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Meiotic role of *SWI6* in *Saccharomyces cerevisiae*

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ABSTRACT

The transcript levels of DNA replication genes and some recombination genes in *Saccharomyces cerevisiae* fluctuate and peak at the G1/S boundary in the mitotic cell cycle. This fluctuation is regulated by MCB (*Mlu*I cell cycle box) elements which are bound by the DSC1/MBF1 complex consisting of Swi6 and Mbp1. It is also known that some of the MCB-regulated genes are induced by treatment with DNA damaging agents and in meiosis. In this report, the function of *SWI6* in meiosis was investigated. Δ*swi6* cells underwent sporulation as did wild-type cells. However, the deletion mutant cells showed reduced spore viability and lower frequency of recombination. The transcript levels of the recombination genes *RAD51* and *RAD54*, which have MCB elements, were reduced in Δ*swi6* cells. The transcript levels of *SWI6* itself were also induced and declined in meiosis. Furthermore, an increased dosage of *SWI6* enhanced the transcript level of the *RAD51* gene and also the recombination frequency in meiosis. These results suggest that *SWI6* enhances the expression level of the recombination genes in meiosis in a dosage-dependent manner, which results in an effect on the frequency of meiotic recombination.

INTRODUCTION

In *Saccharomyces cerevisiae*, the DNA replication genes as well as some of the recombination genes are regulated transcriptionally during the cell cycle. Their transcript levels fluctuate and peak at the G1/S boundary during the mitotic cell cycle. These genes have MCB (*Mlu*I cell cycle box) elements in the upstream region. The DSC1/MBF (DNA synthesis control/MCB binding factor) complex binds at MCBs in a cell cycle-dependent manner and activates these genes (1). It is known that the DSC1 complex consists of Swi6 and Mbp1 and Mbp1 directly interacts with MCB (2). In a deletion mutant of the *SWI6* or *MBP1* genes, genes having MCBs are constitutively expressed at a reduced level (1,2). Swi6 also forms the SBF complex (SCB binding factor) with another protein, Swi4, which binds at SCB (Swi4/Swi6 cell cycle box) elements and activates genes bearing SCBs in the upstream region.

Some of the genes whose transcript levels are regulated by MCBs in mitosis are also induced in early meiosis (3–7) and by DNA damaging agents (3,4,6,8). The regulatory sequence required for the response to DNA damaging agents was examined in the *RAD54* gene, whose transcript level fluctuates during the cell cycle and has MCB elements. It has been shown that a 26 bp sequence containing the MCBs is required for induction by UV and MMS (9). However, MCB elements have no essential role in the DNA damage response, while they enhance the transcript level (10). Moreover, deleting this 26 bp regulatory sequence does not affect induction in meiosis (9). Thus, the regulatory sequences required for induction by DNA damaging agents and in meiosis differ. The transcript levels of genes bearing MCB elements peak at exactly the same time in mitosis, while, in contrast, their transcript levels increase with different kinetics during meiosis (5).

Although the function of the *SWI6* gene in the mitotic cell cycle is known to some extent, the involvement of this gene in the regulation of meiosis has not been examined. Therefore, we studied the effect of the deletion of *SWI6* on meiosis and the meiotic transcript levels of recombination genes. The transcript levels of the *RAD51* and *RAD54* genes were reduced by deletion of *SWI6*. Moreover, the spore viability and recombination frequency decreased in Δ*swi6* mutants. These observations indicate an important role for *SWI6* in meiosis.

MATERIALS AND METHODS

Microorganisms and plasmids

Escherichia coli DH5α (11) was used for all plasmid DNA manipulations. *Saccharomyces cerevisiae* SK1 derivative strains used in this study were as follows: SLH105 (MAT**a** *lys2 ho*::*LYS2 ura3 leu2*::*hisG his4-X trp1*::*hisG*), SLH108 (MATα *lys2 ho*::*LYS2 ura3 leu2*::*hisG his4-B trp1*::*hisG*), SLD101 (MAT**a**/MATα *lys2/lys2 ho*::*LYS2/ho*::*LYS2 ura3/ura3 leu2*::*hisG/leu2*::*hisG his4-X/his4-B trp1*::*hisG/trp1*::*hisG*), SLD102 (as SLD101 except for Δ*ime1*::*LEU2*), SLD109 (as SLD101 except for Δ*swi4*::*LEU2*), SLD113 (as SLD101 except for Δ*swi6*::*TRP1*), NKY857 (MAT**a** *lys2 ho*::*LYS2 ura3 leu2*::*hisG his4-X*) and NKY860 (MATα *lys2 ho*::*LYS2 ura3 leu2*::*hisG his4-B*). SLH105 and SLH108 strains were constructed as follows. A 4.7 kb DNA fragment of pNKY1009 (12) containing the *TRP1* disruption (*trp1*::*hisG-URA3-hisG*) was digested with *Eco*RI and *Bgl*II and was used for transformation of NKY857 and NKY860. Ura$^-$ derivatives of Ura$^+$ Trp$^-$ transformants were initially obtained by

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patching onto 5-fluoroorotic acid (5-FOA) plates, which are selective for Ura$^-$ strains. To construct SLD109 and SLD113, Δ*swi4*::*LEU2* or Δ*swi6*::*TRP1* derivatives of SLH105 and SLH108 obtained by a one-step disruption method (13) using Bd194 and Bd197 plasmids (14) were mated and the diploid strain was selected by the inability to mate with the **a** or α haploid strain. As the Δ*swi4* and Δ*swi6* diploid strains are unstable and often lose the ability to sporulate, the resultant diploid was immediately used for further experiments. To construct SLD102, SLH105 and SLH108 were transformed with a linearized Δ*ime1*::*LEU2* plasmid in which the 1.3 kb *Hin*dIII–*Nru*I fragment of *IME1* (15) was replaced by a 1.6 kb *Bam*HI fragment spanning *LEU2* DNA, obtained from plasmid YDp-L (16), and the resultant Δ*ime1* derivatives were mated.

SLD252 (MAT**a**/α *leu2/leu2 his4-4/his4-290 CAN1/can1*^{r} *ura3/ura3 ho*::*LYS2/ho*::*LYS2 trp1/trp1 CYH2/cyh2*^{r} *ADE6/ade6 ade2/ade2 lys2/lys2*) and SLD330 (SLD252 Δ*swi6*::*TRP1*) are not SK1 derivatives and were used to measure intergenic recombination. SLD330 was constructed as SLD113.

YEpSWI6 is identical to BA354 (14). YCpSWI6 was constructed by connecting a *Sal*I–*Bam*HI fragment spanning *SWI6* (14) with YCplac33 (17).

Procedure of sporulation

The diploid SK1 strains were streaked on YPG plates (1% yeast extract, 2% polypeptone, 3% glycerol, 2% agar) and incubated at 30°C for 12 h (18,19). A single colony on a YPG plate was streaked on YPD (1% yeast extract, 2% polypeptone, 2% glucose, 2% agar) and incubated for 2–3 days. In the case of cells harbouring plasmids, the cells were streaked on SD-Leu or SD-Ura (2% glucose, 0.67% yeast nitrogen base, 2% agar, supplemented with the necessary amino acids lacking leucine or uracil) and incubated for 2–3 days. A single colony from the plate was grown in YPD or SD liquid medium for 18–24 h. Each culture was diluted 100-fold into YPA (1% yeast extract, 2% polypeptone, 2% potassium acetate) or 10-fold into SA (2% potassium acetate, 0.67% yeast nitrogen base, supplemented with the necessary amino acids lacking leucine or uracil) medium, for cells harboring plasmids, and incubated for 12 h. Cells (1–2 × 10^7 cells/ml) were harvested, washed twice with sterile water and resuspended at the same density in SPM (0.3% potassium acetate, 0.03% raffinose). Then, the suspension was incubated at 30°C with vigorous shaking.

Northern blot analysis

To analyse the transcripts during meiosis, cells were sporulated in 1 l SPM, samples were removed at intervals and total RNA was prepared as previously described (18). About 10 µg total RNA was heat denatured at 65°C for 10 min and run on a 6% formaldehyde gel (1.2% Sea-kem agarose gel; FMC BioProducts) with MOPS buffer. After electrophoresis, the gel was washed with distilled water and blotted onto a nylon membrane (Hybond-N; Amersham). The RNA on the membrane was cross-linked by UV, prehybridized and hybridized with [^{32}P]dCTP-labeled DNA probes. The DNA probes used were as follows: *SWI4* probe; 1.9 kb *Bam*HI–*Pst*I fragment (20), *SWI6* probe; 1.4 kb *Bgl*II–*Pvu*II fragment (14), *RAD51* probe; 1.2 kb *Stu*I–*Bst*EII fragment (8), *RAD54* probe; 1.9 kb *Bam*HI fragment (21), *ACT1* probe; 1 kb *Xho*I–*Hin*dIII fragment (22).

Table 1. Spore viability of the *swi4*::*LEU2* and *swi6*::*TRP1* strains

Strain	Total asci dissected	Viable spores/ascus 0	1	2	3	4	Spore viability (%)
Wild-type	44	0	0	1	3	40	97.2
Δ*swi4*	113	13	38	37	14	11	43.8
Δ*swi6*	72	23	23	17	9	0	27.3

Flow cytometry

To prepare meiotic samples for flow cytometry, 2 ml sporulating culture were withdrawn at each time point and the cells were fixed by addition of ethanol to a final concentration of 70%. Flow cytometry was carried out as previously described (23).

RESULTS

Sporulation of a Δ*swi6* mutant

To elucidate the role of *SWI6* in meiosis, we first observed sporulation of a *swi6* deletion mutant in synchronous culture. It is known that Swi4 makes a complex, SBF, with Swi6 that binds to SCB elements. Thus, the effect of *swi4* deletion was also examined. Microscopic observation revealed that the Δ*swi6* mutant formed asci at a frequency (88%) better than the wild-type (72%) after 12 h in sporulation medium and mature asci appeared ~2–3 h earlier than in the wild-type. A Δ*swi4* homozygous diploid mutant showed almost the same phenotype as Δ*swi6*, suggesting that both genes are not essential for spore formation.

Several mutants defective in meiosis show a low viability of spores, although they apparently form normal asci (24). Thus, we examined the spore viability of Δ*swi4* and Δ*swi6* strains by dissecting asci after sporulation. As shown in Table 1, only 27% of Δ*swi6* spores and 44% of Δ*swi4* spores made viable colonies, whereas the wild-type strain showed 97% spore viability. Further microscopic observation revealed that half of the inviable spore clones did not germinate, while the remaining half germinated but cell growth arrested with various cell shapes. This result clearly indicates that Swi4 and Swi6 are required for normal meiosis.

Premeiotic DNA replication in Δ*swi6* mutant cells

We examined premeiotic DNA replication in a Δ*swi6* strain, as Swi6 and Mbp1 form the MBF complex that regulates expression of DNA replication genes during mitotic growth, although three viable spore clones in an ascus from the Δ*swi6* strain suggested that premeiotic DNA replication must occur in Δ*swi6*. Cells transferred to sporulation medium were withdrawn, sonicated, stained with propidium iodide and subjected to flow cytometry. As shown in Figure 1, the DNA content in Δ*swi6* cells increased to 4C faster than in wild-type cells, whereas Δ*ime1* cells did not increase their DNA content, as previously reported (15). These results suggest that premeiotic DNA replication is almost complete in Δ*swi6* strains. Thus, the low viability of spores in Δ*swi6* cells seems not to be caused by the absence of premeiotic DNA replication.

SWI6 is required for meiotic recombination

As most of the mutants defective in meiotic recombination show low spore viability (24), we examined meiotic intragenic and intergenic recombination in a Δ*swi6* strain. Diploid cells were

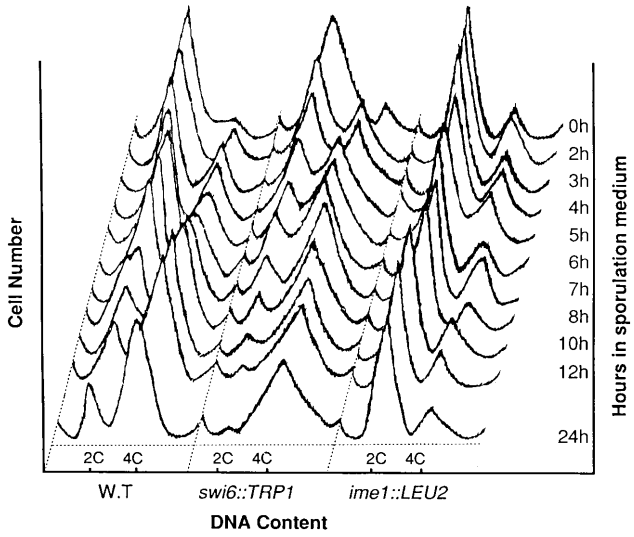

Figure 1. Flow cytometry of Δ*swi6* cells during meiosis. SLD101(*SWI6*), SLD113(Δ*swi6*) and SLD102(Δ*ime1*) cells taken at various time after transfer to sporulation medium were treated with RNase, stained with propidium iodide and subjected to flow cytometry (3). Δ*ime1* cells were used as a control, as they cannot initiate premeiotic DNA replication.

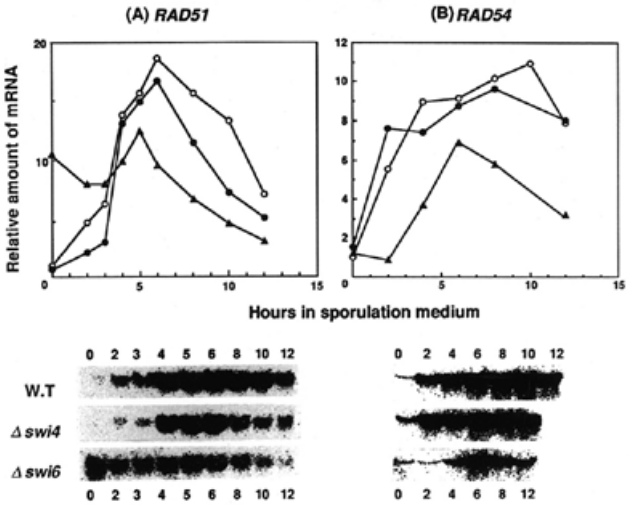

Figure 2. Northern blotting of the recombination genes. Isogenic wild-type (SLD101; ○), Δ*swi4* (SLD109; ●) and Δ*swi6* (SLD113; ▲) cells were sporulated and cells were withdrawn at various times. RNA extracted from these cells at the indicated times was subjected to northern blotting. The same amount of RNA was loaded into each lane and this was confirmed by staining the gel with ethidium bromide before blotting. The direct image of each analysis is shown in the lower panel with the time in sporulation medium. The intensity of the signal was measured using a Fuji image analyzer and normalized such that the intensity of each transcript at 0 h in the wild-type cells is assigned a value of 1. The membrane used in this experiment is that already described in Leem *et al.* (19).

transferred to sporulation medium and aliquots were withdrawn and spread onto YPD and selective plates after appropriate dilution ('return-to-growth'). The colonies that appeared on selective plates were counted as recombinants. In strain SLD330 (Δ*swi6*), the frequencies of intragenic (*his4-4/his4-290*) and intergenic (*cyh2-ade6*) recombination were reduced to 14 and 31% respectively of those in wild-type cells (Table 2). The recombination frequency was also reduced in SLD113 (Δ*swi6*), with a different background. The recombination frequency was also measured in the meiotic products by dissecting asci formed by strains SLD101 and SLD113. In strain Δ*swi6*, only one ascus among 420 dissected had a His^+ spore clone, while six out of 115 in wild-type cells had His^+ clones (wild-type, 5.2×10^{-2}; Δ*swi6*, 4.6×10^{-3}). Thus, *SWI6* is required for meiotic recombination. The reduced recombination frequency probably accounts for the low spore viability in Δ*swi6* cells,

Table 2. Meiotic recombination in the Δ*swi6* mutant

Strain	Relevant genotype	*his4*[a] (×10^{-4})			*cyh2-ade6*[a] (×10^{-4})		
		0 h	6 h	12 h	0 h	6 h	12 h
SLD252	*SWI6*	2.5	520	970	3.8	180	4900
SLD330	Δ*swi6*	1.6	57	140	4.2	20	1500
SLD101	*SWI6*	0.41	37	110	–	–	–
SLD113	Δ*swi6*	0.18	0.83	10	–	–	–
SLD113	Δ*swi6* [YCpSWI6]	0.75	52	160	–	–	–
SLD113	Δ*swi6* [YEpSWI6]	0.66	200	340	–	–	–

[a]Intragenic recombination frequencies between *his4-4* and *his4-290* for SLD252 and SLD330 strains and between *his4-X* and *his4-B* for SLD101 and SLD113 strains and intergenic recombination between *cyh2* and *ade6* were examined as described (18).

SWI6 enhances expression of genes required for meiotic recombination

The reduced frequency of recombination in Δ*swi6* may be caused by reduced expression levels of the recombination genes, since Swi6 works as a transcription factor in the mitotic cell cycle. To examine this possibility, we measured the transcript levels of the *RAD51* and *RAD54* genes during meiosis in a Δ*swi6* strain. Both genes have MCB elements in the upstream sequence and are required for meiotic recombination (3,8,10,24,25).

RNA extracted from sporulating cells was subjected to northern analysis and levels of *RAD51* and *RAD54* transcripts were examined (Fig. 2). The *RAD51* and *RAD54* transcript levels increased 20-fold during meiosis in the wild-type cells, as previously reported (3,8). In Δ*swi6* cells, the maximal level of the *RAD51* and *RAD54* transcripts was reduced to 60% of that in wild-type cells. In Δ*swi4* cells, on the other hand, the maximal level of the *RAD51* and *RAD54* transcripts was almost the same as in wild-type cells. These results suggest that Swi6 also functions in meiosis as a transcription factor.

The *SWI6* transcript is induced in early meiosis

The level of the *SWI6* transcript during meiosis has not been examined. Thus, we performed northern analysis of the *SWI6* transcript during meiosis. As shown in Figure 3A, the *SWI6* transcript level declined and then increased between 2 and 4 h after medium transfer. This is consistent with a role for Swi6 as a transcription factor for the genes containing MCB elements, including the *RAD51* and *RAD54* genes. We also examined the transcript level of *SWI4* in meiosis. Interestingly, the *SWI4*

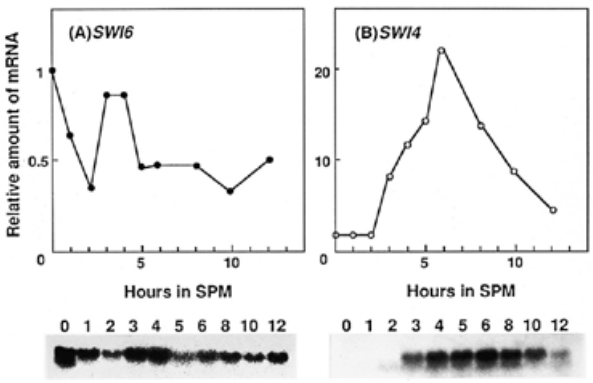

Figure 3. Induction of *SWI6* (**A**) and *SWI4* (**B**) transcripts in meiosis. RNA extracted from sporulating SLD101 cells was transferred to a nylon membrane after electrophoresis and hybridized with *SWI6* and *SWI4* DNA. The membrane used in this experiment is that used in Figure 2 for wild-type cells.

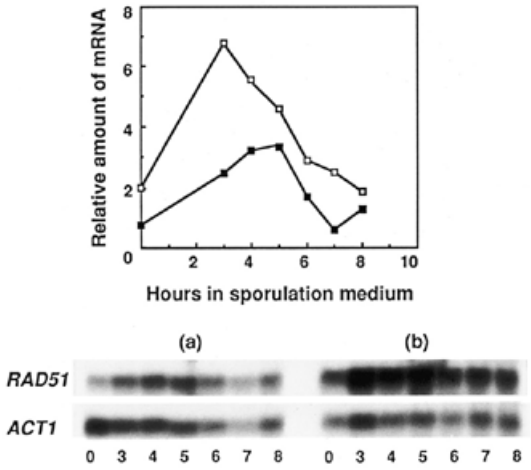

Figure 4. Increased dosage of *SWI6* enhances the level of *RAD51* transcript. Δ*swi6* diploid cells (SLD113) harboring YCpSWI6 (**a**) (■) and YEpSWI6 (**b**) (□) were sporulated. RNA extracted from sporulated cells was subjected to northern blotting using the same probe as in Figure 2. As a control, actin transcript was analyzed using *ACT1* DNA. The image of a northern blot is shown with the time in sporulation medium and the intensity of *RAD51* transcript is normalized to *ACT1* transcript.

transcript was also induced in meiosis (Fig. 3B). However, the timing of its induction is different from that of *SWI6*. The kinetics of induction are consistent with the meiotic function suggested by spore viability (Table 1).

The meiotic recombination frequency depends on dosage of *SWI6*

The lack of *SWI6* decreased the recombination frequency, presumably by reducing the expression of certain recombination genes. The *SWI6* gene on a multicopy or low copy plasmid was introduced into Δ*swi6* diploid cells. The transcript level of *SWI6* in plasmid YEp was 3-fold higher than that in plasmid YCp (data not shown). These strains were subjected to sporulation and aliquots were withdrawn for measurement of recombinants (His^+) and for northern blot analysis. The recombination frequency of the cells with *SWI6* on a multicopy plasmid increased 2-fold over the cells having *SWI6* on a low copy plasmid (Table 2). The *RAD51* transcript level in the same cells increased almost 2-fold (Fig. 4). This result strongly suggests that an increased dosage of *SWI6* enhances transcription of recombination genes and in consequence increases the recombination frequency.

DISCUSSION

In this study, we have shown that the frequency of meiotic recombination and the transcript level of the recombination genes *RAD51* and *RAD54* is reduced in Δ*swi6* mutants. Thus, it is likely that recombination frequency in meiosis depends on the expression level of the recombination genes. This view is strengthened by the observation that an increased dosage of Swi6 enhanced recombination frequency. Increasing the dosage of the *RAD51* or *RAD54* gene alone could not enhance recombination frequency (our unpublished results). Thus, the reduced level of several transcripts seems to additively affect recombination frequency or a lack of *SWI6* reduces the transcript level of an as yet unknown gene(s) which encodes a limiting factor for meiotic recombination.

Δ*swi6* cells form asci earlier and at a slightly higher frequency than the wild-type and go through premeiotic DNA replication faster than mitotic DNA replication (Fig. 1). These facts suggest that *SWI6* also regulates expression of the genes which repress meiosis. The low spore viability and normal recombination frequency in Δ*swi4* cells (Table 1 and our unpublished results) show that Swi4 is probably involved in a sporulation process other than recombination. However, no gene whose meiotic transcript level is regulated by *SWI4* is as yet known.

It was reported that the transcript levels of *CDC8*, *9* and *21*, which have MCBs, increased during meiosis with different kinetics from one another while their transcript levels peaked at exactly the same time in mitosis (5). Our results also show that the transcript levels of *RAD51* and *RAD54* increase with different kinetics. Moreover, deletion of *SWI6* changed the induction pattern of each gene differently; the *RAD51* transcript level was high at 0 h in sporulation medium, while induction of the *RAD54* transcript was delayed. This may be caused by a complex regulation of these transcripts during meiosis which includes the *SWI6* system and other unknown regulation systems.

The *RAD51* and *RAD54* genes were reported to be induced by DNA damaging agents (3,4,10). In this induction, a lack of MCB elements decreases the induction level, whereas the induction kinetics are similar to wild-type cells. Moreover, Δ*swi6* strains show weak sensitivity to MMS but not to UV (10). As most of the lesions caused by MMS are healed by recombinational repair (26), the reduced expression of recombination genes in Δ*swi6* cells could account for the increased sensitivity to MMS. Therefore, it is likely that the same group of recombination genes regulated by *SWI6* affect meiotic recombination and the repair of MMS damage. Moreover, Δ*mbp1* and $swi4^{ts}$ do not affect sensitivity to MMS, suggesting that some unknown factor(s) forms a complex with Swi6 and controls an MCB element (10). Therefore, an unknown factor(s) may interact with the *SWI6* system to regulate gene induction also in meiosis.

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