Cell, Vol. 119, 469–480, November 12, 2004, Copyright ©2004 by Cell Press

# Heterochromatin Regulates Cell Type-Specific Long-Range Chromatin Interactions Essential for Directed Recombination

**Songtao Jia, Takatomi Yamada, and Shiv I.S. Grewal***
Laboratory of Molecular Cell Biology
National Cancer Institute
National Institutes of Health
Bethesda, Maryland 20892

## Summary

**Mating-type switching in *Schizosaccharomyces pombe* involves replacing genetic information at the expressed *mat1* locus with sequences copied from one of two silent donor loci, *mat2-P* or *mat3-M*, located within a 20-kb heterochromatic domain. Donor selection is dictated by cell type: *mat2* is the preferred donor in *M* cells, and *mat3* is the preferred donor in *P* cells. Here we show that a recombination-promoting complex (RPC) containing Swi2 and Swi5 proteins exhibits cell type-specific localization pattern at the silent mating-type region and this differential localization modulates donor preference during mating-type switching. In *P* cells, RPC localization is restricted to a recombination enhancer located adjacent to *mat3*, but in *M* cells, RPC spreads in *cis* across the entire silent mating-type interval in a heterochromatin-dependent manner. Our analyses implicate heterochromatin in long-range regulatory interactions and suggest that heterochromatin imposes at the mating-type region structural organization that is important for the donor-choice mechanism.**

## Introduction

Directed recombination events play pivotal roles in cellular differentiation and development in a wide range of organisms. For example, during the maturation of the mammalian immune system, recombination between exons of the V(D)J sequences leads to the assembly of a diverse repertoire of immunoglobulin and T cell receptors, which are both lineage and developmental stage specific (reviewed in Gellert [2002]). Similarly, mating-type switching in two distantly related yeast species, *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*, involves a highly ordered recombination event in which the mating-type locus switches between two alternative alleles by copying information from one of the two silent donor loci (reviewed in Klar [1989]; Haber [1998]). Although the proteins involved in these processes are quite divergent, each instance involves developmentally regulated long-range interactions between the acceptor locus and the selected donor locus embedded within distinct regions of the genome. Similar long-range interaction between distant chromosomal regions is also used in transcriptional regulation, with transcriptional enhancer and silencer elements activating or silencing genes at a distance, respectively (Blackwood and Kadonaga, 1998; Courey and Jia, 2001). Despite the fundamental importance of these and other long-range chromatin interactions, the mechanisms that govern communication between distal chromosomal regions remain to be fully explored.

Mating-type switching in the fission yeast *S. pombe* occurs efficiently in mitotically dividing cells, providing a unique opportunity for exploring the mechanism of directed recombination and long-range interactions between distant regions of the genome. *S. pombe* contains three mating-type loci, *mat1, mat2*, and *mat3,* located in the middle of the right arm of chromosome II. In wild-type homothallic strains, designated $h^{90}$, *mat2* is separated from *mat1* by an approximately 15-kb interval, named *L*-region, whereas *mat3* is separated from *mat2* by an approximately 11-kb interval, called *K*-region (Beach, 1983; Grewal and Klar, 1997). The mating type of a haploid cell is determined by the presence of *P* (*plus*) or *M* (*minus*) information at the *mat1* locus. The *mat2* and *mat3* loci contain the same genetic information as the *mat1-P* and *mat1-M* alleles, respectively, but are maintained in a transcriptionally silent state (Egel and Gutz, 1981). These silent loci serve as donors of genetic information for the *mat1* locus during mating-type switching (reviewed in Grewal [2000]).

Mating-type switching is initiated by a strand-specific imprint adjacent to the *mat1* locus that is converted to a double-strand break (DSB) in the next S phase (Arcangioli, 1998; Dalgaard and Klar, 1999). Cells repair the broken DNA by a gene conversion event in which the mating-type allele sequence present at the *mat1* locus is replaced with that copied from *mat2* or *mat3* (Beach, 1983). A remarkable feature of mating-type switching is that the choice of the donor locus during each switch is nonrandom. By a process referred to as directionality of switching, cells predominantly switch to the opposite *mat1* allele such that *mat2* is the preferred donor in *mat1-M* cells, whereas *mat3* is the preferred donor in *mat1-P* cells (Miyata and Miyata, 1981). Therefore, cell type (*M* or *P*) dictates which of the silent mating-type loci will be used as a donor. By using strains in which genetic contents of the *mat2* and *mat3* loci were swapped, Thon and Klar (1993) showed that the location of the donor loci on the chromosome, rather than their DNA sequence, directs the choice of donor in each cell type. Therefore, *cis*-acting sequences outside of the donor loci themselves must selectively activate or repress one of the donors in a cell type-specific manner.

It has been shown that heterochromatin within the 20-kb domain containing the *mat2* and *mat3* loci (*mat2/3* interval) is essential for both transcriptional silencing and directionality of switching (Thon and Klar, 1993; Grewal and Klar, 1997; Noma et al., 2001). Heterochromatin assembly involves histone deacetylation and methylation of histone H3 lysine 9 (H3-K9), which is essential for localization of Swi6 (a homolog of mammalian HP1) protein (Nakayama et al., 2001b). H3-K9 methylation and Swi6 are preferentially enriched throughout the *mat2/3* interval, which is flanked by inverted repeat

*Correspondence: grewals@mail.nih.gov

(IR-L and IR-R) boundary elements (Noma et al., 2001). Heterochromatin is nucleated at *cenH*, a repeat element residing within the *K*-region that shares homology to centromeric repeats (Grewal and Klar, 1997), in an RNAi-dependent manner and then spreads to the entire silent domain (Hall et al., 2002). In addition, members of the ATF/CREB protein family, Atf1 and Pcr1, bind to the silent mating-type region and promote heterochromatin assembly (Jia et al., 2004). Factors essential for heterochromatin assembly at the *mat2/3* region, such as Swi6, the H3-K9 methyltransferase Clr4, and Rik1 (a factor required for H3-K9 methylation), are also required for directionality of switching (Thon and Klar, 1993; Ivanova et al., 1998; Nakayama et al., 2001b; Tuzon et al., 2004). Moreover, mutations in histone deacetylases Clr3 and Sir2 affect both mating-type switching and silencing at the *mat2/3* region (reviewed in Grewal [2000]; Shankaranarayana et al. [2003]). It is believed that heterochromatin at the *mat2/3* region preferentially brings one donor locus close to the *mat1* locus in a cell type-specific manner. However, very little is known about the mechanism through which heterochromatin promotes directionality of switching.

Apart from the factors involved in heterochromatin assembly, genetic screens have identified at least three classes of genes that, when mutated, result in a reduced rate of mating-type switching. Among these loci, *swi1*, *swi3*, and *swi7* are implicated in formation of the strand-specific imprint and in generation of the DSB at the *mat1* locus, while *swi4*, *swi8*, *swi9/rad16*, *swi10*, and *rad22* are believed to be important for the resolution of recombination intermediates during the switching process (Egel et al., 1984; Grewal, 2000; Dalgaard and Klar, 2000; Kaykov et al., 2004). A third class of genes which include *swi2* and *swi5* along with *swi6* is believed to be involved in efficient utilization of the DSB during switching process since their mutations result in reduced levels of switching to the opposite mating type without affecting generation of the DSB at the *mat1* locus or recombination resolution steps (Egel et al., 1984). Swi5, a conserved protein that is partially homologous to *S. cerevisiae* Sae3, has been also implicated in general recombination and forms a complex with Rhp51 (Akamatsu et al., 2003), a homolog of mammalian Rad51 (Baumann and West, 1998). However, the exact function of *swi2* and *swi5* in mating-type switching remains obscure. The resemblance of switching defects in *swi2* and *swi5* mutants to those of *swi6* mutants nonetheless suggests an intriguing possibility that these factors have a role in heterochromatin-mediated control of the donor-choice mechanism.

In this article, we present results showing that Swi2 exhibits cell type-specific localization pattern at the silent mating-type region and regulates directionality of mating-type switching. Whereas Swi2 binds specifically to a recombination enhancer located adjacent to the *mat3* locus in *P* cells, it spreads throughout the entire *mat2/3* interval in *M* cells. The spreading of Swi2 is mediated by heterochromatin complexes and depends upon direct binding of Swi2 to Swi6 protein. The presence of Swi2 at the mating-type region also permits Swi5 recombination protein to localize and distribute throughout the *mat2/3* interval in a cell type-specific manner. These analyses provide insights into the mechanism of donor selection during mating-type switching and suggest a novel mode of long-range communication between regulatory DNA elements and the target loci via spreading of protein complexes along heterochromatin.

## Results

### Swi2 Regulates Directionality of Mating-Type Switching

To investigate the role of Swi2 in directionality of mating-type switching, we analyzed the effect of Δ*swi2* on mating-type interconversion in a switching-competent homothallic $h^{90}$ strain (Figure 1A). We monitored by staining with iodine vapors the switching efficiency of individual colonies grown on sporulation medium (Bresch et al., 1968). In this assay, a starch-like compound produced by sporulating cells results in black staining of colonies after exposure to iodine vapors. Cells that switch efficiently to the opposite mating type form colonies with a homogenous distribution of *M* and *P* cells and thereby can readily mate and sporulate, resulting in dark iodine staining. In contrast, cells that switch their mating type with a lower efficiency form lightly staining colonies due to poor mating and sporulation within the colony, caused by the lack of cells of opposite mating type in the vicinity. Our analysis revealed that Δ*swi2* cells switch to the opposite mating type at a reduced frequency. While efficiently switching wild-type cells form uniformly dark-staining colonies, Δ*swi2* strain produced lightly staining colonies with a sectored/mottled staining pattern (Figure 1A). We next investigated whether the reduced iodine staining in Δ*swi2* strain was due to defective utilization of one or of both *mat2* and *mat3* donor loci. We therefore analyzed the utilization of the two donors by determining the *mat1* genetic content. Mating-type allele-specific (i.e., *mat1-P* or *mat1-M*) primers were used to perform quantitative multiplex PCR on either wild-type or Δ*swi2* strain genomic DNA. As expected, the wild-type strain contains an equal number of *P* and *M* cells as indicated by the almost equal intensity bands representing *mat1-P* and *mat1-M* (Figure 1B), suggesting efficient utilization of both donors. However, cells carrying the Δ*swi2* allele were predominantly of the *P* mating type (Figure 1B). These data imply that *mat2* is the preferred donor in Δ*swi2* cells and *mat3* is used relatively inefficiently.

Mutations in *swi6* severely reduce the efficiency of mating-type switching in $h^{90}$ cells, leading to formation of colonies stained lightly with iodine vapors (Thon and Klar, 1993) (Figure 1A). However, in contrast to Δ*swi2* cells, the switching defect in *swi6* mutant cells is attributed to their inability to utilize *mat2* as a donor (Thon and Klar, 1993). Consequently, cells carrying *swi6* mutation are predominantly of the *M* mating type (Figure 1B). We tested whether Δ*swi2* would suppress the switching phenotype of a *swi6* mutation. Remarkably, *swi2swi6* double mutant showed a significant increase in switching to the opposite mating type compared to *swi2* or *swi6* single mutants (Figure 1B). As predicted by this increase in heterologous switching that presumably reflects random selection of donor loci, *swi2swi6* double mutant colonies stained darker with iodine vapors than the single mutant colonies (Figure 1A). These data

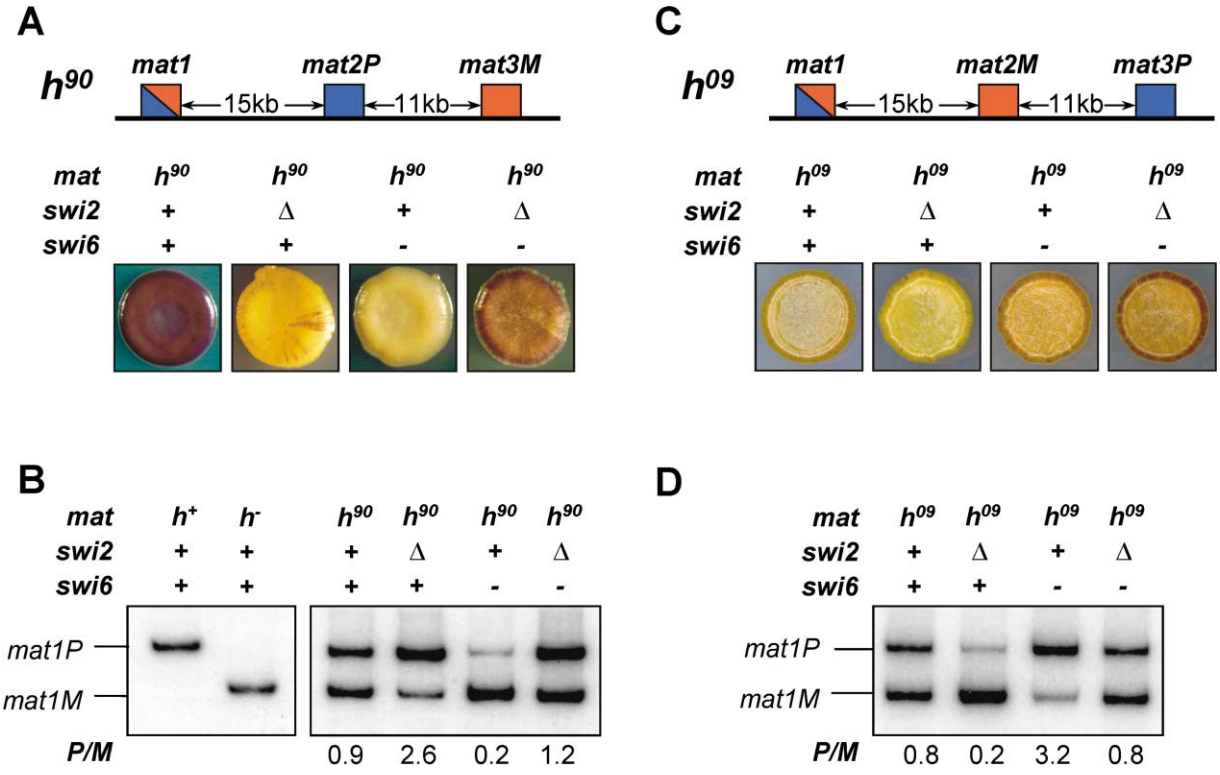


Figure 1. Swi2 Regulates Directionality of Switching

(A and C) Effects of *swi2* and *swi6* mutations on mating-type switching in $h^{90}$ and $h^{09}$ backgrounds. Schematic diagrams of the mating-type region in $h^{90}$ and $h^{09}$ strains (top panel). Iodine-staining phenotypes of *swi2*, *swi6*, or *swi2swi6* double mutant colonies (bottom panel). Colonies were replicated onto sporulation (EMM) medium and allowed to grow at 25°C for 3 days before staining with iodine vapors.

(B and D) Effects of *swi2*, *swi6*, or *swi2swi6* mutations on predominant mating type adopted by $h^{90}$ and $h^{09}$ cells. PCR-based assay was used to determine the *mat1* genetic content. Genomic DNA was isolated from the indicated strains and the *mat1* content was measured with multiplex PCR using primers to amplify *mat1-P* and *mat1-M* sequences simultaneously. The *P/M* ratio is shown beneath each lane. Stable *P* ($h^+$) and *M* ($h^-$) mating-type strains were used as controls to demonstrate the specificity of primer sets.

clearly demonstrate a genetic interaction between Swi2 and Swi6 and suggest that these proteins cooperate to regulate the directionality of switching.

To confirm these results, we also examined the effects of mutations in *swi2* and *swi6* on switching in $h^{09}$ strain background. In $h^{09}$ cells, the contents of the donor loci are swapped such that *mat2* contains *M* information and *mat3* contains *P* information (Thon and Klar, 1993). Since the location of donor loci on the chromosome rather than their genetic content is a deciding factor for donor choice, switching in $h^{09}$ cells results predominantly in futile replacement of the *mat1-P* or *-M* allele with the same allele (Thon and Klar, 1993). As a result, colonies formed by $h^{09}$ cells stain lightly with iodine vapors (Figure 1C). Mutations in *swi2, swi6*, and *swi2swi6* had the predicted effect on donor preference in $h^{09}$ strains. As shown in Figures 1C and 1D, we observed a decrease in utilization of the *mat3* locus as a donor in *swi2* mutant strain, whereas *swi6* mutant strain showed a decrease in the utilization of the *mat2* locus. Moreover, *swi2* and *swi6* mutations each reciprocally suppressed the switching defect caused by the other, resulting in random utilization of *mat2* and *mat3* loci. As a result, *swi2swi6* double mutant $h^{09}$ and $h^{90}$ strains showed comparable levels of sporulation (43%–47%) and iodine staining (Figures 1A and 1C). These results reinforce the conclusion that Swi2 and Swi6 function in nonrandom choice of donors during mating-type switching, irrespective of the mating-type configuration.

### Swi2 Physically Interacts with Swi6

To further elucidate the function of *swi2*, we constructed a strain expressing Swi2 fused with a quadruple myc epitope tag at the C terminus (Swi2-myc). The tagged $swi2^+$ is under the control of its native regulatory elements so as to achieve wild-type levels of expression. Western analysis with anti-myc antibody revealed a single band representing Swi2-myc in the tagged strain that was absent in control wild-type extracts (Figure 2A). Cells expressing Swi2-myc switched normally (data not shown), indicating that Swi2-myc can functionally replace the wild-type protein. Immunofluorescence analysis with anti-myc antibody showed that Swi2 localizes to the nucleus (Figure 2B). Interestingly, we observed one to three bright Swi2 foci present near the nuclear periphery, in addition to the minutely speckled to diffuse distribution throughout the nucleus. Since Swi6 is known to localize at the nuclear periphery to discrete foci, which correspond to heterochromatin found at the centromeres, telomeres, and the mating-type region (Ekwall et al., 1995), we also investigated whether Swi2 colocalizes with Swi6. Coimmunofluorescence with anti-

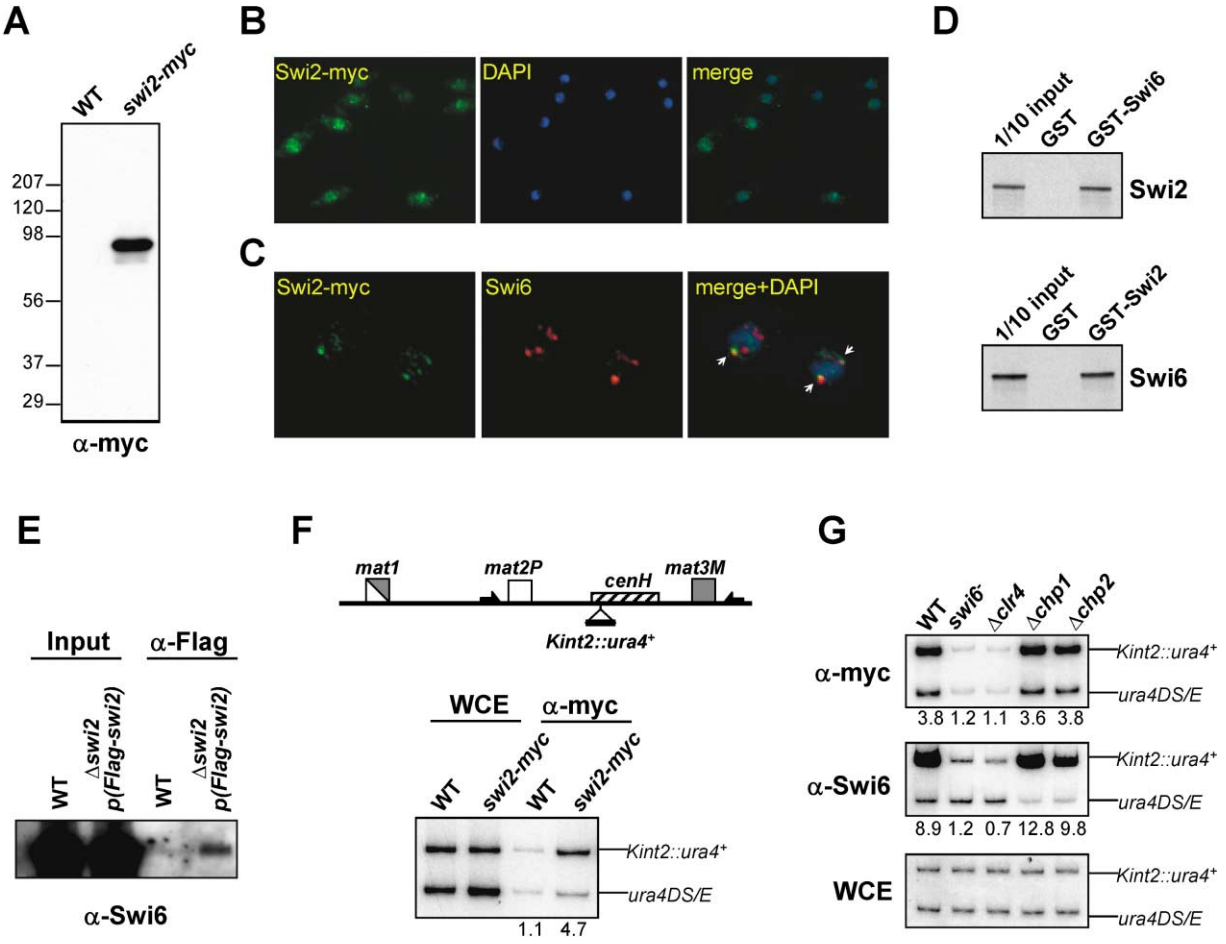


Figure 2. Swi2 Interacts with Swi6

(A) Tagging of Swi2. Extracts prepared from the indicated strains were subjected to Western blot analysis with myc (9E10) antibody.

(B) Swi2 is a nuclear protein. *swi2-myc* cells were stained with anti-myc antibody. DNA was visualized by DAPI. Merged images are also shown.

(C) Swi2 partially colocalizes with Swi6. *swi2-myc* cells were doubly stained with antibodies to myc and Swi6. Merged images of two antibodies together with DAPI are also shown. Arrows indicate colocalization of Swi2 and Swi6.

(D) Swi2 interacts directly with Swi6 in vitro. In vitro translated $^{35}$S-labeled Swi2 was incubated with equal amounts of GST or GST-Swi6 immobilized on glutathione beads (top). In vitro translated $^{35}$S-labeled Swi6 was incubated with equal amounts of GST or GST-Swi2 immobilized on glutathione beads (bottom). The beads were washed extensively and eluted proteins were resolved by SDS-PAGE and imaged by autoradiography. One-tenth of the in vitro translated proteins used for the binding assay are shown for comparison.

(E) Swi2 and Swi6 interact in vivo. Extracts prepared from wild-type cells or Δ*swi2* cells expressing Flag-Swi2 were incubated with a Flag antibody. The IPed fractions were analyzed by immunoblotting with a Swi6 antibody.

(F) Swi2 localizes to the mating-type region. Top panel: a schematic diagram of the *mat* locus indicating the position of *Kint2::ura4*$^+$. Bottom panel: levels of Swi2-myc at *Kint2::ura4*$^+$ were determined by ChIPs with a myc antibody. DNA isolated from ChIP fractions or from whole-cell extract (WCE) was quantitatively analyzed using PCR. The ratios of intensities of *Kint2::ura4*$^+$ and the control *ura4DS/E* minigene in ChIP and WCE lanes were used to calculate the relative fold enrichment, shown below each lane.

(G) Localization of Swi2 at the *mat* locus depends on Swi6. Levels of Swi2-myc and Swi6 at *Kint2::ura4*$^+$ were determined by ChIP in wild-type (WT) or indicated mutant backgrounds.

myc and Swi6 antibodies by using the Swi2-myc strain showed that Swi2 partially colocalized with Swi6 (Figure 2C). In particular, at least one of the bright Swi2 foci overlapped with Swi6 in most cells (Figure 2C). Therefore, in addition to a broad distribution of Swi2 throughout the nucleus, it appears to be preferentially enriched at certain heterochromatic foci, along with Swi6 protein.

Based on two-hybrid analysis, it has recently been suggested that Swi2 might directly bind to Swi6 (Akamatsu et al., 2003). To address this possibility, we investigated the potential interaction between these proteins by using in vitro and in vivo assays. A glutathione S-transferase (GST) pull-down assay was used to examine interactions in vitro. When in vitro translated Swi2 was incubated with GST-Swi6 or GST alone, Swi2 specifically bound to GST-Swi6 (Figure 2D), suggesting that these two proteins interact directly. Moreover, we found that GST-Swi2 bound to in vitro translated Swi6 protein (Figure 2D). We next investigated Swi2 interaction with Swi6 in vivo. For this purpose, we expressed FLAG epitope-tagged *swi2*$^+$ under the control of an inducible promoter from a plasmid (pREP41-Flag-*swi2*$^+$) in the Δ*swi2* strain. The amino-terminal tagged Swi2 (FLAG-Swi2) was functional, as it rescued the switching defect of Δ*swi2* (data

not shown). Extracts prepared from cells expressing FLAG-Swi2 or wild-type cells were used to perform immunoprecipitation (IP) analysis with anti-FLAG antibody. Western analysis revealed that Swi6 associated with FLAG-Swi2, whereas no band was detected in the wild-type control fraction (Figure 2E). It should be noted however that only a small proportion of the total Swi6 bound to Swi2, consistent with results from immunofluorescence analysis showing partial colocalization of Swi2 and Swi6. Collectively, these analyses suggest that Swi2 physically interacts with Swi6, presumably in a locus-specific manner (see below).

### Swi2 Is Not Required for Heterochromatin Formation

The effect of Swi6 on directionality of switching is tightly linked to its role in heterochromatin formation at the *mat2/3* region (Thon and Klar, 1993; Grewal and Klar, 1997). Considering that Swi2 interacts with Swi6, it seemed possible that Swi2 is required for heterochromatin assembly as well. We tested this possibility by examining the effect of Δ*swi2* on silencing and heterochromatin formation at centromeric repeats and the mating-type region. Deletion of *swi2* had no effect on the silencing of *ura4*$^+$ inserted at the inner (*imr1::ura4*$^+$) or outer (*otr1::ura4*$^+$) centromeric repeats and at the silent mating-type region (*Kint2::ura4*$^+$) (Supplemental Figure S1 at http://www.cell.com/cgi/content/full/119/4/469/DC1/). Moreover, the levels of Swi6 and H3-K9 methylation at these heterochromatic loci were comparable in wild-type and Δ*swi2* cells, as shown by chromatin immunoprecipitation (ChIP) analysis (Supplemental Figure S1 on the *Cell* website). Based on these data, we concluded that Swi2 is dispensable for heterochromatin formation.

### Swi2 Binds to the Mating-Type Region

We next assayed by ChiP analysis with strains expressing Swi2-myc for the presence of Swi2 at heterochromatic loci such as centromeric repeats and the *mat* locus. Although Swi2 was not detected at *imr1::ura4*$^+$ or *otr1::ura4*$^+$ reporter genes inserted at the centromeric repeats (Supplemental Figure S2 on the *Cell* website), it was clearly localized to the *Kint2::ura4*$^+$ reporter gene at the silent mating-type region (Figure 2F). Moreover, we found that a mutation in *swi6* or deletion of the H3-K9-specific methyltransferase *clr4* essential for Swi6 localization to the *mat* locus abolished Swi2 localization to *Kint2::ura4*$^+$, concurrent with the loss of Swi6 signal at this region (Figure 2G). In contrast, deletion of two other chromodomain proteins, *chp1* and *chp2* (Thon and Verhein-Hansen, 2000), which are involved in silencing and heterochromatin assembly but do not affect Swi6 localization at the *mat2/3* interval, did not affect Swi2 localization at the *mat* locus (Figure 2G). This analysis suggests that Swi2 localizes to the mating-type region in a Swi6-dependent manner. The localization of Swi2 to the *mat* locus but not to the centromeres, even though Swi6 is present at both loci (Nakayama et al., 2001b; Noma et al., 2001), suggests that additional factors are involved in recruiting Swi2 to the *mat* locus.

### Cell Type-Specific Distribution of Swi2 at the Mating-Type Region

The Swi6-dependent localization of Swi2 at the *mat* locus prompted us to create a high-resolution map of the Swi2 distribution across the entire mating-type interval using ChIPs, as described previously (Noma et al., 2001). Since cell type is the critical factor in donor preference during mating-type switching, we examined Swi2 localization at the mating-type region of both *mat1-P* and *-M* strains. In a stable *M* (*mat1smto*; Engelke et al., 1987) strain, Swi2 was preferentially enriched throughout 20 kb of the silent mating-type region, which includes *mat2* and *mat3* donor loci (Figure 3). The pattern of Swi2 localization coincided precisely with that of histone H3-K9 methylation and Swi6 protein, both of which are restricted to the silent domain by the IR-L and IR-R boundary elements (Noma et al., 2001). Interestingly, in the *swi6* mutant background, Swi2 failed to spread across the *mat2/3* interval and its localization was restricted specifically to a DNA element located 0.5 kb to the right side of the *mat3* locus (Figure 3). We have named this DNA element SRE, *S*wi2-dependent *r*ecombinational enhancer, for its role in promoting utilization of donor loci during mating-type switching. Swi2 targeting to the SRE occurred normally in Δ*clr4* cells (Supplemental Figure S3), further suggesting that Swi2 can localize to the SRE element independent of heterochromatin complexes. Taken together, these results suggest that Swi2 is recruited to the SRE independent of Swi6 but the Swi6-containing heterochromatin is required for spreading of Swi2 in *cis* across the entire *mat2/3* region.

We next mapped Swi2 at the mating-type region of a stable *P* (*mat1PΔ17*; Arcangioli and Klar, 1991) strain. Surprisingly, we found that Swi2 localization was restricted to the SRE element in both wild-type and the *swi6* mutant strain (Figure 3). The failure of Swi2 to spread across the *mat2/3* region in wild-type *P* cells was not attributable to differences in Swi2 levels because Swi2-myc was expressed at equivalent levels in both stable *P* and *M* cells (Supplemental Figure S4 on the *Cell* website). We find it remarkable that Swi2 exhibits a cell type-specific localization pattern at the mating-type region. This differential localization of Swi2 at the *mat2/3* region of *P* and *M* cells is reminiscent of parental imprinting in mammals and might provide the basis for cell type-specific utilization of donors during mating-type switching (see Discussion).

### Deletion of Swi2-Dependent Recombination Enhancer Affects Directionality of Switching

The results presented above suggested that the SRE element serves as an entry site for the recruitment of Swi2 protein to the mating-type locus. To test this directly, we deleted a 454 bp sequence that lies immediately to the right side of the *mat3* locus (Figure 4). Deletion of the SRE completely abolished the binding of Swi2 to the mating-type region (Figures 4A and 4B) and resulted in altered choice of donors during mating-type switching in a pattern that was indistinguishable from the Δ*swi2* strain. *h*$^{90}$ cells carrying deletion of the SRE element were predominantly of the *P* mating type, suggesting that ΔSRE cells utilize *mat2* donor more frequently than *mat3* donor, similar to Δ*swi2* cells (Figure

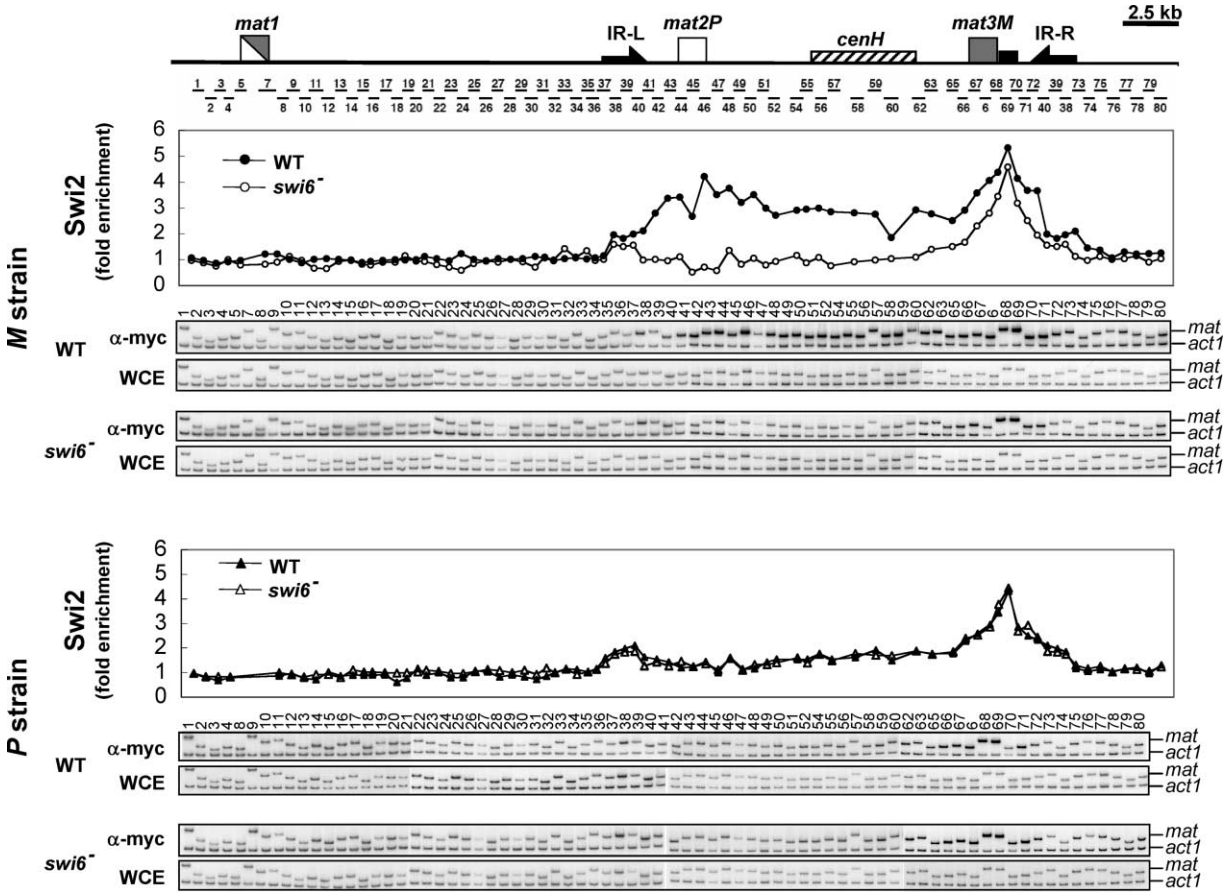


Figure 3. High-Resolution Mapping of Swi2 Levels at the Mating-Type Region

A diagram of the *mat* locus is shown on top. IR-L and IR-R indicate heterochromatin boundary elements. Hatched box represents *cenH* sequence. Black rectangle adjacent to *mat3* represents SRE. ChIP analysis with a myc (9E10) antibody was performed with indicated strains to measure Swi2-myc levels throughout the *mat* locus. DNA isolated from ChIP (α-myc) and whole-cell crude extract (WCE) were subjected to multiplex PCR to amplify DNA fragments from the *mat* locus, indicated by numbered bars (1 to 80), together with an *act1* fragment as an internal control. The ratios of intensities of *mat* and *act1* signals in the α-myc and WCE lanes were used to calculate the relative fold enrichment (see Noma et al., 2001 for details). Quantification of these results is plotted in alignment with the map of the *mat* locus.

4C). Moreover, we observed that switching defect caused by ΔSRE was suppressed by mutation in *swi6*, as reflected by almost equal utilization of *mat2* and *mat3* loci as donors in ΔSRE *swi6* double mutant cells. We conclude from these analyses that the SRE is the de novo binding site of Swi2 that serves to enhance nonrandom utilization of donor loci during mating-type switching.

### Swi2 Is Required for Cell Type-Specific Distribution of Swi5 at the Mating-Type Region

It has been recently reported that Swi2 mediates interaction between the recombination proteins Swi5 and Rhp51 (Akamatsu et al., 2003). Since Swi5 is also known to be important for mating-type switching and belongs to the same epistasis group as Swi2, we explored the possibility that Swi5 localizes to the mating-type region. We performed ChIPs with an anti-Swi5 antibody (a gift from H. Iwasaki) to map the binding of Swi5 across the entire *mat2/3* interval. Interestingly, in *M* cells, Swi5 was found to localize throughout the 20-kb silent mating-type domain; whereas in *P* cells, Swi5 binding was attenuated and restricted to the SRE element (Figure 5). This Swi5 localization pattern closely resembled that of Swi2, suggesting that Swi5 might be recruited to the mating-type region through its interaction with Swi2. Supporting this possibility, we found that the localization of Swi5 to the mating-type region was completely abolished in the Δ*swi2* mutant background in both *P* and *M* cell types (Figure 5). Moreover, ΔSRE also abolished the binding of Swi5 across the *mat2/3* interval in *M* cells (Supplemental Figure S5 online), concomitant with the loss of Swi2 binding at this region (Figure 4A). Thus, Swi5 also displays at the silent mating-type region a cell type-specific localization pattern that is dependent upon Swi2.

### Effect of a *swi2* Deletion on Chromatin Structure at the SRE Element

Donor mating-type loci are embedded in a heterochromatin domain that exhibits severe recombination suppression, in addition to transcriptional silencing (Klar and Bonaduce, 1991). Despite the dramatic suppression of recombination within the *mat2/3* region, recombination required for utilization of the donor loci during mating-type switching occurs efficiently. As it was possible that Swi2 is required for chromatin remodeling at the

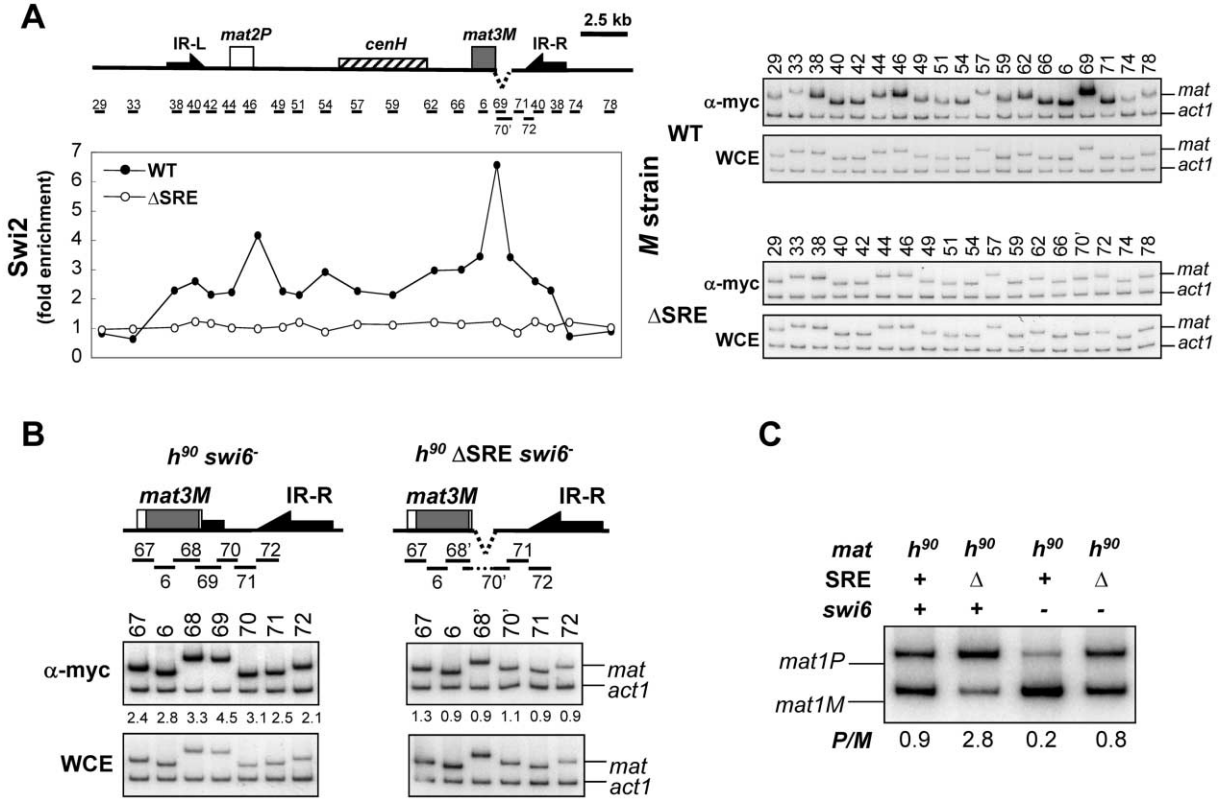


Figure 4. SRE Is a Recombination Enhancer that Recruits Swi2

(A) Deletion of the SRE element abolished the binding of Swi2 to the entire silent mating-type region in *M* cells. A diagram of the *mat2/3* region is shown. Deletion of a 454 bp sequence to the right of *mat3* corresponding to SRE is indicated by a dotted line. Numbered bars indicate primers used for ChIP. ChIP analysis was performed with the indicated strains to measure Swi2-myc levels at the *mat* locus (right). Quantification of the relative fold enrichment of Swi2 is plotted in alignment with the map of the *mat* locus (left).

(B) Swi2 binding to the *mat3* region in a *swi6* mutant requires the SRE element. Schematic diagrams of the sequence around *mat3* are shown (top). ChIP analysis was performed to examine the localization of Swi2-myc to selected regions of the *mat* locus in the presence or absence of SRE in a *swi6-115* background (bottom).

(C) Deletion of SRE affects donor choice similar to Δ*swi2* (see Figure 1B). The genetic content at *mat1* was quantified with multiplex PCR using genomic DNA isolated from indicated strains. The *P/M* ratio is shown beneath each lane.

donor loci making them accessible to the recombination machinery, we compared chromatin structure around the SRE element in wild-type and Δ*swi2* strains. Micrococcal nuclease (MNase) digestion and end-labeling methods were applied to probe nucleosome positioning in vivo (Mizuno et al., 1997). Although loss of Swi2 resulted in altered nucleosome positioning at the SRE element, as indicated by the appearance of two new MNase cleavage sites in this region, no change in chromatin structure at the nearby *mat3* locus was observed (Figure 6). Similarly, comparison of chromatin structure in wild-type and Δ*swi2* cells did not reveal any significant differences in local chromatin at the *mat2* locus (data not shown). Thus, Swi2 does not appear to act by regulating the local chromatin structure of the donor loci. The significance of the changes in nucleosome positioning observed at the SRE in Δ*swi2* cells remains to be explored, but most likely is a manifestation of loss of Swi2 binding to this region.

## Discussion

The process of directionality of switching in fission yeast, wherein cell type dictates which of the silent mating-type loci will serve as donor during mating-type switching, provides a striking example of developmentally regulated interactions between distant chromosomal regions. It has been suggested that directionality is regulated by heterochromatic structures present at the silent mating-type region (Thon and Klar, 1993; Grewal and Klar, 1997), but how heterochromatin could mediate the nonrandom choice of donors has remained unclear. In this study, we discovered that Swi2 protein is required for directionality of switching and is the critical factor that connects heterochromatin at the silent mating-type region to donor choice preference. A remarkable finding is that Swi2 exhibits cell type-specific localization patterns at the mating-type region. Swi2 binds specifically to a *cis*-acting recombination enhancer, SRE, located adjacent to *mat3* in *P* cells, but it spreads throughout the entire 20-kb heterochromatic *mat2/3* region in *M* cells. The spreading of Swi2 from the SRE site to across the *mat2/3* interval depends upon heterochromatin complexes, in particular Swi6 protein coating this entire region. We demonstrate that Swi2 is required for the localization of a recombination repair protein Swi5, which also displays cell type-specific distribution at the *mat2/3* region, and together these proteins activate do-

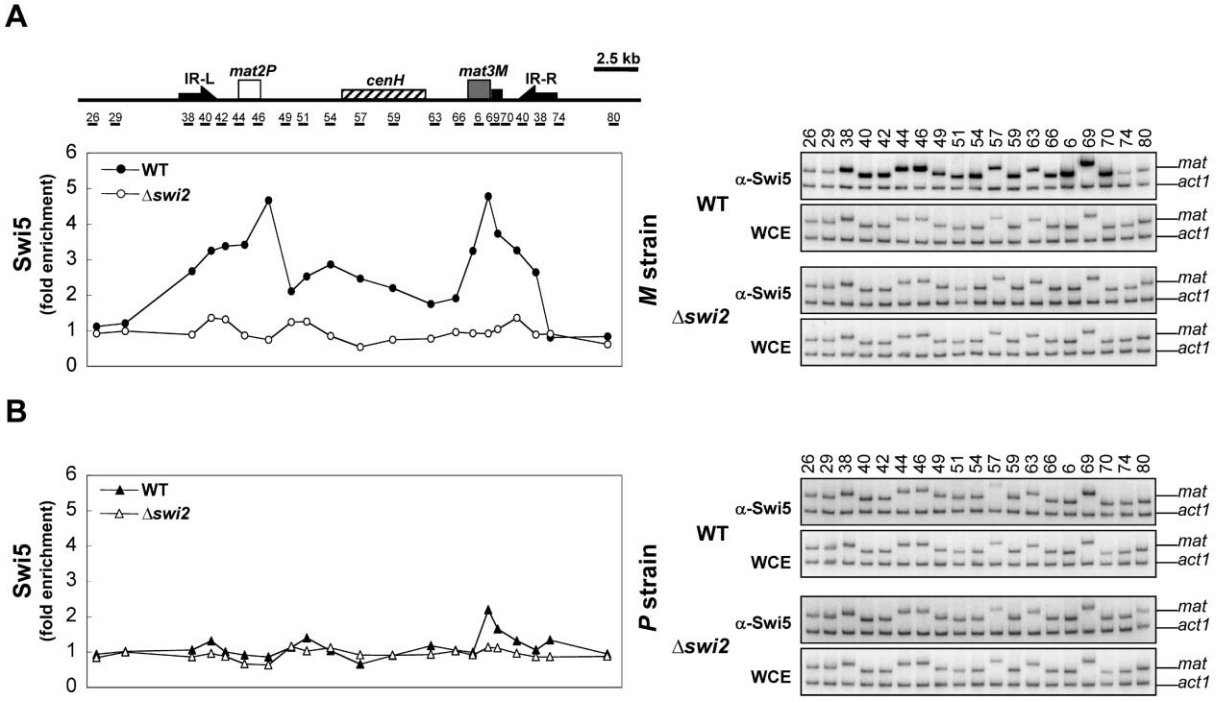


Figure 5. Mapping of Swi5 Levels across the Silent Mating-Type Region

(A) Localization of Swi5 in *M* strain. ChIP analysis with a Swi5 antibody was performed with the indicated strains to measure Swi5 levels throughout the *mat2/3* region (right). Quantification of these results is plotted in alignment with the map of the *mat* locus (left).

(B) Localization of Swi5 in *P* strains as performed in (A).

nors for recombination during mating-type switching. Independent of its role in the spreading of Swi2, heterochromatin seems to impose at the mating-type region structural organization that is also important for directionality of switching. We discuss the implications of our findings for long-range regulatory interactions in yeast and in higher eukaryotes.

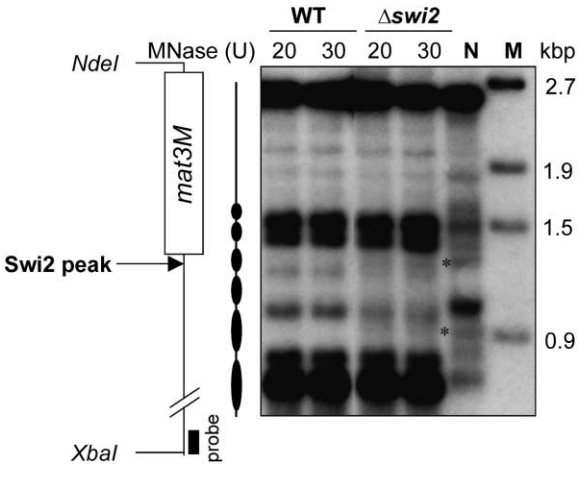


Figure 6. Effect of Δ*swi2* on Chromatin Structure Surrounding the SRE Element

Chromatin fractions were prepared from wild-type and Δ*swi2* cells and treated with 20 or 30 units/ml of MNase at 37°C for 5 min. Purified DNA was digested with NdeI and XbaI and displayed on Southern blots. The diagram shows the positions of restriction enzyme sites, Swi2 binding peak, and *mat3M* locus. Filled ovals represent positions of nucleosomes in wild-type cells. Lanes N and M represent naked DNA treated with MNase and DNA size markers, respectively. Asterisks indicate Mnase-sensitive sites in Δ*swi2* cells.

### The Recruitment and Cell Type-Specific Spreading of Swi2: A Heterochromatin Connection

Our analyses suggest that the localization of Swi2 to the *mat* locus is a highly regulated event. Swi2 can bind to the SRE element regardless of the cell type or the presence of heterochromatin, but the spreading of Swi2 across the silent mating-type interval is strictly dependent upon the heterochromatin complexes distributed throughout this region. More importantly, the spreading of Swi2 occurs in *M* cells but not in *P* cells, even though heterochromatin is present at the *mat2/3* region in both cell types. The exact mechanism by which Swi2 localizes to the SRE element and spreads in a cell type-specific manner remains to be elucidated. However, we note that Swi2 contains two putative AT hook motifs, which are characteristic of high mobility group (HMG) proteins that bind preferentially to AT-rich sequences. It is therefore possible that Swi2 binds directly to the SRE DNA, which has high AT content. This initial binding of Swi2 to the SRE is a prerequisite for the subsequent heterochromatin-mediated spreading of Swi2 (Figure 4) and might explain the lack of Swi2 localization at the centromeres, which presumably are deficient in Swi2 binding sites. As a result, Swi2 cannot be recruited to centromeres even though heterochromatin is highly enriched at these loci.

Among the components of heterochromatin, we find

that Swi6 is a critical structural element required for the spreading of Swi2 at the mating-type region. Mutations in Swi6 or factors that affect Swi6 localization to the heterochromatic loci, such as Clr4 histone methyltransferase, abolish Swi2 spreading. Several lines of evidence suggest that Swi2 physically interacts with Swi6. First, immunofluorescence analysis shows that Swi2 and Swi6 colocalize at specific foci in the nucleus. Second, Swi2 binds directly to Swi6 in vitro, and these proteins form a complex in vivo. Third, Swi2 interacts with Swi6 in a two-hybrid screen (Akamatsu et al., 2003). Despite the strong interaction between these proteins in vitro, we observed that only a small proportion of Swi2 is associated with Swi6 in vivo (Figure 2). Moreover, Swi2 selectively colocalizes with Swi6. These results suggest that additional factors contribute to the interaction between Swi2 and Swi6. The loading of Swi2 to heterochromatic loci by binding to specific DNA elements, such as SRE, is likely an important requirement for its interaction with Swi6. More importantly, the interaction between Swi2 and Swi6 is in all likelihood regulated by cell type-specific factors. It is possible that an *M* cell type-specific factor(s) cooperates with Swi6 to actively promote spreading of Swi2. Alternatively, *P* cells encode factor(s) that adversely affects Swi2 interaction with Swi6, thus prohibiting the spreading of Swi2.

Based on our analyses, Swi2 acts downstream from Swi6 localization at heterochromatic loci. Consistent with this idea, the loss of Swi2 does not affect heterochromatin assembly at the mating-type region nor at centromeres. A possibility remains, however, that selective interactions between Swi2 and Swi6 in *M* cells stabilize heterochromatic structures and might account for subtle differences in silencing at the mating-type region of *M* versus *P* cells (Ayoub et al., 1999). In this respect, we previously demonstrated that *M* cells contain relatively higher levels of Swi6 throughout the *mat2/3* interval than *P* cells, even though levels of H3-K9 methylation are comparable in each cell type (Noma et al., 2001).

### The Role of Swi2 in Donor Preference

How does Swi2 regulate the directionality of switching? We have demonstrated that Swi2 is required for the recruitment of Swi5 to the mating-type region. Moreover, the presence of Swi5 at the *mat2/3* region in *M* and *P* cells coincides with the distribution of Swi2 across this interval. Since Swi5 is known to be a component of the recombination and DNA repair complex that includes Rhp51 (Akamatsu et al., 2003), it is conceivable that Swi2 could mediate the localization of Rhp51 together with the rest of the recombination machinery to the *mat2/3* region to form a recombination complex that can promote activation of specific donor loci for recombination during switching. Consistent with this idea, Swi2 has been shown to serve as a molecular bridge that can promote the interaction between Swi5 and Rhp51 (Akamatsu et al., 2003), and both Swi5 and Rhp51 are required for mating-type switching (Akamatsu et al., 2003; Grishchuk et al., 2004). Moreover, switching defects caused by Δ*swi5* are indistinguishable from defects observed in Δ*swi2* strains (our unpublished data). The recruitment of Rhp51 and other recombination proteins, triggered by the generation of a DSB as the DNA replication fork passes through *mat1* region (Arcangioli, 1998; Dalgaard and Klar, 1999), is probably transient and occurs only at a specific window of S phase. Predictably, our ChIP assays have not been able to capture the presence of Rhp51 at the *mat* locus (our unpublished data). Nevertheless, our ability to detect Swi5 at the *mat2/3* region in an unsynchronized cell population composed primarily of G2 cells indicates that Swi2 could recruit Swi5 first to the mating-type region independent of Rhp51, and that Swi2 and Swi5 subsequently cooperate to recruit Rhp51 and the rest of the recombination apparatus briefly to the *mat* locus during switching. While Swi5 and Rhp51 mutants are sensitive to DNA-damaging agents, Swi2 mutants do not display any obvious sensitivity (Akamatsu et al., 2003; our unpublished data). This apparent paradox was solved recently in a study showing the existence of Swi5-Rhp1 in another complex containing a second Swi2-like protein, named Sfr1, that function in general recombination pathway (Akamatsu et al., 2003).

### A Model for Directionality of Switching

Our analyses suggest that directionality of switching in fission yeast consists of two primary components, namely heterochromatin and the Swi2-Swi5-containing recombination-promoting (RPC) complex, and together their orchestration ensures switching to the opposite mating type in each cell type (see Figure 7). Analyses of mutant strains defective in heterochromatin formation at the *mat2/3* region indicate that the presence of heterochromatin at the silent mating-type domain performs two distinct functions to ensure nonrandom utilization of donor loci. First, heterochromatin makes the *mat2* locus more accessible, and thus more likely than the *mat3* locus to serve as the donor during mating-type switching. This is most likely accomplished by heterochromatin-mediated organization of the silent mating-type region into a higher-order structure that brings *mat2* locus in close proximity to the *mat1* locus (Grewal and Klar, 1997). Second, heterochromatin promotes directionality by regulating the cell type-specific spreading of RPC from the SRE element to across the *mat2/3* interval. Importantly, the location of where RPC binds has a great influence on which silent mating-type locus will be selected as the donor site.

Switching is initiated by formation of the DSB at the *mat1* locus, which triggers the recruitment of DNA repair factors that facilitate the search for homologous donor sequences to repair the DSB. Heterochromatin-mediated higher-order folding of the *mat2/3* region serves to guide the search process such that the repair machinery scans the nearby *mat2* for accessibility as a donor before *mat3*. However, the actual choice of donor is predominantly determined by the localization of RPC whose presence abets the DNA repair factors in recognizing and selecting the corresponding donor site for recombination. This two-component directionality model accounts for the preferential utilization of donors seen in *M* and *P* cell types (Figure 7A). First, in *M* cells where RPC is distributed throughout the silent mating-type region, the favored interaction between *mat1* and *mat2* that is attributed to higher-order chromatin structure at the *mat2/3* region ensures efficient utilization of *mat2*

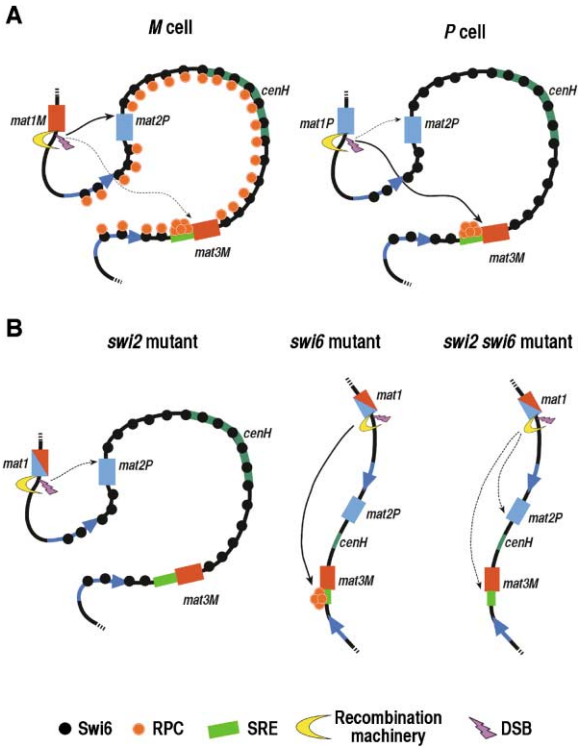


Figure 7. A Model for Directionality of Switching

(A) Directionality control in wild-type cells. Mating-type switching is initiated by the generation of a DSB adjacent to *mat1*, which is recognized by the recombination machinery. Heterochromatin at the *mat2/3* interval mediates formation of a higher-order structure that ensures that DSB will encounter *mat2* before *mat3* during search for donor loci. Recombination occurs at the first donor where RPC is present. Therefore, in *M* cells the presence of RPC at *mat2* makes this locus a preferred donor, whereas in *P* cells the restricted binding of RPC to the SRE element makes *mat3* the preferred donor (solid line arrows). The utilization of the wrong donor at a low frequency (thin dotted line arrows) likely accounts for the leakiness in directionality control.

(B) Altered directionality control in switching mutants. Left, the loss of RPC in *swi2* mutant reduces recombination frequency at both donors, nonetheless *mat2* is preferentially utilized as a donor because of the favored interaction between *mat1* and *mat2* mediated by heterochromatin. Middle, mutation in *swi6* abolishes higher-order chromatin organization at *mat2/3* interval as well as prevents the spreading of RPC. The presence of RPC at the SRE in *swi6* mutant cells makes *mat3* the preferred donor. Right, the loss of both heterochromatin and RPC in *swi2swi6* double mutant cells results in random utilization of donor loci. Solid and dotted line arrows denote efficient and inefficient switching, respectively.

donor during switching. In contrast, in *P* cells, the restricted binding of RPC to the SRE element makes *mat3* the preferred donor. In this case, the absence of RPC at the *mat2* locus makes this site less accessible for recombination, so the DNA repair machinery continues to search until it encounters *mat3*, where the RPC bound to the SRE promotes efficient recombination. Therefore, heterochromatin and RPC ensure efficient switching to the opposite mating type in both cell types. It is possible that in *M* cells, the homology search mechanism occasionally bypasses the *mat2* locus and instead uses *mat3* as a donor, and similarly in *P* cells, *mat2* might be used as a donor at low levels even in the absence of the RPC. This leakiness in directionality control might result in nonproductive switching events in a small fraction of cells. Indeed, fission yeast cells switch to the opposite mating type in about 72%–90% of the cell divisions (Miyata and Miyata, 1981).

The model proposed above is fully compatible with the phenotypes seen in different directionality mutants (Figure 7B). For example, in *swi2* mutant strains, the absence of RPC reduces the recombination efficiency at both donors, allowing the influence of heterochromatin-mediated preferential interaction between *mat1* and *mat2* to dominate. As a result, *mat2* is utilized more frequently as the donor site than *mat3*. In contrast, in *swi6* mutant strains, impaired heterochromatin abolishes the preferential interaction between *mat1* and *mat2* loci and prevents the spreading of RPC throughout the *mat2/3* region in *M* cells. Consequently, RPC localization is restricted to the SRE element in both *M* and *P* cells, making *mat3* the preferred donor site in both cell types. Importantly, confirming the key prediction of our model, the loss of both heterochromatin and RPC in a *swi2swi6* double mutant strain confers equal probabilities on both *mat2* and *mat3* to serve as donors. However, in contrast to highly regulated donor choice in wild-type cells, the choice of donor in the *swi2swi6* double mutant is random regardless of the cell type, causing relatively inefficient conversion of mating type. Consistent with this idea, *swi2swi6* double mutant colonies do not completely recapitulate the dark iodine-staining pattern of wild-type strain colonies (Figure 1A). Taken together, the observations described here argue that donor choice is a highly dynamic process that not only reduces futile homologous donor search but also improves the precision of DNA recombination.

### Possible Relation of Donor Preference Mechanism in Fission Yeast to Long-Range Regulatory Interactions in Other Systems

The ability of the SRE element to activate recombination at a distance through a mechanism that involves cell type-specific spreading of RPC from the SRE site to across the *mat2/3* domain has important implications for developmentally controlled long-range regulatory interactions. Eukaryotic genomes are known to contain small regulatory DNA elements that can exert regional control in *cis* over considerable distances (West et al., 2002). Such DNA elements have been suggested to regulate gene expression, recombination, and/or DNA replication in diverse species (Blackwood and Kadonaga, 1998; Haber, 1998; Dorsett, 1999; Park and Kuroda, 2001; Stam et al., 2002). In *S. cerevisiae*, a 700 bp recombination enhancer that regulates the recombination potential of an entire chromosome arm is required for directionality of mating-type switching (Wu and Haber, 1996; Szeto et al., 1997). However, the exact mechanism of how this element activates recombination is not fully understood. By analogy to the SRE element, it is possible that the recombination enhancer in *S. cerevisiae* also serves as a loading site for a protein complex similar to RPC, which then spreads outward across the entire chromosome arm. The existence of long-range recombination enhancers in distantly related budding and fission yeasts indicate that this mode of recombination control

might be conserved in other species. Indeed, site-specific recombination in V(D)J regions in the immune system of mammals, which involves cell type-specific changes in chromatin structure (Stanhope-Baker et al., 1996), is stimulated by enhancer elements that also regulate transcription of the immunoglobulin genes in B and T cells (Capone et al., 1993). Another well-studied example of long-range control is the locus control region (LCR) in humans that regulates expression of β-globin genes from a distance of 50 kb and participates in initiation of DNA replication (Aladjem et al., 1995). The mechanisms proposed for long-range interactions in this case include looping between the LCR and the target gene promoter, or that the LCR serves as an entry site for factors that then track along the chromosome to the target promoter and create a favorable environment for gene transcription (Blackwood and Kadonaga, 1998; Carter et al., 2002; Tolhuis et al., 2002). In this regard, we note that donor preference in fission yeast depends upon both the spreading of RPC from the SRE entry site across the silent mating-type domain and the preferential interaction between the *mat1* and *mat2* loci mediated by heterochromatin.

Long-range regulatory interactions provide the flexibility for the coregulation of related genes during development or to modulate various other chromosomal properties over long distances. However, this convenience is achieved at the risk of selecting the wrong targets. Cells have evolved mechanisms to protect individual loci or extended chromosomal domains from such inappropriate signals, which in some cases involve specialized DNA elements termed insulators or boundary elements (West et al., 2002). In this regard, it is noteworthy that the spreading of RPC at the mating-type region of fission yeast is limited to the heterochromatic domain surrounded by IR-L and IR-R boundary elements (Figure 3). Our finding that heterochromatin promotes spreading of RPC and regulate donor preference suggests a novel role for this specialized form of chromatin in long-range regulatory interactions. Such interactions might be important for proper expression in *Drosophila* of *light* and *rolled* genes, which require heterochromatin for their proper expression (Lu et al., 2000). This fits into a growing body of evidence that heterochromatin acts as a platform for the recruitment of other factors that are important for fundamental chromosomal processes such as kinetochore formation, sister chromatid cohesion, and meiotic chromosome pairing, instead of passively serving as a dead-end pathway to permanently inactivate genes.

## Experimental Procedures

### Strains

*swi2* deletion strain as well as *swi2-myc* strain were constructed with a PCR-based module method (Bahler et al., 1998). *swi2-myc* strain was created by fusing four copies of the myc epitope tag to the C terminus of Swi2. To generate ΔSRE strain, we first inserted the *ura4*$^+$ gene at the EcoRV site near *mat3* and then replaced the *ura4*$^+$ with a DNA fragment containing the deletion of 454 bp sequences to the right of *mat3* by homologous recombination.

A natural variant of an uncharacterized locus *swi6-mod* has been shown to suppress the switching defect in *swi6* mutant cells, similar to Δ*swi2*. Our analyses showed that *swi2* and *swi6-mod* are two distinct loci (our unpublished data). First, sequencing of the *swi2* coding region and surrounding sequences from a strain carrying *swi6-mod* did not reveal any mutation. Second, *swi2* and *swi6-mod* segregate as independent markers in genetic crosses. Strains used in this study do not contain *swi6-mod*.

### Mating-Type Switching Assays

Iodine-staining procedure was used to estimate the efficiency of switching as described previously (Grewal and Klar, 1997). Switching efficiency was also examined by a PCR-based assay. In this assay, genomic DNA prepared from wild-type and mutant strains was subjected to quantitative multiplex PCR to determine the genetic content at *mat1*. The primers used were MT1 (common to *mat1-P* and *mat1-M*) 5′-AGAAGAGAGAGTAGTTGAAG-3′; MP (*mat1-P* specific) 5′-ACGGTAGTCATCGGTCTTCC-3′; and MM (*mat1-M* specific) 5′-TACGTTCAGTAGACGTAGTG-3′. *mat1-P*- and *mat1-M*-specific PCR fragments were simultaneously amplified, resolved on polyacrylamide gels, and quantified using phosphoimager.

### Immunofluorescence and ChIP

Immunofluorescence analysis was carried out as previously described (Nakayama et al., 2001a). All samples were analyzed with a Zeiss Axioplan2 fluorescent microscope. For deconvolution, images were collected at 0.2 μm intervals and subjected to volume deconvolution using the nearest three neighbors. OPENLAB software (Improvision) was used for all analyses.

High-resolution ChIP analysis was carried out essentially as previously described (Noma et al., 2001). IPs were performed with Swi5, Swi6, H3-K9 di-methyl (Upstate Biotechnology), and myc (9E10) antibodies.

### Chromatin Analysis

Chromatin structure surrounding donor loci was analyzed by MNase digestion assay essentially as described (Mizuno et al., 1997). Briefly, genomic DNA was purified from Mnase-treated chromatin fractions and digested with NdeI and XbaI. The hybridization probe was made by PCR amplification, using plasmid pIRRD::kanMX as a template with primers M1007 (CTTTGCTCCAGATAGCAAATCTG) and M1008 (CTTTTCACGGAAATCTCCTGCCGA). The amplified fragment was gel purified and labeled with $^{32}$P-α-dCTP by random prime method.

### Acknowledgments

We thank H. Iwasaki for providing the Swi5 and Rhp51 antibodies. We also thank H. Cam, I. Hall, M. Lichten, C. Wu, and Y. Rong for critical reading of the manuscript and members of the Grewal laboratory for helpful discussions.

Received: August 3, 2004
Revised: September 24, 2004
Accepted: October 7, 2004
Published: November 11, 2004

## References

Akamatsu, Y., Dziadkowiec, D., Ikeguchi, M., Shinagawa, H., and Iwasaki, H. (2003). Two different Swi5-containing protein complexes are involved in mating-type switching and recombination repair in fission yeast. Proc. Natl. Acad. Sci. USA *100*, 15770–15775.

Aladjem, M.I., Groudine, M., Brody, L.L., Dieken, E.S., Fournier, R.E., Wahl, G.M., and Epner, E.M. (1995). Participation of the human beta-globin locus control region in initiation of DNA replication. Science *270*, 815–819.

Arcangioli, B. (1998). A site- and strand-specific DNA break confers asymmetric switching potential in fission yeast. EMBO J. *17*, 4503–4510.

Arcangioli, B., and Klar, A.J. (1991). A novel switch-activating site (*SAS1*) and its cognate binding factor (SAP1) required for efficient mat1 switching in *Schizosaccharomyces pombe*. EMBO J. *10*, 3025–3032.

Ayoub, N., Goldshmidt, I., and Cohen, A. (1999). Position effect variegation at the mating-type locus of fission yeast: a cis-acting

element inhibits covariegated expression of genes in the silent and expressed domains. Genetics *152*, 495–508.

Bahler, J., Wu, J.Q., Longtine, M.S., Shah, N.G., McKenzie, A., 3rd, Steever, A.B., Wach, A., Philippsen, P., and Pringle, J.R. (1998). Heterologous modules for efficient and versatile PCR-based gene targeting in *Schizosaccharomyces pombe*. Yeast *14*, 943–951.

Baumann, P., and West, S.C. (1998). Role of the human RAD51 protein in homologous recombination and double-stranded-break repair. Trends Biochem. Sci. *23*, 247–251.

Beach, D.H. (1983). Cell type switching by DNA transposition in fission yeast. Nature *305*, 682–688.

Blackwood, E.M., and Kadonaga, J.T. (1998). Going the distance: a current view of enhancer action. Science *281*, 61–63.

Bresch, C., Muller, G., and Egel, R. (1968). Genes involved in meiosis and sporulation of a yeast. Mol. Gen. Genet. *102*, 301–306.

Capone, M., Watrin, F., Fernex, C., Horvat, B., Krippl, B., Wu, L., Scollay, R., and Ferrier, P. (1993). TCR beta and TCR alpha gene enhancers confer tissue- and stage-specificity on V(D)J recombination events. EMBO J. *12*, 4335–4346.

Carter, D., Chakalova, L., Osborne, C.S., Dai, Y.F., and Fraser, P. (2002). Long-range chromatin regulatory interactions in vivo. Nat. Genet. *32*, 623–626.

Courey, A.J., and Jia, S. (2001). Transcriptional repression: the long and the short of it. Genes Dev. *15*, 2786–2796.

Dalgaard, J.Z., and Klar, A.J.S. (1999). Orientation of DNA replication establishes mating-type switching pattern in *S. pombe*. Nature *400*, 181–184.

Dalgaard, J.Z., and Klar, A.J.S. (2000). *swi1* and *swi3* perform imprinting, pausing, and termination of DNA replication in *S. pombe*. Cell *102*, 745–751.

Dorsett, D. (1999). Distant liaisons: long-range enhancer-promoter interactions in *Drosophila*. Curr. Opin. Genet. Dev. *9*, 505–514.

Egel, R., and Gutz, H. (1981). Gene actiation by copy transposition in mating-type switching of a homothallic fission yeast. Curr. Genet. *12*, 429–433.

Egel, R., Beach, D.H., and Klar, A.J.S. (1984). Genes required for initiation and resolution steps of mating-type switching in fission yeast. Proc. Natl. Acad. Sci. USA *81*, 3481–3485.

Ekwall, K., Javerzat, J.P., Lorentz, A., Schmidt, H., Cranston, G., and Allshire, R. (1995). The chromodomain protein Swi6: a key component at fission yeast centromeres. Science *269*, 1429–1431.

Engelke, U., Grabowski, L., Gutz, H., Heim, L., and Schmidt, H. (1987). Molecular characterization of $h^-$ mutants of *Schizosaccharomyces pombe*. Curr. Genet. *12*, 535–542.

Gellert, M. (2002). V(D)J recombination: RAG proteins, repair factors, and regulation. Annu. Rev. Biochem. *71*, 101–132.

Grewal, S.I.S. (2000). Transcriptional silencing in fission yeast. J. Cell. Physiol. *184*, 311–318.

Grewal, S.I., and Klar, A.J.S. (1997). A recombinationally repressed region between *mat2* and *mat3* loci shares homology to centromeric repeats and regulates directionality of mating-type switching in fission yeast. Genetics *146*, 1221–1238.

Grishchuk, A.L., Kraehenbuehl, R., Molnar, M., Fleck, O., and Kohli, J. (2004). Genetic and cytological characterization of the RecA-homologous proteins Rad51 and Dmc1 of *Schizosaccharomyces pombe*. Curr. Genet. *44*, 317–328.

Haber, J.E. (1998). Mating-type gene switching in *Saccharomyces cerevisiae*. Annu. Rev. Genet. *32*, 561–599.

Hall, I.M., Shankaranarayana, G.D., Noma, K., Ayoub, N., Cohen, A., and Grewal, S.I.S. (2002). Establishment and maintenance of a heterochromatin domain. Science *297*, 2232–2237.

Ivanova, A.V., Bonaduce, M.J., Ivanov, S.V., and Klar, A.J.S. (1998). The chromo and SET domains of the Clr4 protein are essential for silencing in fission yeast. Nat. Genet. *19*, 192–195.

Jia, S., Noma, K., and Grewal, S.I.S. (2004). RNAi-independent heterochromatin nucleation by the stress-activated ATF/CREB family proteins. Science *304*, 1971–1976.

Kaykov, A., Holmes, A.M., and Arcangioli, B. (2004). Formation, maintenance and consequences of the imprint at the mating-type locus in fission yeast. EMBO J. *23*, 930–938.

Klar, A.J.S. (1989). The interconversion of yeast mating type: *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*. In Mobile DNA, D.E. Berg and M.W. Howe, eds. (Washington, D.C.: American Society for Microbiology), pp. 671–691.

Klar, A.J.S., and Bonaduce, M.J. (1991). *swi6*, a gene required for mating-type switching, prohibits meiotic recombination in the *mat2-mat3* "cold spot" of fission yeast. Genetics *129*, 1033–1042.

Lu, B.Y., Emtage, P.C., Duyf, B.J., Hilliker, A.J., and Eissenberg, J.C. (2000). Heterochromatin protein 1 is required for the normal expression of two heterochromatin genes in *Drosophila*. Genetics *155*, 699–708.

Miyata, H., and Miyata, M. (1981). Mode of conjugation in homothallic cells of *Schizosaccharomyces pombe*. J. Gen. Appl. Microbiol. *27*, 365–371.

Mizuno, K., Emura, Y., Baur, M., Kohli, J., Ohta, K., and Shibata, T. (1997). The meiotic recombination hot spot created by the single-base substitution ade6–M26 results in remodeling of chromatin structure in fission yeast. Genes Dev. *11*, 876–886.

Nakayama, J., Allshire, R.C., Klar, A.J., and Grewal, S.I.S. (2001a). A role for DNA polymerase $\alpha$ in epigenetic control of transcriptional silencing in fission yeast. EMBO J. *20*, 2857–2866.

Nakayama, J., Rice, J.C., Strahl, B.D., Allis, C.D., and Grewal, S.I.S. (2001b). Role of histone H3 lysine 9 methylation in epigenetic control of heterochromatin assembly. Science *292*, 110–113.

Noma, K., Allis, C.D., and Grewal, S.I.S. (2001). Transitions in distinct histone H3 methylation patterns at the heterochromatin domain boundaries. Science *293*, 1150–1155.

Park, Y., and Kuroda, M.I. (2001). Epigenetic aspects of X-chromosome dosage compensation. Science *293*, 1083–1085.

Shankaranarayana, G.D., Motamedi, M.R., Moazed, D., and Grewal, S.I.S. (2003). Sir2 regulates histone H3 lysine 9 methylation and heterochromatin assembly in fission yeast. Curr. Biol. *13*, 1240–1246.

Stam, M., Belele, C., Dorweiler, J.E., and Chandler, V.L. (2002). Differential chromatin structure within a tandem array 100 kb upstream of the maize *b1* locus is associated with paramutation. Genes Dev. *16*, 1906–1918.

Stanhope-Baker, P., Hudson, K.M., Shaffer, A.L., Constantinescu, A., and Schlissel, M.S. (1996). Cell type-specific chromatin structure determines the targeting of V(D)J recombinase activity in vitro. Cell *85*, 887–897.

Szeto, L., Fafalios, M.K., Zhong, H., Vershon, A.K., and Broach, J.R. (1997). Alpha2p controls donor preference during mating type interconversion in yeast by inactivating a recombinational enhancer of chromosome III. Genes Dev. *11*, 1899–1911.

Thon, G., and Klar, A.J.S. (1993). Directionality of fission yeast mating-type interconversion is controlled by the location of the donor loci. Genetics *134*, 1045–1054.

Thon, G., and Verhein-Hansen, J. (2000). Four chromo-domain proteins of *Schizosaccharomyces pombe* differentially repress transcription at various chromosomal locations. Genetics *155*, 551–568.

Tolhuis, B., Palstra, R.J., Splinter, E., Grosveld, F., and de Laat, W. (2002). Looping and interaction between hypersensitive sites in the active beta-globin locus. Mol. Cell *10*, 1453–1465.

Tuzon, C.T., Borgstrom, B., Weilguny, D., Egel, R., Cooper, J.P., and Nielsen, O. (2004). The fission yeast heterochromatin protein Rik1 is required for telomere clustering during meiosis. J. Cell Biol. *165*, 759–765.

West, A.G., Gaszner, M., and Felsenfeld, G. (2002). Insulators: many functions, many mechanisms. Genes Dev. *16*, 271–288.

Wu, X., and Haber, J.E. (1996). A 700 bp cis-acting region controls mating-type dependent recombination along the entire left arm of yeast chromosome III. Cell *87*, 277–285.