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swi1 and *swi3* Perform Imprinting, Pausing, and Termination of DNA Replication in *S. pombe*

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Summary

The developmental program of cell-type switching of *S. pombe* requires a strand-specific imprinting event at the mating-type locus (*mat1*). Imprinting occurs only when *mat1* is replicated in a specific direction and requires several *trans*-acting factors. This work shows (1) that the factors swi1p and swi3p act by pausing the replication fork at the imprinting site; and (2) that swi1p and swi3p are involved in termination at the *mat1*-proximal polar-terminator of replication (*RTS1*). A genetic screen to identify termination factors identified an allele that separated pausing/imprinting and termination functions of swi1p. These results suggest that swi1p and swi3p promote imprinting in novel ways both by pausing replication at *mat1* and by terminating replication at *RTS1*.

Introduction

Schizosaccaromyces pombe is a fission yeast whose cells exhibit two different mating types, called plus (P) and minus (M) (Figure 1a; reviewed in Egel, 1989, and Klar et al., 1998). During starvation, haploid cells of opposite mating type mate, and the resulting zygote then undergoes meiosis and sporulation to produce four haploid spores. Homothallic haploid cells are able to switch between the two mating types during mitotic growth in a distinct asymmetrical pattern: When a newly switched cell divides, it creates a "switchable" cell and an "unswitchable" cell (Miyata and Miata, 1981; Egel and Eie, 1987; Klar, 1987, 1990). A switchable cell undergoes asymmetrical division producing a switched and a switchable cell, while an unswitchable cell forms an unswitchable and a switchable cell (Figure 1b, bold letters). Thus, only one in four related granddaughter cells of an unswitchable cell ever switches.

The mating type of *S. pombe* is determined by the *mat1* locus located at chromosome II (Figure 1a). Mating-type switching occurs when the DNA cassette at *mat1* is replaced with a cassette that contains the opposite mating-type information (Beach, 1983; Beach and Klar, 1984; Kelly et al., 1988). The replacement proceeds via a DNA recombination event that utilizes one of two donor cassettes, located distal to *mat1,* as a source of genetic information. These *mat2P* and *mat3M* donor cassettes are transcriptionally silent and only function as storage of the mating-type information (reviewed in Klar et al., 1998). According to the direction of replication model, the asymmetrical switching pattern is established by utilization of the asymmetry of DNA replication (Figure 1b; Dalgaard and Klar, 1999). A chromosomally inherited, strand-specific *mat1* imprint is central in dictating the pattern of switching in cell pedigrees (Egel and Eie, 1987; Klar, 1987, 1990; Klar and Bonaduce, 1993). Lagging-strand replication is proposed to introduce an imprint at *mat1* and the cell that inherits the imprinted chromosome becomes switchable. The imprint is either a strand-specific nick (Arcangioli, 1998) or a site- and strand-specific alkali-labile modification of the DNA, hypothesized to be an RNA residue(s) remaining after priming of lagging-strand synthesis (Dalgaard and Klar, 1999) or another type of DNA modification. During standard DNA purification, the imprint is converted into a double-stranded break (DSB) that can be used to quantify the amount of imprinting at *mat1* (Beach, 1983; Beach and Klar, 1984; Arcangioli, 1998; Dalgaard and Klar, 1999).

According to the model, leading-strand replication induces a break when the replication fork encounters the imprint (Figure 1, shaded intermediate). This transient break induces the recombination event that leads to mating-type switching.

The site of the imprint was mapped to the junction between H1, one of two homology domains flanking *mat1,* and the *mat1* cassette-specific sequences (Nielsen and Egel, 1989; Arcangioli, 1998). A 262 bp *cis*-acting sequence located just distal to *mat1* was identified as necessary for imprinting, and the sap1p protein was shown to bind to two sites within this sequence (Arcangioli and Klar, 1991, 1994). The *swi1*, *swi3*, and *swi7* genes were genetically implicated in formation of the imprint, but the mechanism of their action remains unknown (Egel et al., 1984; Klar and Bonaduce, 1993). *swi7* encodes the catalytic subunit of DNA polymerase α (polα) (Singh and Klar, 1993). polα is predominately involved in lagging-strand synthesis, during which it performs both priming and DNA replication functions. In support of the direction of replication model, imprinting was shown to be dependent on the direction of replication at *mat1* (Dalgaard and Klar, 1999). Genetic rearrangements and analysis of replication intermediates showed that (a) *mat1* is replicated unidirectionally by forks moving in the centromere-proximal direction; (b) imprinting is abolished if *mat1* is inverted so it is only replicated in the nonnative direction; and (c) when competing origins are introduced on the proximal side of the inverted *mat1,* the efficiency of imprinting is partially restored. In summary, imprinting occurs only when the strand to be modified is synthesized by the polα lagging-strand replication complex (Figure 1). In our unpublished work, we have identified and implicated the *RTS1* element, located proximal to *mat1*, in controlling the direction of replication at *mat1* (J. Z. D. and A. J. S. K., unpublished data). This element acts as a polar terminator of replication, terminating forks moving in the distal direction (Figure 1a).

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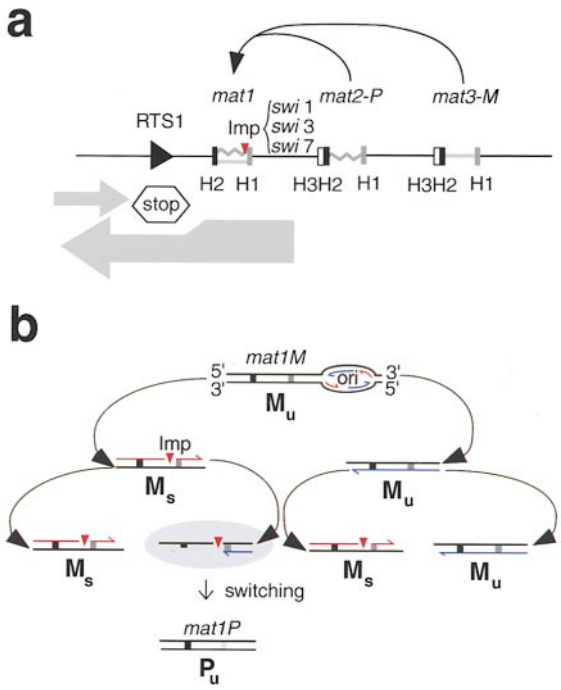

Figure 1. The *mat1* Region and the Switching Pattern of *S. pombe*

(a) Mating-type region. The overall organization of the mating-type cassettes *mat1*, *mat2P*, and *mat3M* is shown. The transfer of DNA cassette from *mat2P* or *mat3M* to *mat1* by recombination is indicated by thin arrows. H1, H2, and H3 are homology domains that flank the mating-type cassettes and provide homology for the recombination event. The position of the imprint is indicated by a red triangle. The *trans*-acting factors, *swi1*, *swi3*, and *swi7,* and the *cis*-acting *SAS* site are involved in imprinting. The polar terminator of replication *RTS1* regulates the direction of replication at *mat1*. *RTS1* arrests replication forks moving in the distal direction while allowing forks to move in the proximal direction (J. Z. D. and A. J. S. K., unpublished observation). Forks are shown by the gray arrows below the line drawing, and the width of the arrows roughly indicates relative contribution of replication in the indicated direction. The distances between different elements are not drawn to scale. (b) The direction of replication model superimposed on the mating-type switching pattern (bold letters). A pedigree starting with a newly switched M cell is shown. The subscripts "u" and "s" indicate unswitchable and switchable cells, respectively. The origin (ori) in the top drawing indicates that *mat1* is replicated by a (centromere-) distal origin(s). Imprinting is proposed to occur during lagging-strand synthesis (red), therefore only modifying one of the two sister chromatids. In a cell that inherited an imprinted chromosome (e.g., M_s) leading-strand replication complex runs into the modification in the DNA (shaded intermediate), resulting in the formation of the break that initiates *mat1* switching. The pattern of inheritance of different DNA chains at *mat1* is indicated by arrows.

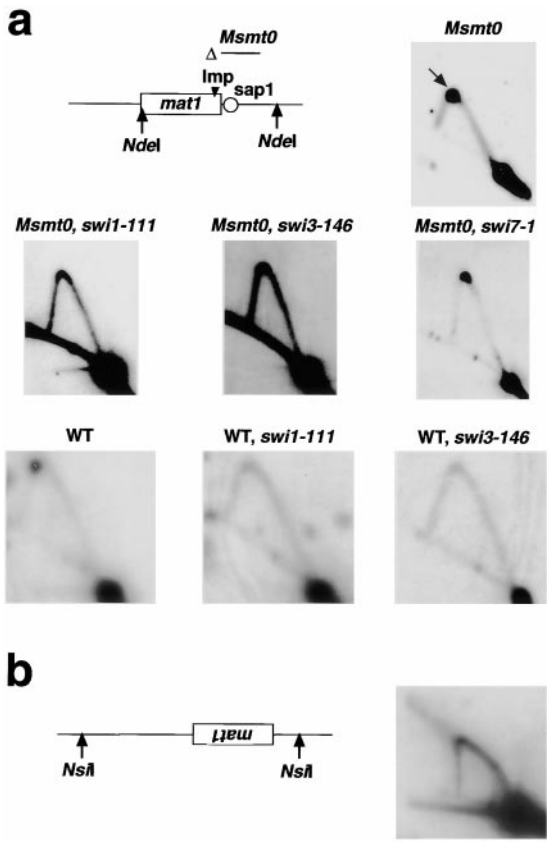

Figure 2. Replication Intermediates of *mat1*

(a) Pausing at *mat1* is dependent on swi1p and swi3p. Line-drawing: The site of imprinting is marked with a triangle (Imp) located approximately in the middle of the 3.2 kb NdeI restriction fragment (Imp). The deletion in the *Msmt0* strain removes the binding sites for the sap1p protein (circle). Autoradiograms of standard 2D gel analysis of replication intermediates are shown below. The following strains were analyzed: JZ108, *Msmt0*; SP814, *Msmt0, swi1–111*; SP815, *Msmt0, swi3–146*; SP840, *Msmt0, swi7–1*; SP918, h^{90} (wild-type), SP785, *swi1–111*; SP918, *swi3–146*; SP848, *swi7–1*. In the *Msmt0* panel, an arrow indicates the pause site observed at a position corresponding to the imprinting site. (b) Analysis of strain JZ148 carrying an inverted *mat1*. The size of the analyzed NsiI fragment is 6.1 kb. The large sized fragment causes the deformed shape of the observed arc. The line drawing indicates positions of relevant restriction sites. The weak barrier, observed as a spot on the ascending part of the arc, is due to the *RTS1* element. Termination of replication occurring at *RTS1* can be observed as a shadow protruding from the apex of the arc.

This paper addresses the cause of sensitivity of imprinting to the direction of replication and defines the molecular function of *swi1* and *swi3* gene products in imprinting. Data presented here show that swi1p and swi3p promote imprinting by pausing the replication forks at the imprinting site in a direction-dependent (polar) manner. Furthermore, *swi1* and *swi3* are also required for polar termination of replication at the *RTS1* site. A genetic screen for factors necessary for termination of replication identified a *swi1* allele that was defective for replication termination but allowed pausing/imprinting and switching. The identification of this allele demonstrated that the two functions of swi1p are separable. Computer searches with the *swi1* sequence identified swi1p as a homolog with the *Saccharomyces cerevisiae* topoisomerase I interacting factor (tof1p; Park and Sternglanz, 1999). Genetic crosses and drug-sensitivity experiments demonstrated a genetic interaction between *top1* and both *swi1* and *swi3*.

Results

Replication Pausing at *mat1* Is Dependent on swi1p and swi3p Functions

Even though the imprint site is present in *Msmt0* strains, *mat1* imprinting is absent due to a deletion of an adjacent *cis*-acting sequence containing the sap1p binding

site (Arcangoli and Klar, 1991; Styrkarsdottir et al., 1993; Figure 1a). Earlier experiments characterizing *mat1* replication intermediates from an *Msmt0* strain detected replication pausing at the site where the imprint is located in wild-type strains (Dalgaard and Klar, 1999; Figure 2a, panel *Msmt0*). This pause site is henceforth referred to as *MPS1* (*mat1* pause site 1). The localization of the pause site at the site of imprinting suggested that the paused replication species could be an intermediate of the imprinting reaction, but that these intermediates were nonproductive due to the *cis*-acting deletion present in the *Msmt0* strain. To test this hypothesis, strains were analyzed where the *Msmt0* deletion was combined with mutant alleles of *swi1*, *swi3*, or *swi7* known to adversely affect imprinting (Egel et al., 1984; Klar and Bonaduce, 1993). The 2D gel analysis showed that the *swi1–111* and *swi3–146* alleles abolished pausing at *MPS1,* while the *swi7–1* mutation had no effect (Figure 2a). These data supported the notion that the pause at *MPS1* was related to the imprinting process and that *MPS1* pausing was dependent on both swi1p and swi3p.

To exclude the possibility that *MPS1* pausing was an artifact due to *Msmt0* deletion, a wild-type strain was analyzed. The *MPS1*-pausing occurred at wild-type *mat1* as well (Figure 2; WT) and it was also dependent on swi1p and swi3p factors (Figure 2; *swi1* and *swi3*). Thus, *cis*-acting sequences containing sap1p binding site and defined by the *Msmt0* deletion do not influence *mat1* pausing. Furthermore, as predicted by the direction of the replication model, a linear fragment was observed of a size corresponding to the distal part of *mat1* (data not shown). The model suggests that this fragment is formed when leading-strand replication in imprinted cells encounters the imprint, resulting in formation of the transient DSB (Figure 1b, shaded intermediate).

Together these findings suggested that swi1p/swi3p-mediated pausing was required but not sufficient for imprinting. Furthermore, presence of the *MPS1* in *swi7* and *Msmt0* strains suggested that the swi7p and sap1p activities act downstream of swi1p and swi3p functions in the imprinting pathway. One possible function of *MSP1* is that it facilitates the lagging-strand replication complex's placement of a postulated RNA primer at a specific position.

Pausing Is Dependent on the Direction of Replication

Polar pausing and termination of replication have been observed in several systems: (a) the *Saccharomyces cerevisiae* rDNA replication barrier (Brewer and Fangman, 1988; Linskens and Huberman, 1988; Kobayashi and Horiuchi 1996); (b) the ter-elements involved in termination of replication in *Ercherichia coli* and *Bacillus subtilis* (reviewed by Bussiere and Bastia, 1999 and Duggin et al., 1999); and (c) the *RTS1* element located distal to *mat1* (J. Z. D. and A. J. S. K., unpublished observation). Thus, one reason why imprinting is influenced by the direction of replication at *mat1* could be that the swi1p/swi3p-pausing site is polar. Strain JZ148 contains an inverted *mat1* allele that is replicated in the direction opposite to that of the wild-type allele (Figure 2b). The analysis showed a much reduced pausing (Figure 2b). Thus, when *mat1* is replicated in the opposite direction, pausing at *MPS1* is absent or much reduced.

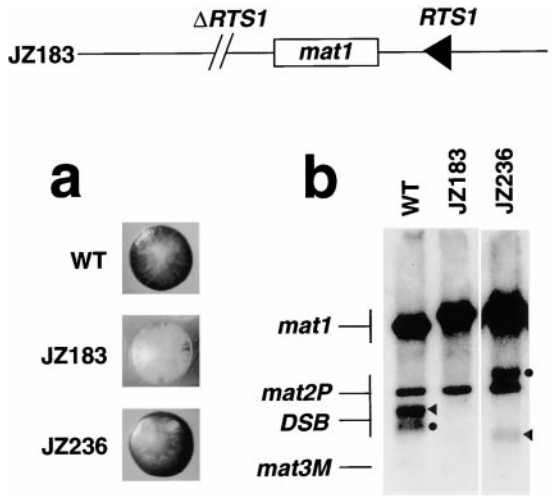

Figure 3. Isolation of Termination-Defective Mutations

The line drawing displays the *mat1* region of strain JZ183 used in the screen. The *RTS1* element was deleted from the proximal side and inserted in the inverted orientation on the distal side of *mat1*. (a) Iodine-staining phenotype of colonies following sporulation. WT indicates wild-type strain SP976; JZ183 is described above; JZ236 is a derivative of JZ183 containing a *swi1-rtf3* mutation restoring imprinting and switching. (b) Quantification of the imprint/DSB at *mat1* using Southern analysis (Dalgaard and Klar, 1999). A *mat1P*-specific probe was used. *mat2P* and *mat3M* can be seen due to homology with *mat1* sequences in the probe. "DSB" indicates the two fragments generated when the imprint is converted into a double-stranded break during the purification of DNA; a dot marks the proximal fragment, while a triangle marks the distal fragment. Quantification of different bands showed that the JZ236 mutation restored the level of imprinting to ~85% of wild-type level.

Genetic Screen for Factors Involved in Termination of Replication at *RTS1*

We have shown that imprinting was abolished when *RTS1* was removed from the proximal side and inserted in the inverted orientation at the distal side of *mat1* due to *mat1* replication in the opposite direction (J. Z. D. and A. J. S. K., unpublished observation). This genetic rearrangement in the subsequent text will be referred to as a translocated *RTS1* element (Figure 1a). This very strong effect made it possible to screen for factors involved in termination of replication at the *RTS1* element. While colonies of nonsporulating strains remain yellow, colonies of sporulating strains stain black when exposed to iodine vapors because spores synthesize a starch-like compound (Figure 3a, WT; Meade and Gutz, 1975). The strain carrying the translocated *RTS1* element was inhibited from imprinting and switching, and therefore its colonies displayed a decreased level of sporulation and a yellow iodine-staining phenotype (Figure 3a, JZ183). This reduction in iodine staining was utilized to screen for *trans*-acting UV-induced mutations that allowed replication forks to pass through the *RTS1* element leading to increased imprinting/switching and iodine staining. Thirty mutants that stained black were isolated. One of the three complementation groups identified was characterized representing a single *rtf3–1* allele (replication termination factor 3; Figure 3a, JZ236) is described here. Detection of the DSB at *mat1* showed that the *rtf3–1* mutation restored the imprint at *mat1* (Figure 3b). However, when the *rtf3* allele was combined

Table 1. Genotypes of Strains

Strain	Genotype	Reference
E111	*h90*, his2, ade6-M210, swi1-111*	Gutz and Schmidt, 1985
E146	*h90, his2, ade6-M210, swi3-146*	Gutz and Schmidt, 1985
E148	*h90, his2, ade6-M210, swi1-148*	Gutz and Schmidt, 1985
E157	*h90, his2, ade6-M210, swi3-157*	Gutz and Schmidt, 1985
E159	*h90, his2, ade6-M210, swi1-159*	Gutz and Schmidt, 1985
JZ108	*Msmt0, Δmat2,mat3::LEU2, his2, leu1-32*	Dalgaard and Klar, 1999
JZ148	*mat1-inv, ade6-M210, ura4-D18, leu1-32*	Dalgaard and Klar, 1999
JZ183	*ΔRTS1-mat1-SspI::RTS1, ade6-M210, ura4-D18, leu1-32*	This study
JZ236	*ΔRTS1-mat1-SspI::RTS1, ade6-M210, ura4-D18, leu1-32, rtf3*	This study
JZ277	*h90, his2, ade6-M210, rtf3-1*	This study
JZ423	*ΔRTS1-mat1-SspI::RTS1, ade6-210, ura4-D18, Δswi1::ura4*	This study
JZ449	*h90, ade6-M210, swi1-111*	This study
JZ450	*h90, ade6-M210, leu1-32, Δtop1::LEU2, swi1-111*	This study
JZ452	*h90, ade6-M210, leu1-32, Δtop1::LEU2*	This study
JZ454	*h90, ade6-M216, leu1-32, Δtop1::LEU2, swi3-157*	This study
JZ458	*ΔRTS1-mat1-SspI::RTS1, ade6-210, ura4-D18, Dtop1::LEU2*	This study
HE384	*h90, ura4-D18, Δswi1::ura4*	Schmidt, 1987
SP785	*h90, his2, ade6-M216, swi1-111*	This study
SP814	*Msmt0, ade6-M210, leu1-32, swi1-111*	This study
SP815	*Msmt0, ade6-M216, leu1-32, swi3-146*	This study
SP848	*Msmt0, ade6-M210, leu1-32, swi7-1*	This study
SP918	*h90, ade6-M216, leu1-32, swi3-157*	This study
SP976	*h90, ade6-M210, ura4-D18, leu1-32*	This study
SP981	*h90, ade6-M210*	This study
SP982	*h90, his2, ade6-M216*	This study
pHE6	*swi1* containing Sau3A fragment cloned in plasmid pWH5	Schmidt, 1987

* h^{90} is the locus containing wild-type *mat1, mat2,* and *mat3* genes; Δ indicates deletion

with a wild-type *mat1* allele, the level of imprinting was slightly decreased (~20% reduction) relative to wild-type levels (data not shown). This decrease in imprinting was comparable to that observed in the *RTS1*-deleted strain (J. Z. D. and A. J. S. K., unpublished observation), also suggestive of a loss of *RTS1* function.

Genetic crosses with candidate genes identified the *rtf3–1* mutation as a *swi1* allele. A cross between strain JZ236 and strain JZ423 containing the *swi1*::*ura4* deletion (Table 1; Schmidt, 1987) produced no recombinants between *rtf3* and the *swi1* in 27 tetrads analyzed. Furthermore, the *rtf3* allele was fully complemented by a *swi1* encoding plasmid (pHE6; Schmidt, 1987). The identification of a *swi1* mutation causing increased imprinting and switching was surprising, given that all previously identified *swi1* mutations reduced imprinting and switching (Gutz and Schmidt, 1985). Moreover, this discovery suggested that *swi1* was involved in termination of replication at *RTS1* as well as in pausing and imprinting at *MPS1*.

Termination of Replication at *RTS1* Is Dependent on *swi1* and *swi3*

We determined whether the known alleles of *swi1* and *swi3,* that abolish pausing at *MPS1*, also affect termination of replication at *RTS1*. The NsiI *mat1*-fragment from *swi1–111* and *swi3–146* strains was therefore analyzed by 2D gel analysis (Figure 4). The *RTS1* element was located in one end of this fragment. Consequently, *RTS1* can be observed in wild-type strains as a replication fork barrier on the descending part of the arc (Figure 4, left panel). The *swi1–111* and *swi3–146* mutations totally abolished the barrier signal (Figure 4, center and right panel), which verified that termination of replication at *RTS1* depends on both swi1p and swi3p functions.

Pausing at *MPS1* Is Not Affected in the *swi1-rtf3* Mutant

The *swi1-rtf3* mutant was proficient in imprinting; therefore if, as proposed earlier, pausing was essential for switching, it would be expected that pausing at *MPS1* should not be affected by this mutation. This expectation was confirmed. Analysis of the *mat1*-containing NdeI fragment from strain JZ277 showed that *MPS1* pausing was unaffected (Figure 5, right panel). To verify that the *swi1-rtf3* mutation indeed affected termination at the

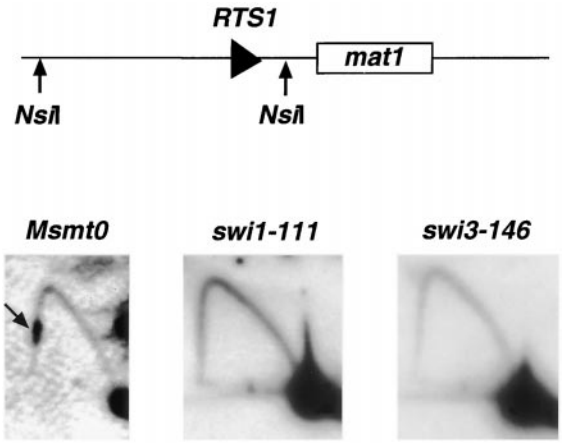

Figure 4. Termination of Replication at *RTS1* Is Dependent on swi1p and swi3p

The *RTS1* element is located approximately ~700 bp from the distal *Nsi*I site in the 4.0 kb NsiI fragment. An arrow points to the *RTS1* barrier in the panel marked *Msmt0* (strain JZ108). This barrier is absent in *swi1–111* (SP785) and the *swi3–146* (SP918) mutants.

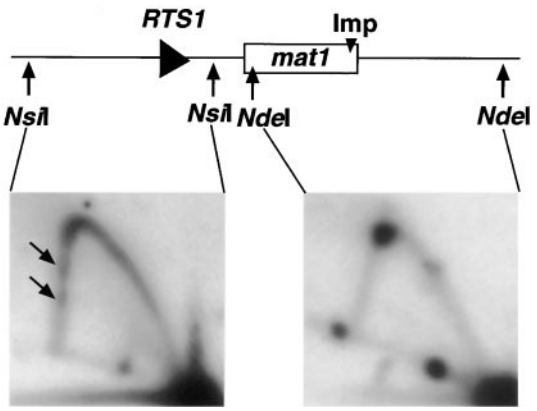

Figure 5. The *rtf3* Allele of *swi1* Affects Termination at *RTS1* but Not *MPS1* Pausing

A line drawing of the *mat1* region of JZ277 strain is shown above the 2D gel panels. Two separate 2D gel analyses of fragments covering the *MPS1* or the *RTS1* sites are displayed. The left panel shows that the *swi1-rtf3* mutation abolishes the strong termination of replication signal at *RTS1* (compare it to Figure 4 left panel as a control). Two weaker barriers observed are marked by arrows. The right panel depicts *MPS1* site pausing is unaffected by the *swi1-rtf3* mutation.

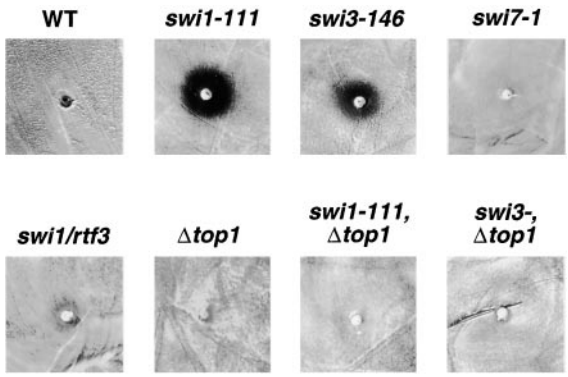

Figure 6. Effect of *swi1* Mutations on Camptothecin Sensitivity

Growth inhibition by camptothecin treatment (see Experimental Procedures). WT, wild-type.

indigenous *RTS1* element, the proximal *RTS1*-containing NsiI fragment was analyzed. The *RTS1* signal in the control (Figure 4; panel *Msmt0*) was significantly reduced to two more subtle barriers (Figure 5, left panel); therefore, the *swi1-rtf3* mutation demonstrated separation of the swi1p pausing and imprinting functions from those involved in termination of replication. This finding suggests that swi1p is involved in at least two independent pathways (see Discussion).

Sequence Analysis of *swi1* Mutations

All earlier described alleles of *swi1* affect imprinting (Egel et al., 1984, Schmidt et al., 1987). Sequencing of three of these showed the following mutations: *swi-111*, 1749G→C; *swi1-148*, 1849C→T; *swi1–159*, 1645C→T; *swi1-rtf3*, 2785G→A. This showed that the *swi1–159* allele contains an opal, and *swi1–148* allele an amber, nonsense mutation, while the *swi1–111* allele carried a mutation in the acceptor sequence in the only putative intron present in the gene (accession number CAB44362). This corresponds with the observation that *swi1–159* is fully, while the *swi1–148* is partially, suppressed by an opal suppressor *sup9–169* (Schmidt et al., 1987). Interestingly, all three mutations located in the first third of the protein cause severe truncations of the encoded protein. In contrast, the *swi1-rtf3* allele contains a missense mutation changing amino acid 662 in the C-terminal region of the protein from a negatively charged glutamic acid to a positively charged lysine residue.

Genetic Interactions between *swi1, swi3*, and *top1*

Computer searches with the *swi1* sequence suggested that it is a homolog of the *S. cerevisiae tof1* gene (data not shown). tof1p was identified in a two-hybrid screen as interacting with top1p (topoisomerase I; Park and Sternglanz, 1999). To examine if top1p was essential for imprinting or for mating-type switching, a *top1* deletion (Uemura et al., 1987) was combined with a wild-type *mat1* allele. This strain displayed only a small reduction in switching as measured by sporulation efficiency and iodine-staining assays (Strain JZ452; data not shown). top1p is therefore not essential for imprinting and mating-type switching. We also combined the Δ*top1* allele with the transposed *RTS1* allele (strain JZ458). If *RTS1* function in imprinting and sporulation had been affected because of top1p deficiency, an increase in imprinting and sporulation would be expected. However, no increase was observed, showing that top1p was not involved in termination of replication.

However, a *swi1–111,* Δ*top1* double mutant revealed a decreased growth rate, suggesting a *swi1* and *top1* interaction. Determination of the growth rates in liquid media showed that the double mutant (JZ240) had a significantly longer doubling time (3 hr 15 min) than wild-type SP982 (2 hr 20 min) or single mutants [JZ449, *swi1–111* (2 hr 45 min); JZ452, Δ*top1* (2 hr 20 min)]. A similar slow growth phenotype was also observed for a Δ*top1*, *swi3* double mutant (JZ454) when compared to a *swi3* mutant strain (E157). This finding suggested that both swi3p and swi1p might interact genetically with top1p.

To examine this possibility further, the sensitivity of the yeast strains to camptothecin was tested. Camptothecin is an inhibitor of top1p that inhibits the second step of the topoisomerase reaction, leaving top1p covalently attached to the nicked DNA (Morham and Shuman, 1992). A drug-diffusion assay showed that the growth inhibition of camptothecin was much greater in the *swi1–111* (strain E111) than in wild-type strains (SP976) (Figure 6). The same phenotype (data not shown) was observed for two other *swi1–148* (E148) and *swi1–159* (E159) alleles. Hypersensitivity was also observed in *swi3–146* (E146) and *swi3–157* (E157) mutants but not in a *swi7–1* (SP855) mutant. The hypersensitivity was not due to swi1p/swi3p involvement in termination of replication at *RTS1* nor to imprinting and switching at *mat1*, as strains remained hypersensitive when *swi1–111* was combined with Δ*RTS1*, *Msmt0*, or Δ*mat2,3* alleles. To test whether the hypersensitivity to camptothecin was dependent on top1p activity, double mutants (JZ450 *swi1–111,* Δ*top1* and JZ456 *swi3–146,* Δ*top1*) were found to be insensitive to the drug, showing that the hypersensitivity observed in *swi1* and *swi3* mutants is dependent on top1p activity (Figure 6).

The *swi1-rtf3* allele was used to determine if the *swi1* mutant hypersensitivity to camptothecin could be caused by a defect only in termination activity. Interestingly, the *swi1-rtf3* mutant showed a reduced sensitivity that was close to that of wild-type strains (Figure 6). This result suggested that hypersensitivity might be caused by swi1p/swi3p pausing defect and/or by other functions of swi1p/swi3p but not due to replication termination defect. One possibility we favor is that in the absence of swi1p/swi3p-dependent pausing an increased amount of substrate may accumulate for top1p to resolve (see Discussion).

These data presented above strongly suggest that both swi1p and sw3p interact with topoisomerase I and that this interaction is unrelated to the developmental program of *S. pombe.* In this context, we note that some evidence exists linking swi1p/swi3p function to DNA replication. Both *swi1,* polα-*swi7* and *swi3,* polα-*swi7* double mutants are inviable. Furthermore, like the camptothecin sensitivity, this synthetic lethality is independent of whether the strains are able to switch (Schmidt et al., 1987), implying that the lethality is not related to mating-type switching. Our results supported and extended this observation, suggesting that swi1p/swi3p might be involved in general DNA replication and that the top1p interaction could play an important role there.

Discussion

This paper provides evidence for a unique molecular mechanism of imprinting exquisitely depending on the replication process. Specifically, the swi1p and swi3p proteins, necessary for imprinting, were shown to pause the replication fork at the site of imprinting. More importantly, this pause signal is only recognized by replication forks moving in one of the directions. As such, *S. pombe* has evolved a mechanism to utilize the asymmetry of DNA replication and a polar replication-pause site to establish an asymmetrical pattern of cell differentiation. This discovery raises the possibility that other biological systems might utilize the asymmetry of DNA replication to establish asymmetric differentiation. In addition, the data presented here add further support to the direction of a replication model that explains the asymmetrical mating-type switching pattern of *S. pombe* (Dalgaard and Klar, 1999). A central axiom of this model is that imprinting involves the lagging-strand replication complex. Earlier results have supported this model by showing that imprinting at *mat1* is sensitive to changes in the direction of replication at *mat1,* and that *cis*-acting elements ensure a specific direction of replication (summarized in Figure 1a; Dalgaard and Klar, 1999).

On the basis of the data presented here, it is possible to propose a pathway for imprinting at *mat1*. The pathway consists of three steps: (1) The first step in the imprinting pathway is likely to be swi1p- and swi3p-dependent pausing of replication at the site of imprinting. Since both *swi7* and *Msmt0* strains lack the imprint, yet pausing is observed, these elements must be working downstream of the swi1p/swi3p function. (2) Synthesis of the imprint. polα with its associated primase activity is proposed to synthesize a primer at the imprint site. (3) The RNA/imprint is maintained perhaps by preventing DNA-repair enzymes from removing it before it is utilized to initiate switching during the next round of replication (Figure 1b). This function is proposed to involve the *mat1* distal *cis*-acting sequences and sap1p that binds to them. The proposed function of swi1p in imprinting might explain the speckled phenotype of the *swi1*-deletion strain (Schmidt, 1987). Perhaps the swi1p (and swi3p) increase efficiency by pausing the replication fork at the specific position.

Data presented in this paper also show that swi1p and swi3p promote polar replication fork pausing at *MPS1* both by interactions with that site and by preventing replication forks with the wrong polarity from passing through *MPS1* by blocking them at *RTS1.* One possibility being tested is that swi1p/swi3p first catalyze pausing at *RTS1*, and subsequently other factors are recruited to dislodge the paused replication complex. Such a mechanism would differ from that operating at the *ter*-elements in bacterial genomes where only one protein is required for termination (reviewed by Bussiere and Bastia, 1999; Duggin et al., 1999). In such a scenario, the *swi1-rtf3* mutation could be disrupting the recruitment of these additional factors. In some support of this model, the genetic screen that identified the *swi1-rtf3* allele also identified other genetic complementation groups that potentially could be the factors interacting with swi1p at *RTS1*.

Finally, we show that *swi1*/*swi3* have an additional function that involves *top1.* This function is unrelated to pausing, imprinting, and termination of replication in the *mat1* region, but could involve *swi1*- and *swi3*-pause activity at other positions in the genome. swi1p/swi3p could act during general replication by providing pausing sites allowing time for the unwinding of supercoils formed in front of the replication fork. In this context, a top1-swi1p/swi3p interaction could play an important role in recruitment of top1p to the replication fork to further assist unwinding of supercoils. If this model is correct, one would imagine that in the absence of swi1p/swi3p local supercoiling could increase. This would result in an increase in top1p substrate concentration and could explain the observed hypersensitivity to camptothecin.

Interestingly, several other proteins in the database showed significant sequence similarity to *swi1.* These include a multidrug resistance protein of *Aspergillus nidulans* (accession # AAD41625) and the *tim1* gene family involved in clock formation (reviewed by Lakin-Thomas, 2000). In *D. melanogaster* tim1p interacts with per1p and both proteins exhibit a circadian expression pattern. However, in mammals and *Caenorhabditis elegans* only per1p expression cycles (Jeon et al., 1999). It is tempting to suggest that mammalian tim1p could have functions similar to *swi1*. Interestingly, the *swi1-rtf3* mutation that affects termination is in a conserved region suggesting that DNA termination could be a conserved function among this family of proteins.

Experimental Procedures

2D Gels

The 2D gels were performed as described by Brewer and Fangman (1989), except that strains were grown in rich YEA media (Moreno et al., 1991) supplemented with 70 mg/l each of uracil, adenine, histidine, and leucine. 50 μg of DNA was digested with restriction enzymes. Replication intermediates were purified as described by using BND cellulose. The electrophoresis gels contained 0.4% agarose for the first dimension and 1.1% for the second dimension.

Camthothecin Growth Inhibition Assay

Ten microliters of a saturated solution of Camthothecin in DMSO was applied in a small well indented in the middle of the YEA plate

seeded with 100 μl of 1:10 diluted overnight cultures. The plates were incubated for 2 days at 33°C before taking pictures.

S. pombe Strains and Crosses

Strains were constructed using standard methods (Moreno et al., 1991). Strains and the plasmid used in this study along with genotypes and references are given in Table 1.

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