). Furthermore, the amount of *swi2S* transcript was about twice the amount of *swi2L* in M cultures. Most interestingly, the *swi2S* species was absent in the mc^- mutant. This result showed that expression of the *swi2S* transcript was Mc dependent. Furthermore, these modes of expression were unaffected whether or not the cells contained donor loci (Figure 3B). In addition, since Mc is partially repressed in nitrogen-rich medium (Kelly *et al.* 1988), the *swi2S* transcript level was reduced in rich medium in comparison to the level found in nitrogen-starved conditions (Figure 3B). We concluded that *Mc* controls the *swi2S* mRNA expression.

It was previously known that Abp1 regulates switching directionality, but the molecular mechanism of its action is unknown (Aguilar-Arnal *et al.* 2008). We found that the *swi2S* transcript was absent in the Δ*abp1* mutant. Collectively, these results led us to entertain a model in which both Mc and Abp1 factors affect directionality by regulating the *swi2S* gene expression.

Next we determined the ends of both transcripts. We conducted rapid amplification experiments of cDNA ends (RACE) and cloned the PCR products (Scotto-Lavino *et al.* 2006) (Figure 3C). Sequencing results showed that the common *swi2L* forms found in both P and M cells were identical. *swi2L* is a 2476-nt-long transcript capable of encoding a 722-amino-acid-long open reading frame (ORF) (Figure 3A). The *swi2S* is 1962-nt-long transcript capable of encoding a predicted 518-amino-acid-long ORF. The 3′ ends of both forms were identical. Overall, the *swi2S* form is a 5′-end truncated species of the *swi2L* form, and these species share the same ORF in overlapping regions of the gene.

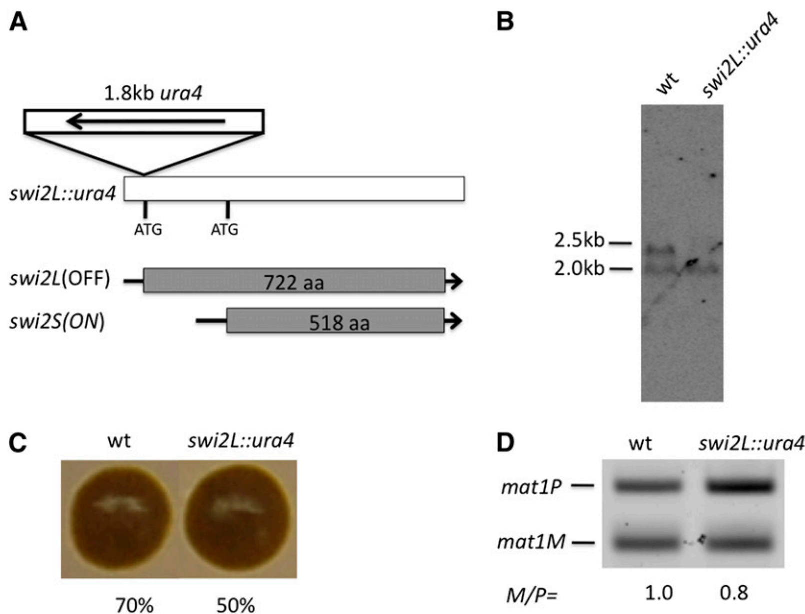

Figure 4 Effect of Δ*swi2L* on mating-type switching. (A) Structure of the *swi2L*::*ura4* allele is diagrammed. A 1.8-kb *ura4 Hin*dIII fragment was inserted in the translation initiation site (ATG) of the swi2L gene. (B) Northern blot analysis of wild-type (SP976) and the *swi2L*::*ura4* (CY345) cultures. RNAs prepared from cultures grown in PMA medium were analyzed with the Swi2P probe (Figure 3A). (C) Iodine-staining colony phenotype. Numbers below each colony reflect their efficiency of sporulation. (D) The *mat1* M/P ratio of cultures was determined with the method described in Figure 2B.

Northern blot analysis with the 5′-end-specific probe from the *swi2L* form (Swi2 5′P) did not detect the *swi2S* signal (Figure 3B), confirming our RACE results.

Swi2S and Swi2L proteins detected when expressed from a strong promoter

We tested whether the Swi2S form is expressed as an ORF predicted protein in yeast cells. We could not detect hemagglutinin (HA)-tagged Swi2 protein when it was expressed at the genomic locus from its indigenous promoter (Figure 3D), a result in accord with a previous report suggesting that the level of expression is too low to be detected by Western blot analysis (Akamatsu *et al.* 2003). Then we attempted to express both *swi2L* and *swi2S* genes with a stronger *nmt1* promoter of a pREP3 plasmid vector (Maundrell 1993). Two bands of about 80 kDa and 60 kDa, corresponding to the 722-aa and 518-aa predicted proteins, respectively, were detected (Figure 3D). These results suggested that the *swi2S* encodes an N-terminal truncated protein form of the Swi2L protein when these genes were expressed from the *nmt1* promoter.

swi2S gene plays a key role in regulating directionality

Our molecular work predicted that *swi2S* is essential for directionality of switching. We genetically tested this possibility. We inserted the 1.8-kb DNA fragment, containing the *ura4* gene in a transcription orientation reversed with that of the *swi2* gene, into the *swi2L* translation initiation site in the genome (Figure 4A). The resulting strain was named *swi2L*::*ura4*. Northern blot analysis showed that the DNA insert did not alter the *swi2S* expression, but the *swi2L* form was missing (Figure 4B). This result demonstrated that the *swi2S* mRNA is independently transcribed from the *swi2L* form and that the *swi2S* promoter is probably located in the 5′-coding region of the *swi2L* gene. The iodine vapor dark-staining phenotype of the *swi2L*::*ura4* strain was indistinguishable from that of the wild-type genotype, but the sporulation frequency of strains was somewhat reduced (50% *vs.* 70%) (Figure 4C). Also, the *mat1P* content was increased relative to the *mat1M* content in the *swi2L*::*ura4*, in comparison to that of the wild-type cultures (Figure 4D). A colony of the *swi2L*::*ura4* genotype stains dark (Figure 4C), and in comparison, the Δ*swi2* or Δ*abp1* colonies were reported previously to stain lightly (Akamatsu *et al.* 2003; Aguilar-Arnal *et al.* 2008). These results indicated that the *swi2S* form indeed provides a *mat1* switching function and that it facilitates *mat2* donor utilization over *mat3*.

Mc and abp1 genes regulate directionality specifically by inducing swi2S expression

Do Mc and Abp1 control directionality specifically by promoting the expression of *swi2S*? We conducted complementation experiments to address this question. We expressed *swi2S* through the *nmt1* promoter from an ectopic pREP3 vector (Maundrell 1993). Ectopic expression of *swi2S* led to increased *mat1P* content over *mat1M* (Figure 5A), and consequently, otherwise wild-type colonies stained lighter and a reduced level of sporulation was observed in them (Figure 5B). *swi2S* expression in mc^- or Δ*abp1* mutants led to a dramatic *mat1* M/P ratio reversal from >4.0 to 0.6. These results indicated that increased *swi2S* expression greatly suppressed the mc^- and Δ*abp1* mutants' switching defect. Specifically, the *mat2P* donor utilization defect of mc^- and Δ*abp1* mutants was suppressed through *swi2S* expression (Figure 5B). Expression from the *nmt1* promoter can be inhibited by adding 0.05 μM thiamine to the medium (Javerzat *et al.* 1996). Moderating *swi2S* expression with the thiamine addition partially complemented the Δ*abp1*-staining phenotype, and consequently, the *mat1* M/P ratio changed from 4.4 to 2.3 (Figure 5A). These results strongly suggest that *Mc* and *abp1* genes regulate directionality specifically, or predominantly, by inducing *swi2S* expression.

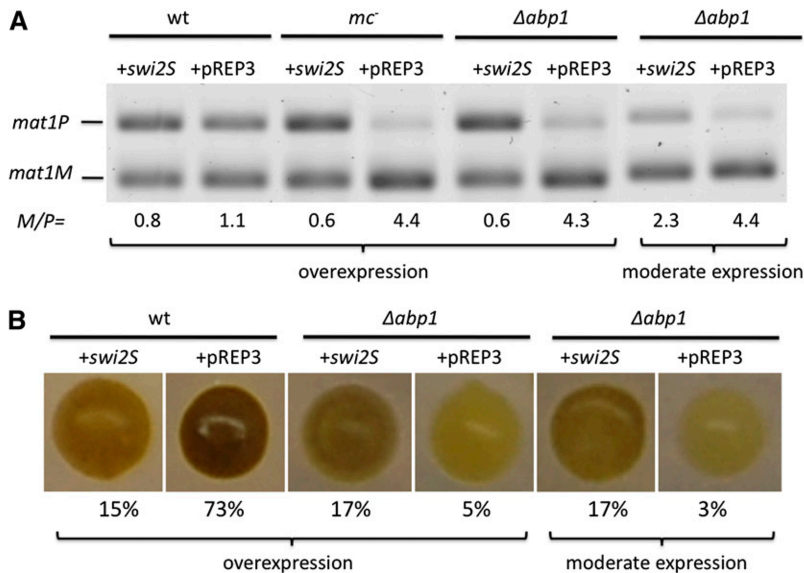

Figure 5 Ectopically expressed *swi2S* suppresses *mc*⁻ (CY195) and Δ*abp1* (CY327) mutants' switching defect. (A) The *mat1* M/P ratio of cultures was determined with the method described in Figure 2B. Analysis of an empty *pREP3* vector transformant was included for comparison, as a negative control (wt, SP976). (B) Iodine-staining colony phenotype. Moderate *swi2S* gene expression was achieved by 0.05 μM thiamine addition to the medium and thiamine was not added to achieve overexpression.

As shown above, Δ*ste11* did not influence the P/M cell ratio in both h^{90} and h^{09} cells (Figure 2C). As we have implicated *swi2S* in directionality, we tested whether Ste11 is required for *swi2S* expression. The Northern blot result showed that the Δ*ste11* mutation reduced the *swi2S* mRNA level as compared to that of the *swi2L* form in both h^{90} and h^{09} background (Supporting Information, Figure S1). The reduction of *swi2S* is more likely due to reduction of the Mc gene expression, which is regulated by Ste11 (Kjaerulff *et al.* 1997; Xue-Franzen *et al.* 2006). Although *swi2S* expression is partially reduced, the P/M cell ratio is not affected by the *ste11* mutation (Figure 2C). We think that there are two possible explanations for it: one is that the reduction of *swi2S* amount is not enough to affect switch directionality; the other is that Ste11 may control other target genes that can compensate for the *swi2S* reduction.

A mat2 cis-acting SRE2 element implicated in donor preference

A previous report identified an SRE (Swi2-dependent recombinational enhancer) element, located centromere-distal to the *mat3* locus as the Swi2/Swi5 complex loading site (Jia *et al.* 2004). Here we refer to it as the SRE1 element. Similarly, we discovered a sequence located centromere-distal to the *mat2* locus that promotes its utilization as a donor (Figure 6A). A 400-bp deletion mutation of *mat2*:H1 distal region greatly reduced both colony staining and sporulation (Figure 6B). We named the region defined by the deletion as the SRE2 element. Analysis of the *mat1* locus composition showed that *mat1P* predominates in ΔSRE1 and *mat1M* predominates in ΔSRE2 strains (Figure 6B). This work demonstrated that both *mat2* and *mat3* loci use flanking elements for their utilization as donors for switching, and they probably help recruit the recombination Swi2/Swi5 complex in a cell-type-specific fashion. This idea is supported by studies reporting the recruitment of Swi2/Swi5 at these two sites (Jia *et al.* 2004; Aguilar-Arnal *et al.* 2008).

Discussion

In this study, we found evidence that the *mat1-Mc* gene dictates directionality in M cells such that *mat2* is preferred over *mat3* as the donor for switching. Moreover, we discovered the mechanism of this preference. Specifically, Mc induces the transcription of a shorter form of *swi2* mRNA by activating a previously uncharacterized promoter, which lies within the 5′-end side of the ORF of the longer form of the *swi2* gene. We also found that Abp1 similarly dictates directionality by inducing *swi2S* mRNA synthesis. Furthermore, a new *cis*-acting element, SRE2, located next to *mat2* was identified, and that also facilitates *mat2* utilization. A diagram of the genetic pathway summarizing our results is presented in Figure 7A.

A model for Swi2L and Swi2S factors controlling directionality

A model for directionality controlled by two forms of the *swi2* gene in *P* and *M* cells is proposed in Figure 7B. In *mat1-M* cells, the directionality is primarily governed by the *mat1-Mc* gene, which induces the *M*-cell-type-specific *swi2S* expression at the transcriptional level. It is not known whether the Mc factor regulates *swi2S* expression directly or indirectly through regulating other gene(s). Similarly, the *abp1* gene is required for *swi2S* expression, also by acting either directly or indirectly. We postulate that, owing to their protein sequence differences, the Swi2L and Swi2S proteins possess different Swi2/Swi5 complex-spreading abilities at the donor-loci region. The Swi2L form contains a strong self-interaction domain at its N-terminal end, and it was postulated that this form determines the cell-type specific pattern of Swi2/Swi5 complex distribution (Akamatsu *et al.* 2003; Haruta *et al.* 2008). The Swi2L protein prefers to accumulate at the *mat3* region, and it does not spread to other regions. The Swi2S protein lacks the strong self-interaction domain, and it might facilitate the spread to the entire donor

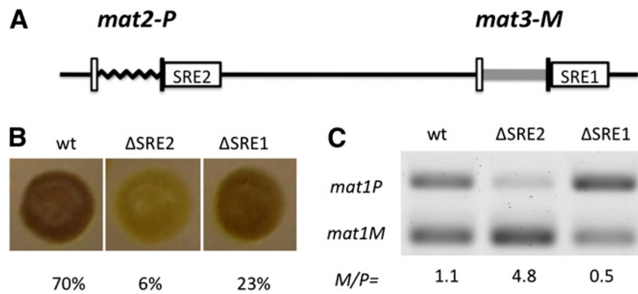

Figure 6 Effect of ΔSRE (switching recombinational enhancer) deletion on mating-type switching. (A) The diagram shows location of SRE1 and SRE2 elements with respect to donor loci. ΔSRE2 (CY212) comprises a 400-bp deletion located distal to the H1 region of *mat2P*. The SRE1 is defined by a 454-bp deletion of sequences (ΔSRE1, CY294) located distal to H1 region of *mat3M* (Jia *et al.* 2004). The iodine staining colony phenotype (B) and the *mat1* M/P ratio (C) of indicated strains are presented.

loci heterochromatic region. It is also possible that the Swi2S helps bind the complex, preferably to the *mat2* locus, by binding to the SRE2 element (Figure 7B). The proposed model is in accord with our observations and those of others, including: (1) in Δ*abp1* background, the Swi2L is the only form expressed in *P* as well as in *M* cells, and the Swi2/Swi5 complex binds to the *mat3* locus, as shown previously by chromatin immunoprecipitation experiments (Jia *et al.* 2004; Aguilar-Arnal *et al.* 2008); (2) the Swi2S is the predominant form in *M* cells, and the Swi2/Swi5 complex is assembled on the entire region to make *mat2* as the preferred donor; (3) in the Δ*swi2L* (*swi2L*::*ura4*) strain, where only the Swi2S form is expressed, the *mat2* is a preferred donor when compared with wild-type cells; (4) in the Δ*swi2* strain, where both Swi2L and Swi2S forms are lacking, the *mat3* donor is not preferred because Swi2L is absent. In this case, however, it is not possible to comment on the donor locus preference because of the very low rate of switching (Jia *et al.* 2004; Aguilar-Arnal *et al.* 2008). In Δ*swi2* cells, the *mat1-P* allele predominates, perhaps because the nearby *mat2* is at an advantage over the more distant *mat3* locus for recombination with *mat1*.

Mc and Abp1 may physically bind to a putative swi2S promoter to activate expression

We learned here that the *swi2* gene expresses two *swi2S* and *swi2L* forms. The *swi2S* is more crucial than *swi2L* for efficient mating-type switching. The *swi2S* expression requires Mc and Abp1. Mc is a transcriptional factor and regulates M-cell-specific genes in conjunction with Ste11 (Kjaerulff *et al.* 1997). Our study showed that Δ*ste11* reduced *swi2S* expression, perhaps indirectly through controlling *mat1-Mc* expression, rather than by directly controlling *swi2S* expression. We do not think the Ste11 directly regulate *swi2S* expression because Δ*ste11* still show reduced levels of *swi2S* expression. We investigated the sequence upstream of the *swi2S* transcription start site to look for a putative promoter element that might exist there. A short 12-bp motif (5′-ACAATGCCCATTGT) was found 45 bp upstream of the *swi2S* transcript start site, and this motif contains two standard, inverted Mc-binding sites (ACAATG) (Kjaerulff *et al.* 1997). This finding reminds us of the mating-type switching-directionality regulation of the distantly related budding yeast, *Saccharomyces cerevisiae*. Budding yeast mating-type *MATα2* factor binds to two closely located recognition motifs at RE (recombinational enhancer) region as a diamer to repress the RE function for donor choice during mating-type switching (Houston *et al.* 2004; Coic *et al.* 2006). The Mc binding to this putative promoter might activate *swi2S* transcription. A recent chromatin immunoprecipitation analysis showed that Mc physically interacts with the *swi2S* promoter region, as predicted here (Matsuda *et al.* 2011). Whether Mc binding depends on the two inverted presumptive recognition sites needs further investigation.

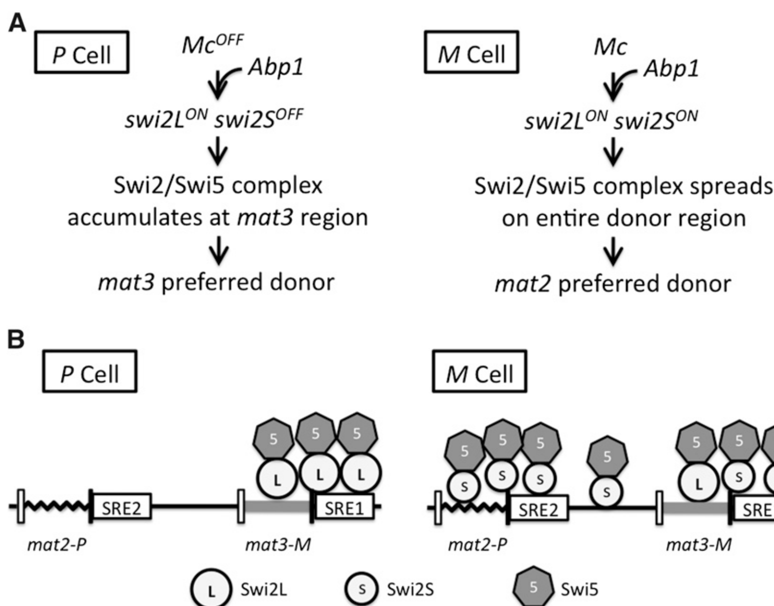

Figure 7 The mechanism directionality of switching. (A) Summary of genetic determinants controlling directionality of *mat1* switching. In P cells, because Mc factor is absent, only *swi2L* form is expressed ON. In *M* cells, the Mc factor, in combination with the Abp1 factor, activates the *swi2S* gene. (B) Proposed role of Swi2L and Swi2S in directionality of switching. In P cells, the Swi2L protein promotes localization of the Swi2/Swi5 complex to the *mat3* region to make it as the preferred donor. In M cells, perhaps Swi2S and Swi2L cooperate to promote assembly of the Swi2/Swi5 complex across the entire silenced region to make *mat2* as the preferred donor. The two SRE elements might facilitate enrichment of Swi2/Swi5 complexes at donor loci in cell-type specific fashion.

The *abp1* gene was originally identified as the ARS-binding protein (Murakami *et al.* 1996) and it is probably involved in DNA replication initiation. Abp1 and the other two CENPB homologs (Cbh1 and Cbh2) bind to the centromere, helping centromeric heterochromatin assembly to promote centromere function (Nakagawa *et al.* 2002). More recently, genome-wide Abp1 distribution mapping showed that Abp1 also binds to retrotransposons to help maintain genome stability by silencing transposons and by controlling replication fork pause release (Cam *et al.* 2008; Zaratiegui *et al.* 2011). The Abp1 was previously implicated in the directionality of switching by promoting Swi2/Swi5 distribution at donor loci (Aguilar-Arnal *et al.* 2008). Since there is a strong Abp1 binding signal near the CenH sequence in the *mat2/3*-region, it was postulated that Abp1 might control Swi2/Swi5 binding by directly binding to this site (Aguilar-Arnal *et al.* 2008). We deleted the region containing the putative Abp1-binding site, but no obvious colony-staining defect was observed (Figure S2). This result indicated that the putative *Abp1* binding site is not required for switching directionality. Instead, we found that *Abp1* regulates directionality of switching primarily by regulating *swi2S* expression. According to the high-resolution mapping data (*S. pombe* epigenome website: http://pombe.nci.nih.gov/index.html?org=S.+pombe&db=pombe&hgsid=59268), there is about a 2.5-fold Abp1 enrichment signal found at the putative *swi2S* promoter region. Thus, it is possible that Abp1 induces the *swi2S* transcript by directly binding to our predicted *swi2S* gene promoter region. We speculate that Abp1 helps load Mc to the *swi2S* promoter to activate *swi2S* expression (Figure 7A).

Regulation of the swi2 gene by two separate cell-type-activated promoters

Different-sized transcripts and the encoded proteins are usually produced by alternative mRNA splicing. In the database, the *swi2* gene (GenBank Accession no. NM_001019690) is listed as encoding a 722-amino-acid ORF, lacking introns reflecting the *swi2L* species. We found two *swi2* species resulting from two promoters with overlapping transcripts that differ from each other only at the 5′ end. A precedent for one gene producing two overlapping transcripts, initiated from two different promoters, is found with the *Drosophila sex-lethal* (*Sxl*) gene. Although alternatively spliced products of *Sxl* eventually regulate sexual identity during development, the initial decision for sexual identity occurs through a choice between the maintenance promoter (Pm) and establishment promoter (Pe) (Keyes *et al.* 1992). The Sxl-Pm is transcribed in both sexes, but the Sxl-Pe, located in the region encoding the first intron of the Sxl-Pm transcript, shows female-specific expression. The protein product from Sxl-Pe regulates the Sxl-Pm splicing to produce the SXL protein in female embryos. The Sxl-Pm transcript found in males includes an extra exon, which renders the transcript nonfunctional (Salz and Erickson 2010). Similarly, we found that *swi2L* expresses in both yeast cell types, but the *swi2S* expresses only in *M* cells and at a level higher than that of *swi2L*. In this respect, *swi2* regulation is analogous to that of the *Sxl* gene of *Drosophila*. However, both of the *swi2L* and *swi2S* species are functional and bind to the mating-type donor region with alternative distribution patterns to regulate directionality of switching. In sum, the two *swi2* forms cooperate to regulate donor choice such that most switches to the opposite mating type occur in cells of either mating type.

We note that previously the *swi2S* form has escaped detection by microarray analysis or genome-wide sequence analysis possibly because it was regarded as a partial degradation product of the *swi2L* form. As a consequence, previous studies (Akamatsu *et al.* 2003; Jia *et al.* 2004) performed to define the function of *swi2* in switching considered only the *swi2L* form and here we found that the *swi2S* form is a major determinant of directionality of switching.

While we were preparing this manuscript for publication, it was reported (Matsuda *et al.* 2011) that the Mc and Abp1 genes control the switching recombination directionality, that Mc protein directly binds to the Swi2 gene regulatory region, and that binding requires Abp1. Results of that report are complementary to some parts of our study and support our conclusions. However, the discovery of the Swi2S form and its role in directionality is presented here only in our study.

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Going in the Right Direction: Mating-Type Switching of *Schizosaccharomyces pombe* Is Controlled by Judicious Expression of Two Different *swi2* Transcripts

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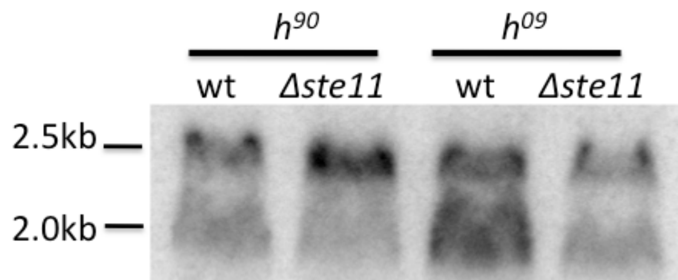

Figure S1 *Δste11* reduced *swi2S* expression relative to *swi2L* form. Northern blot analysis of wild-type (h^{90}, SP976; h^{09}, PG19) and the *Δste11* (h^{90}, CY146; h^{09}, CY148) cultures. RNAs prepared from cultures grown in PMA medium were analyzed with the Swi2P probe (Figure 3A).

A

URA4

CCTTTTTCACAACCAAAATGGATGGCGACAATGTATCCTGCTAACTGTATTTACT
GGTCACGATGCTTAGGCGGTGTAATTAGTGAATTAGTCATATCTCCAATTATATA
ACTTGAAATGGTAACTGTTAGAGACATCGAAACTTGCTATTCCAATTCATTCGC
GTAACGATACTTCTTATCTGTCTTCTCCTCCATAACAGCCAGGTAGCGGCAGTAT
TACTTGTATTTCCAAAACTAGTATTCTGTCGAAATTATCGAAAGCTACATTTTTC
CCTGCTTCAGTTTCGCCAACGTGGAAGAAAAGCTTTAACAACGCATAAATATGG
CTCGGAAGACATGTCCTCAGCTATCATTGTGGTCACAAACACATATTATATGGAT
ATTTGAACAATGTATAACAAGCTGATAAAAAAAAAACAATATAGTGGTGCTGATG
GTACCATTCAACTTGACAATCATTAAAGCTCTCTGCAAGCA**CCCTAAAAAGCAA**
TGAATAGACTCCATTGAGGAATCTGTGCAACTTGAGAGATTCTCTTAAGTGTAT
ATAGATAAATGTTTAAGCTACGCTCTTGCTAACCTTTATACAG

B

C

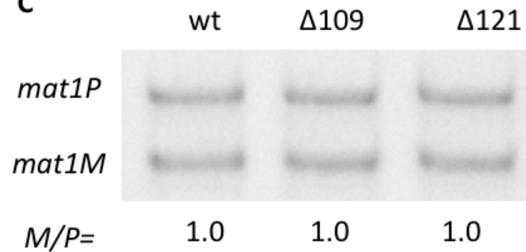

Figure S2 Deletion analysis of the putative Abp1 binding site located in the *mat2/3* region. (A) Deleted sequence. The sequence deleted in Δ109 (CY303) mutation is underlined and that deleted in Δ121 (CY301) mutation is represented in gray color. A putative Abp1 binding site is represented in bold letters. To help create mutation constructs, both mutations contained the *ura4* gene insertion at the indicated site. The iodine-staining phenotype of wild type (wt, CY308 only containing the *ura4* insertion) and mutants (B) and their *mat1* M/P ratios of indicated strains (C) are presented.