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Roles of histone acetylation and chromatin remodeling factor in a meiotic recombination hotspot

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Histone acetyltransferases (HATs) and ATP-dependent chromatin remodeling factors (ADCRs) are involved in selective gene regulation via modulation of local chromatin configuration. Activation of the recombination hotspot *ade6-M26* of *Schizosaccharomyces pombe* is mediated by a cAMP responsive element (CRE)-like sequence, *M26*, and a heterodimeric ATF/CREB transcription factor, Atf1·Pcr1. Chromatin remodeling occurs meiotically around *M26*. We examined the roles of HATs and ADCRs in chromatin remodeling around *M26*. Histones H3 and H4 around *M26* were hyperacetylated in an *M26*- and Atf1-dependent manner early in meiosis. SpGcn5, the *S. pombe* homolog of Gcn5p, was required for the majority of histone H3 acetylation around *M26 in vivo*. Deletion of *gcn5*$^+$ caused a significant delay in chromatin remodeling but only partial reduction of *M26* meiotic recombination frequency. The *snf22*$^+$ (a Swi2/Snf2-ADCR homologue) deletion and *snf22*$^+$*gcn5*$^+$ double deletion abolished chromatin remodeling and significant reduction of meiotic recombination around *M26*. These results suggest that HATs and ADCRs cooperatively alter local chromatin structure, as in selective transcription activation, to activate meiotic recombination at *M26* in a site-specific manner.

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Introduction

Eucaryotic chromosomal DNA is packaged into a highly condensed chromatin structure, which inhibits various DNA-associated processes, such as replication, transcription, and recombination, presumably by preventing the loading of *trans*-acting factors onto target DNA sites. Thus, the local chromatin structure around *cis*-acting DNA sites should be converted into an open configuration before the initiation of DNA-associated processes (Wolffe, 1997).

Chromatin-modifying machineries, such as histone acetyltransferase (HAT) complexes and ATP-dependent chromatin remodeling factors (ADCRs), are often recruited to promoters via sequence-specific DNA binding proteins, bound to their target sites, to activate transcription (Cosma *et al*, 1999; Krebs *et al*, 1999; Agalioti *et al*, 2000). Chromatin-modifying machineries may also activate other DNA-associated reactions. For example, histone acetylation is involved in DNA repair and site-specific V(D)J recombination (McBlane *et al*, 1995; McMurry and Krangel, 2000; Bird *et al*, 2002).

Homologous recombination is elevated markedly in meiosis and contributes to the genetic diversity of the next generation and the proper segregation of meiotic chromosomes. Most meiotic recombination in *Saccharomyces cerevisiae* is initiated by transient, meiosis-specific DNA-double strand breaks (DSBs) that map to hotspots for meiotic gene conversion. Such DSB sites are often found in transcription promoters that show hypersensitivity to nucleases (Ohta *et al*, 1994; Wu and Lichten, 1994). Meiotic DSBs also initiate recombination in fission yeast (Cervantes *et al*, 2000; Zenvirth and Simchen 2000). These observations suggest that the chromatin structure and its modification, possibly mediated by some sequence-specific transcriptional activators, are important to regulate the initiation of meiotic homologous recombination. In support of this idea, certain transcription factors influence DSB formation at 'α-hotspots' in *S. cerevisiae* (Petes, 2001).

The *ade6-M26* (*M26*) locus in the fission yeast *Schizosaccharomyces pombe* is a well-characterized meiotic recombination hotspot that provides a good model system for studies of recombination regulation. The *ade6-M26* allele is a single G/T transversion in the 5′ end of the *ade6* coding region (Ponticelli *et al*, 1988; Szankasi *et al*, 1988). This mutation creates a nonsense codon and also confers an up to 15-fold, meiosis-specific elevation of recombination as compared to control alleles such as *ade6-M375* (*M375*) (Gutz, 1971; Ponticelli *et al*, 1988; Schuchert *et al*, 1991). The *ade6-M375* allele is also a G/T base substitution that creates a nonsense mutation in the codon adjacent to that altered by the *M26* mutation, but *M375* does not show the hotspot activity. Thus, the *ade-M375* allele provides an excellent negative control for studies of hotspot recombination at *ade6-M26*.

Recent studies have revealed a molecular basis for hotspot recombination at *ade6-M26*. Atf1 and Pcr1, which both belong to the ATF/CREB transcription factor family, bind as a heterodimer specifically to a heptameric DNA sequence, ATGACGT, which is created by the *M26* mutation and is similar to the CRE (cAMP Response Element, TGACGT) sequence (Wahls and Smith, 1994; Kon *et al*, 1997). Binding of the Atf1·Pcr1 complex to this '*M26* DNA site' is required for the recombination hotspot activity (Wahls and Smith, 1994; Kon *et al*, 1997). Interestingly, the Atf1·Pcr1 heterodimer is a transcription factor involved in meiotic induction, but it is dispensable for the steady-state levels of *ade6* transcription (Wahls and Smith, 1994; Kon *et al*, 1997). Activation of meiotic recombination at *M26* is at least partly due to meiosis-specific formation of DSBs around *M26*, which are dependent upon Rec12 (the *S. pombe* homologue of Spo11) and Pcr1 (Steiner *et al*, 2002).

We previously reported that the chromatin structure around *ade6-M26* is remodeled meiotically (Mizuno *et al*, 1997). The nucleosome phasing along the *ade6* open reading frame is lost, and in turn a micrococcal nuclease (MNase)-sensitive site appears *de novo*. This process, referred to as *M26* chromatin remodeling, is regulated by meiosis-inducing signaling pathways (Mizuno *et al*, 2001). In addition, similar chromatin remodeling occurs around naturally occurring CRE-related sequences, such as $ctt1^+$ and $fbp1^+$ promoter sequences (Mizuno *et al*, 2001; Hirota *et al*, 2003), controlled by cAMP-dependent kinase (PKA) and stress-activated kinase (SAPK) pathways and fission yeast Tup1-like corepressors (Hirota *et al*, 2003). This suggests that *M26* chromatin remodeling might reflect an intrinsic physiological response occurring at *S. pombe* natural CRE-related sequences.

These findings, together with the dual roles of the Atf1·Pcr1 heterodimer in meiotic development and hotspot activation (Kon *et al*, 1998), suggest that the induction of recombination in meiosis may be partly regulated at the chromatin or DNA accessibility level, and may be coupled in some way to transcriptional regulation in a developmental stage-specific manner. To further understand the mechanisms underlying the induction of meiotic recombination, we have studied the roles of histone acetylation in *M26* chromatin remodeling. Here we report roles of the Atf1·Pcr1-, SpGcn5 HAT-mediated histone acetylation, and SpSnf22 ADCR-like factor in chromatin remodeling and meiotic recombination at the *M26* hotspot. These results provide important insights into molecular mechanisms on site-specific chromatin regulation at CRE-like sequences in the activation of meiotic recombination.

Results

Atf1 and Pcr1 are required for chromatin remodeling at ade6-M26

Activation of the *M26* recombination hotspot requires Atf1·Pcr1 (Kon *et al*, 1997), which is constitutively expressed and binds to the *M26* DNA site, as revealed by a methylation interference assay (Kon *et al*, 1998) and a chromatin immunoprecipitation (Ch-IP) assay (W. Wahls, unpublished results). To study the molecular basis of *M26* chromatin remodeling, we first analyzed the effects of *atf1*Δ and *pcr1*Δ (null) mutations on chromatin structure. Wild-type ($atf1^+$ $pcr1^+$), *atf1*Δ, *pcr1*Δ, and *atf1*Δ *pcr1*Δ mutant cells were cultured in a pre-sporulation medium and were then cultured in a sporulation medium prior to analysis of chromatin structure. In $atf1^+$ $pcr1^+$ cells, the chromatin structure around *ade6-M26* changed by 3 h of meiosis, while little or no change was observed around *M375* (Figure 1A). However, in *atf1*Δ or *pcr1*Δ cells, the *M26* DNA site-dependent chromatin remodeling was not observed at 3 h and even at 6 h (data not shown) after meiotic induction. Similarly, *atf1*Δ *pcr1*Δ double mutant cells did not exhibit chromatin remodeling at *M26* (Figure 1A). We conclude that both Atf1 and Pcr1 are strictly required for the meiotic chromatin remodeling around *ade6-M26*.

As Atf1 and Pcr1 are transcription factors that induce some genes during meiotic differentiation (Takeda *et al*, 1995; Kanoh *et al*, 1996; Watanabe and Yamamoto, 1996), it was possible that the deletion of $atf1^+$ or $pcr1^+$ indirectly affected the chromatin remodeling around *ade6-M26*. Kon *et al*. previously ruled out the possibility that the loss of $atf1^+$ or $pcr1^+$ decreased *ade6* transcription and consequently abolished the chromatin remodeling (Kon *et al*, 1997). The entire loss of the *M26* chromatin remodeling in the *atf1*Δ and the *pcr1*Δ strains is also unlikely to be due to their deficiencies in the meiotic proficiency, since nearly half of the mutant cells underwent sporulation. However, it remained possible that Atf1·Pcr1 regulated the expression of other meiotic factors that mediated the chromatin remodeling.

To test this hypothesis more rigorously, we used two approaches to bypass the requirement for the Atf1·Pcr1 heterodimer in meiotic induction. First, we induced meiosis ectopically by expressing Ste11 (Sugimoto *et al*, 1991) in *pcr1*Δ cells. Second, we induced meiosis ectopically in *atf1*Δ cells by inactivation of the Pat1 kinase (Iino and Yamamoto, 1985). Each approach alleviated the early meiotic defects of *pcr1*Δ and *atf1*Δ cells (Watanabe and Yamamoto, 1996), but did not compensate for the loss of the *M26* chromatin remodeling, even though meiosis was almost fully induced in these mutant cells (Figure 1B). These results, together with the fact that Atf1·Pcr1 occupies the *M26* site in cells, led us to conclude that the *M26* chromatin remodeling is mediated directly *in cis* by the Atf1·Pcr protein bound to the *M26* DNA site.

Histones around ade6-M26 are hyperacetylated

HATs are often recruited by sequence-specific transcriptional activators, and thereafter convert the local chromatin configuration into a state suitable for active transcription (Brown *et al*, 2000). This led us to speculate that the Atf1·Pcr1 heterodimer might recruit HATs to the *M26* site and mediate the chromatin remodeling through histone acetylation. To test this idea, we used Ch-IP for the analysis on the state of histone acetylation around *M26*. Cells were induced to enter meiosis and cell extracts were subjected to Ch-IP using antibodies specific for acetylated isoforms of histones. The DNA from the immunoprecipitated chromatin and the input material was placed on slot blots and was hybridized with either total genomic DNA (serving as a reference for global histone acetylation levels) or with DNA sequences around the *M26* hotspot.

We reproducibly detected stronger signals on the blots of immunoprecipitated chromatin from the *M26* cells as compared to those from the *M375* cells (Figure 2A). Quantitative

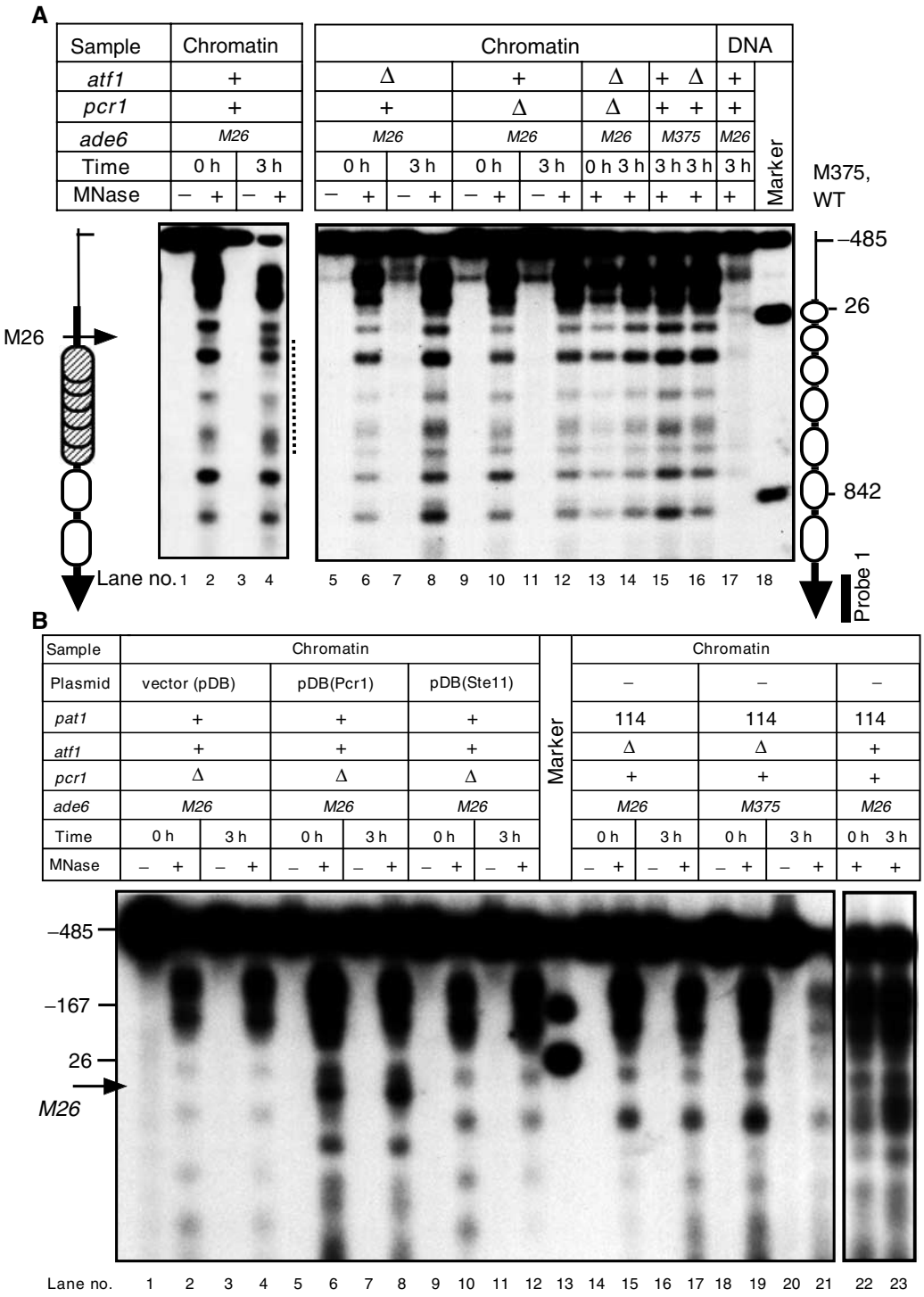

Figure 1 Atf1·Pcr1 is required for meiotic chromatin remodeling around *ade6-M26*. (**A**) Disruption of *atf1*$^+$ or *pcr1*$^+$ abolishes meiotic chromatin remodeling around *ade6-M26*. Diploid strains ELD205 (*ade6-M26*), WSP779 (*ade6-M26, atf1*Δ), WSP857 (*ade6-M26, pcr1*Δ), WSP859 (*ade6-M26, atf1*Δ*, pcr1*Δ), ELD203 (*ade6-M375*), and WSP780 (*ade6-M375, atf1*Δ) were cultured in presporulation medium (lanes 0 h). Cells were then transferred to sporulation medium and cultured further for 3 h (lanes 3 h). Chromatin isolated from cells was digested with 0 (lanes −) or 20 (lanes +) units/ml of MNase and analyzed as described (Mizuno *et al*, 1997). Probe 1 was used for indirect end labeling. The vertical and the horizontal arrows indicate the *ade6* ORF and the position of the *M26* mutation, respectively. Numbers by the right vertical arrow show the positions in nucleotides of the *Xho* I (−485), *Bam* HI (26), and *Hind* III (842) sites relative to the first A of the *ade6* coding region. Open and hatched ovals represent phased and randomly positioned nucleosomes, respectively. The broken line by lane 4 indicates the region where the chromatin structure is remodeled. (**B**) Ectopically induced meiosis does not induce chromatin remodeling in *atf1*Δ and *pcr1*Δ cells. (Lanes 1–12) Chromatin structures of WSP857 (*ade6-M26, atf1*$^+$*, pcr1*Δ) diploids harboring the empty vector (lanes vector(pDB)), the Pcr1 expressing plasmid (lanes pDB(Pcr1)), and the Ste11 expressing plasmid (lanes pDB(Ste11)) were analyzed. Experiments were performed as in Figure 1A. (Lanes 14–23) The K213 (*ade6-M26, atf1*Δ*, pat1-114*), K214 (*ade6-M375, atf1*Δ*, pat1- 114*), and GP1725x (*ade6-M26, pat1-114*) cells were cultured for 24 h in presporulation medium (lanes 0 h). Cells were transferred to sporulation medium and cultured at 34°C for 3 h (lanes 3 h). Chromatin analyses were performed as in Figure 1A. Overexpression of Pcr1 caused chromatin remodeling in premeiotic cells (lane 6). This may be due to artificial activation of chromatin remodeling by enhanced binding of Atf1·Pcr1 to the *M26* site.

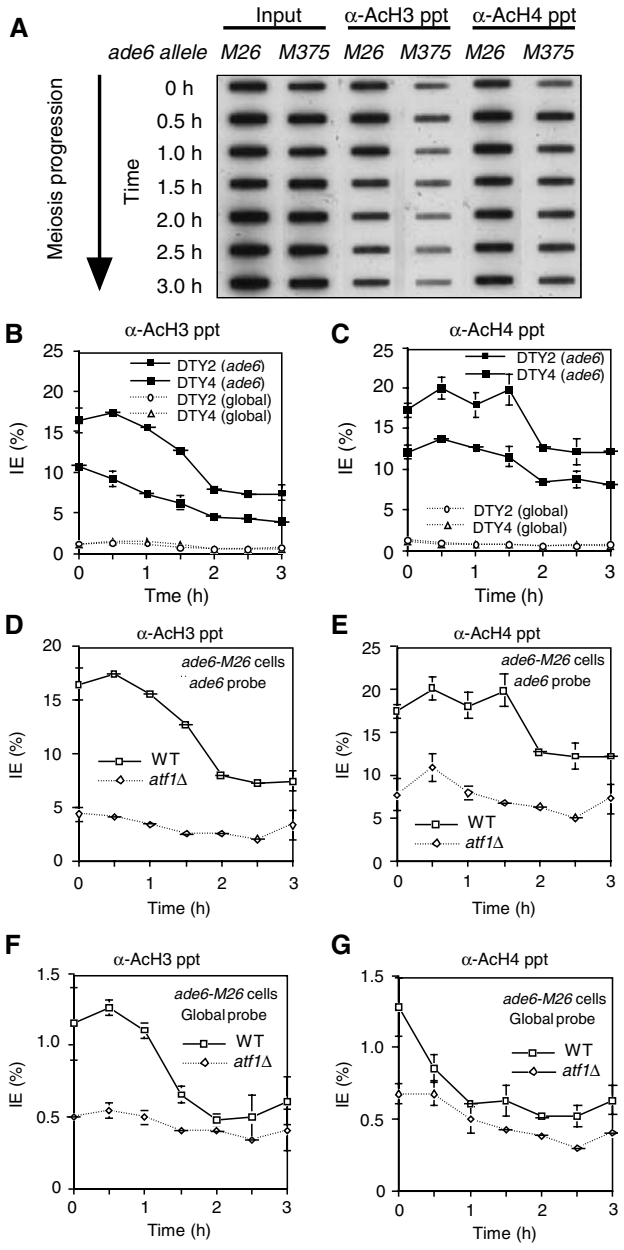

Figure 2 Histones H3 and H4 around *ade6-M26* are highly acetylated in an Atf1-dependent manner during meiosis. Chromatin obtained from DTY2 (*ade6-M26*), DTY4 (*ade6-M375*), and WSP779 (*atf1*Δ, *ade6-M26*) diploid cells was immunoprecipitated with antiacetylated histone H3 and H4 antibodies. DNA obtained from input and immunoprecipitated material was applied to slot blots. (**A**) Example of primary data showing autoradiograms of slot blots probed for the *ade6-M26* or *ade6-M375* region. (**B–G**) Quantitative data are expressed as the ratio of the bound to the input material (immunoprecipitation efficiency: IE). All data were averages of at least three independent experiments. Labels inset into each figure panel indicate the relevant cell genotype and the probe used for each experiment. The data for WT in panels D–G are the same as those in panels B, C (solid line) and B, C (broken line). (B, C) Effects of *ade6* alleles on acetylation of histone H3 and H4, respectively. (D, E) Requirement for Atf1 in histone H3 and H4 acetylation at *ade6-M26*, respectively. (**F, G**) Role of Atf1 in global (genome average) acetylation of histone H3 and H4, respectively.

analysis demonstrated that the histones H3 and H4 around *ade6-M26* were highly acetylated at 0.5 h after the meiotic induction. The histone H3 and H4 acetylation levels at *M26* (about 20% of the total input fraction) were 2- and 1.5-fold higher, respectively, than those at *ade6-M375*, (Figure 2B and C), and were ~20-fold higher than the global (genome average) levels (Figure 2B and C, about 1% of the total input fraction). The relatively high levels of histone acetylation around *ade6-M375* (5–10% of the total input fraction), compared to the global levels, may be due to the active transcription of the *ade6* gene, which is an essential housekeeping gene. The histone acetylation levels gradually decreased to 50% of their maximal values by 2 h after the meiotic induction, a time point when premeiotic DNA synthesis was almost completed and the *M26* chromatin remodeling was initiated (Mizuno *et al*, 1997). Separate experiments revealed that the global levels of histone acetylation in cells harboring *ade6-M26* were similar to those in *ade6-M375* cells (Figure 2B-C, broken lines). These results demonstrate that histones at the *ade6* locus are hyperacetylated in an *M26* DNA site-dependent manner.

We next applied the Ch-IP analysis to *atf1*Δ cells, in which no meiotic chromatin remodeling was observed around *M26* (see Figure 1). As shown in Figure 2D and E, the acetylation of histones H3 and H4 around *ade6-M26* was greatly reduced in the *atf1*Δ cells, indicating that histone acetylation around *M26* requires Atf1. Interestingly, the global levels of histone acetylation were also reduced significantly in *atf1*Δ cells (Figure 2F and G).

S. pombe Gcn5p is a HAT

We next sought to identify the HAT that may be involved in histone acetylation around *M26*. Referring to the amino-acid sequences of the conserved HAT domains and bromodomains of budding yeast and human Gcn5p, we searched for a fission yeast homologue of Gcn5p by a BLAST search. One gene, *SPAC1952.05*, showed the highest similarity to yeast and human Gcn5p (Figure 3A). *SPAC1952.05* was previously identified as a gene encoding a putative *S. pombe* homologue of Gcn5p, and the gene product was found in a SAGA-like complex (Mitsuzawa *et al*, 2001), although it was unknown whether the protein had HAT activity.

To test the hypothesis that *SPAC1952.05* encoded a HAT enzyme, we produced and purified bacterially expressed, wild-type protein and a protein with an alanine substitution at the glycine205. The Gly205 residue is in the putative acetyl CoA-binding site and corresponds to an amino acid required for the HAT activity of budding yeast Gcn5p (Kuo *et al*, 1998) (Figure 3A). We then assayed the potential HAT activities of these proteins, using human histone octamers as substrates (Figure 3B).

The wild-type protein had HAT activity, with preference toward histone H3 compared to H4 (~30-fold, Figure 3B, lane WT), which is similar to the specificity of the budding yeast Gcn5p (Kuo *et al*, 1996). The protein with the G205A substitution exhibited very low HAT activity (Figure 3B, lane G205A). We concluded that *SPAC1952.05* encodes a functional HAT enzyme homologous to human and budding yeast Gcn5, and therefore use the names '*gcn5*$^+$' and 'SpGcn5' to refer to the *S. pombe* gene and protein, respectively.

To determine whether SpGcn5 was responsible for the acetylation of histones at *ade6 in vivo*, we deleted the *gcn5*$^+$ gene. Haploid *gcn5*Δ (null) mutants were viable and grew slightly slower than the *gcn5*$^+$ cells. Diploid *gcn5*Δ mutants underwent meiosis I normally, except that the

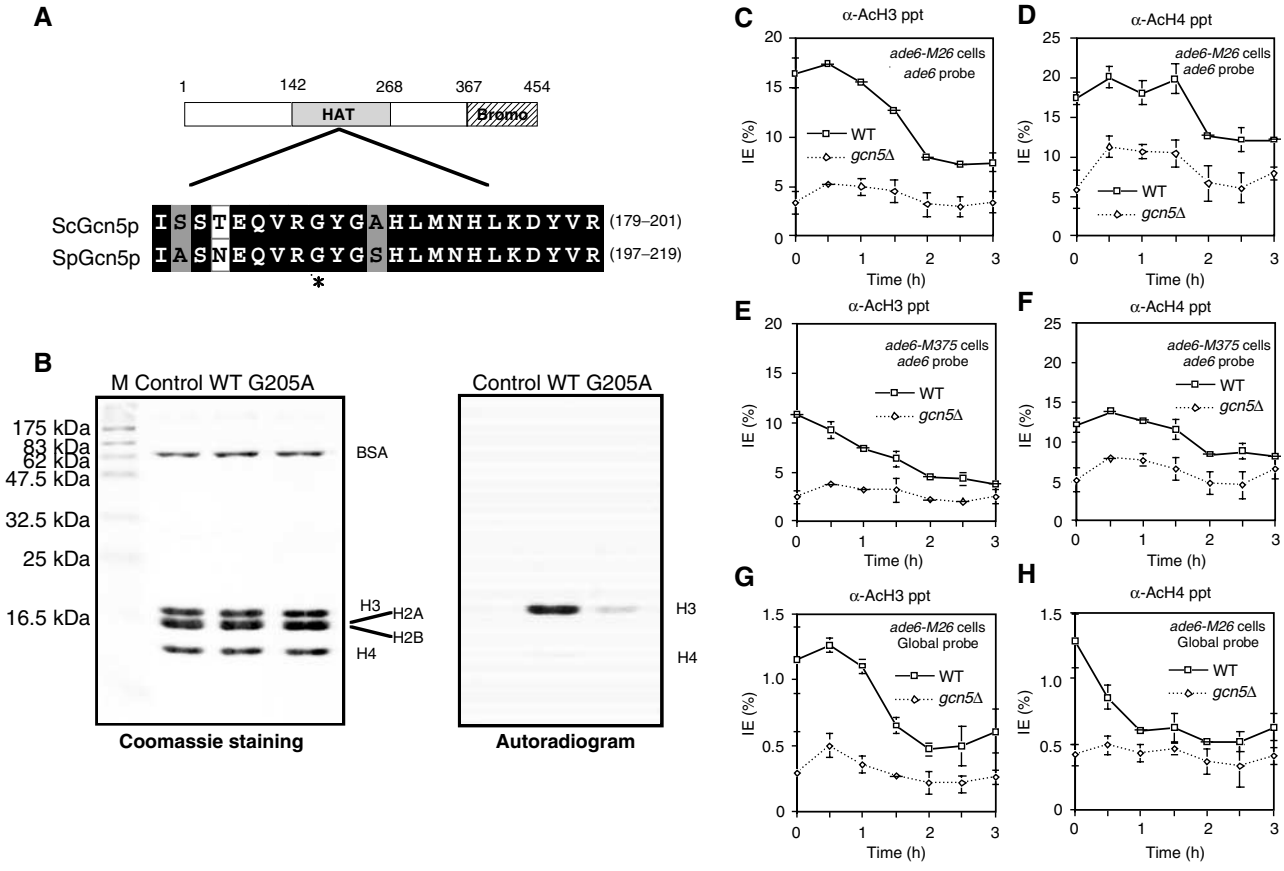

Figure 3 HAT activity of SpGcn5 *in vitro* and *in vivo* around *M26* (**A**) Sequence alignments of ScGcn5p and SpGcn5 showing identical (dark gray) and conserved (light gray) amino acids in motif 'A' of the HAT domain. The asterisk indicates the glycine residue replaced with alanine in SpGcn5(G205A). (**B**) SpGcn5 has histone (H3 > H4) acetyltransferase activity *in vitro*. Recombinant SpGcn5 and SpGcn5(G205A) proteins were purified and assayed for HAT activity by incubation with human histone octamers and [^{14}C] acetyl-CoA. Labeled proteins were separated by SDS–PAGE, stained with Coomassie brilliant blue (left panel), and subjected to autoradiography (right panel) to reveal acetylated histones (control, no enzyme). (**C–H**) Chromatin obtained from DTY9 (*gcn5*Δ, *ade6-M26*) and DTY11 (*gcn5*Δ, *ade6-M375*) cells was analyzed as in Figure 2. All data are averages of at least three independent experiments. Labels inset into each figure panel indicate the relevant cell genotype and the probe used for each experiment. The data for WT in panels C–H are same as those in Figures 2B, C, F and G. Requirement for Gcn5 in histone H3 (C, E) and H4 (D, F) acetylation at *ade6-M26* (C, D) and at *ade6-M375* (E, F). (G, H) Role of Gcn5 in global (genome average) acetylation of histone H3 and H4, respectively.

meiosis I process seems slightly quick and a small fraction of the cells (less than 10%) entered meiosis prematurely when cultured in a presporulation medium (Supplemental data 1). In addition, the *gcn5*Δ cells produced asci with four viable spores (78% spore viability). Moreover, northern analyses revealed that loss of *gcn5*$^+$ did not affect the transcriptional induction of the meiosis-specific recombination genes, *rec6*$^+$ and *rec12*$^+$ (data not shown). These phenotypes differ markedly from those of budding yeast, where the deletion of *GCN5* completely inhibits meiotic events (Burgess *et al*, 1999).

The Ch-IP experiments revealed that histone H3 acetylation levels around *ade6-M26* were much lower in *gcn5*Δ cells than in *gcn5*$^+$ cells (~75% reduction, Figure 3C). A less severe decrease in histone H4 acetylation was observed, consistent with the substrate preference of SpGcn5 toward histone H3 (Figure 3D), while such *in vivo* effects might include an indirect influence on acetylation by other HATs in the absence of SpGcn5. We also observed a significant reduction of histone H3 acetylation around *ade6-M375* (Figure 3E) and in the entire genome (Figure 3G) in *gcn5*Δ cells. Notably, in *gcn5*Δ cells the absolute levels of histone H3 acetylation around *ade6-M26* were very similar to those around *ade6-M375*. These results demonstrate that SpGcn5 is required for the acetylation of histone H3 around *ade6* and in the whole genome (see discussion), and more importantly it is required for the *M26*-specific hyperacetylation.

Effects of gcn5$^+$ *deletion on genome-wide DSB formation and meiotic recombination*

We next examined the effects of *gcn5*$^+$ deletion on the meiotic DSB formation on whole chromosomes (Figure 4A) using haploid strains with the *pat1-114 rad50S* background, which enabled us to detect the accumulation of discrete meiotic DSB bands in highly synchronized meiosis. In *gcn5*$^+$ cells, we detected the accumulation of smeared chromosomal bands, reflecting meiotic DSBs, after 4 h in sporulation culture (Figure 4A lanes 4 and 5). The abundance of these subchromosomal fragments was markedly reduced at 4 h in *gcn5*Δ cells (Figure 4A lane 9), while *gcn5*Δ cells undergo slightly quicker meiosis I (see above). However, the final extent of chromosomal breakage in the *gcn5*$^+$ and *gcn5*Δ

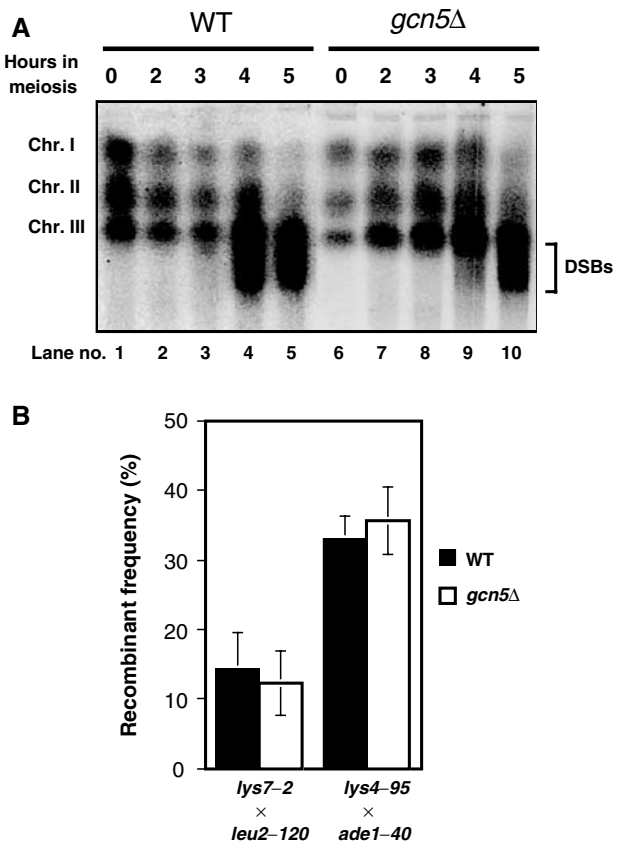

Figure 4 Effects of *gcn5*$^+$ disruption on the genome-wide DSB formation and recombination. (**A**) Effects of *gcn5*Δ mutation upon meiotic DSBs in whole chromosomes. Genomic DNA was prepared from K341 (*ade6-M26*, *rad50S*, lanes WT) and TY24 (*ade6-M26*, *rad50S*, *gcn5*Δ, lanes *gcn5*Δ) cells cultured for the indicated times in sporulation medium, and was analyzed by pulsed-field gel electrophoresis. The electrophoresis image was obtained by an FM BIO II image analyzer (HITACHI) after Syber Green staining. The smeared DNA shows the broken DNA generated during meiosis. (**B**) Effects of the *gcn5*Δ mutation upon intergenic recombination. Standard genetic crosses between haploids containing appropriate markers were performed, and spores were plated on complete medium. Then the colonies arising from the spores were replicated onto appropriate test media to check the prototrophy and to measure the recombination frequencies. Each recombination frequency represents average of five independent crosses.

cells was similar at a later time point (Figure 4A, lane 10). These results indicate that SpGcn5 is required for the proper timing of the DSB formation, but has only a nominal effect on the final level of the genome-wide DSB formation.

To determine whether the *gcn5*Δ mutation influences global recombination, we measured the frequency of intergenic recombination (crossing-over) between *lys7* and *leu2* on chromosome I and between *lys4* and *ade1* on chromosome II (Figure 4B). We found that the *gcn5*$^+$ disruption did not significantly affect the recombinant frequencies. A possibility of overestimating recombinants in *gcn5*Δ mutants was eliminated, since there is no significant effect of the *gcn5*Δ mutation on viable or diploid spore formation. Thus, we concluded that although SpGcn5 is required for proper timing of meiotic DSB formation, it is dispensable for intergenic, nonhotspot recombination, at least for the two intervals tested.

Effects of gcn5$^+$ *disruption on chromatin remodeling, DSB formation, and recombination at the ade6-M26 hotspot*

We studied the effects of the *gcn5*Δ mutation on chromatin remodeling, meiotic DSB formation, and recombination at the *ade6-M26* hotspot. In wild-type diploid cells, the *M26* chromatin remodeling occurred by 2–3 h after meiotic induction (Figures 1 and 5A). However, in *gcn5*Δ diploid cells, chromatin remodeling was not observed after 3 h, but partial remodeling occurred at 4.5 h (data not shown) and at 6 h (Figure 5A). Similar results were obtained with highly synchronized meioses in *pat1-114* cells (data not shown). These results demonstrate that SpGcn5 is involved in the *M26*-dependent chromatin remodeling during meiosis.

We subsequently analyzed meiotic DSB formation around *ade6-M26* using genomic DNA prepared from the *pat1-114 rad50S* haploid cells (Figure 5B). In the *gcn5*$^+$ cells, the meiotic DSB signals around *ade6-M26* could be detected after 4 h in sporulation culture, and accumulated as meiosis proceeded. The DSB frequency at 6 h was $0.62 \pm 0.065\%$ ($n = 3$). On the other hand, no DSB signal could be detected in *gcn5*Δ cells after 4 h, and even after 6 h only reduced levels of meiotic DSBs were detected. The final level of the DSBs around *M26* was $0.32 \pm 0.095\%$ ($n = 3$), almost half of that in *gcn5*$^+$ strain. The delayed rate and the decreased amount of DSB formation in *gcn5*Δ cells paralleled the effects of *gcn5*Δ on chromatin remodeling at *M26* (Figure 5A).

We also analyzed other *M26* hotspot-independent DSB sites located upstream of the *ade6* locus (Figure 5C). A slight delay in the DSB formation was observed at the *M26*-independent DSB sites adjacent to the *ade6* locus, consistent with the delay in the genome-wide DSB formation. However, the final levels of DSB formation at those *M26*-independent sites were not significantly affected in *gcn5*Δ. On the other hand, DSBs around the *M26* site occurred even later than those at the *M26*-independent sites, and were significantly impaired in the final DSB levels; the DSBs around *M26* in *gcn5*$^+$ cells (123% relative to one of the upstream DSB site) exhibited 42% reduction in *gcn5*Δ cells (71% relative to the upstream DSB site). Thus, the reduction in the final DSB level in *gcn5*Δ cells is specific to the *M26* site.

We further tested the effects of the *gcn5*$^+$ disruption on intragenic meiotic recombination at *ade6*. The recombination frequency (gene conversion) was measured by scoring the frequencies of Ade$^+$ recombinants in random spores after zygotic meioses (Figure 6C). Recombination frequencies from crosses harboring *M26* (hotspot) were reproducibly and significantly lower (60.3%) in *gcn5*Δ cells than in *gcn5*$^+$ cells (Figure 6C). The reduction in the gene conversion frequencies was only partial but proportional to the decrease in the DSB frequencies (51.6%) in the *gcn5*Δ mutants. Importantly, no significant effects on recombination at *M375* were detected in *gcn5*Δ cells (Figure 6C).

Effects of snf22$^+$ *disruption on chromatin remodeling and meiotic recombination at the ade6-M26 hotspot*

The *gcn5*$^+$ deletion has only partial effects on meiotic recombination. This may be because other chromatin remodeling mechanisms can partially compensate the Gcn5 function. ADCRs are candidates for such alternative mechanisms. Hence we looked for ADCRs homologues and examined their mutant effects on the chromatin and recombination at *M26*.

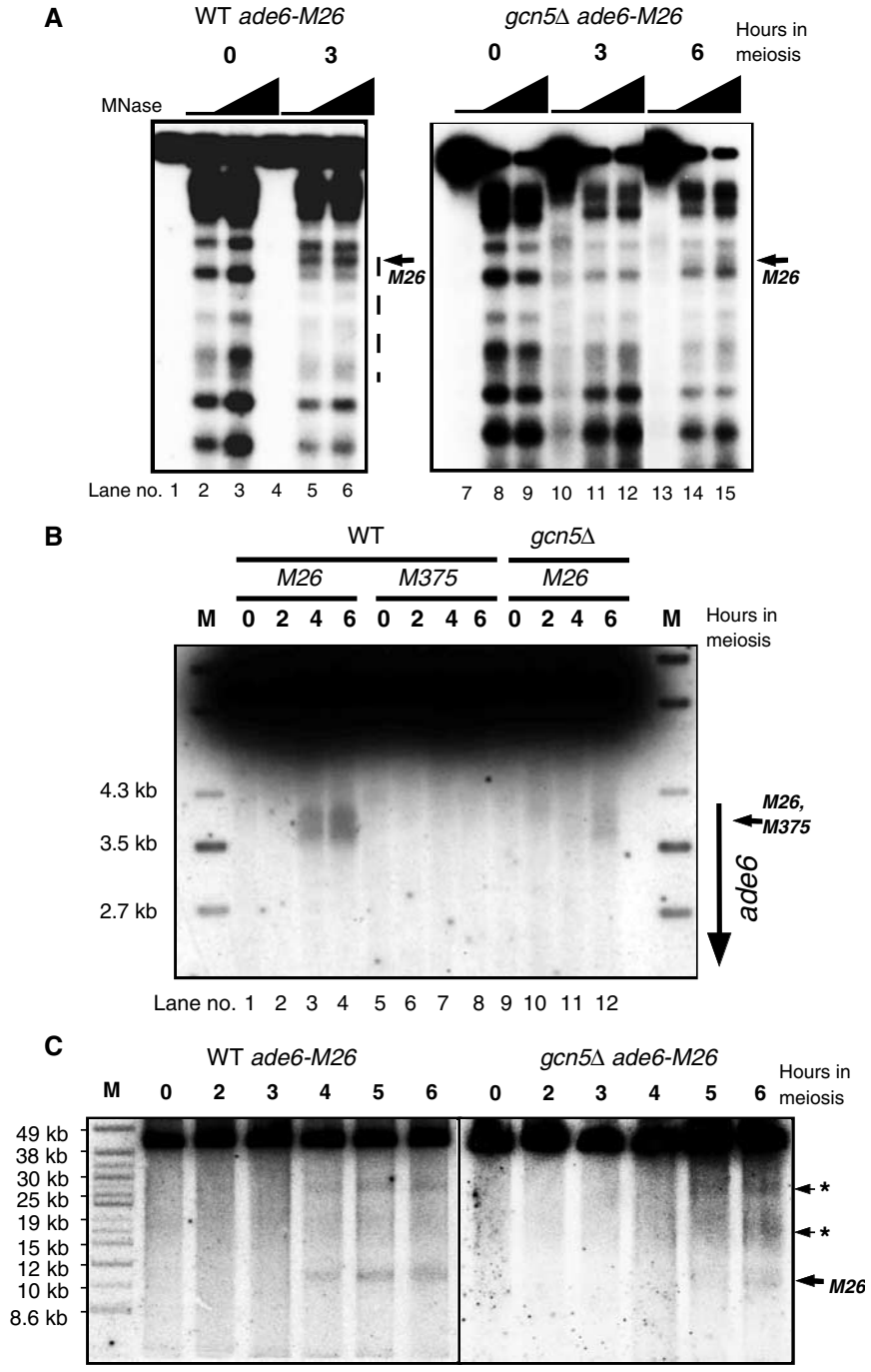

Figure 5 Effects of *gcn5*$^+$ disruption on the chromatin remodeling, DSB formation, and recombination at *M26*. (**A**) Requirement for SpGcn5 in meiotic chromatin remodeling. Meiotic chromatin remodeling at the *ade6* locus in DTY2 (*ade6-M26*, lanes WT) and DTY9 (*ade6-M26*, *gcn5*Δ, lanes *gcn5*Δ) diploids was analyzed as in Figure 1, using 0, 20, and 30 units/ml of MNase. Three independent experiments gave the same results. The broken line by lane 6 indicates the region where the chromatin structure is remodeled. (**B**) Requirement for SpGcn5 in meiotic DSB formation around *M26*. Genomic DNA was prepared as in Figure 4A, was digested with *Afl* II, and analyzed by Southern analysis. Horizontal and vertical arrows indicate the positions of the *M26* (or *M375*) sites and the *ade6* ORF, respectively. (**C**) Meiotic DSBs at the *M26*-independent sites adjacent to *M26* in the *gcn5*Δ strain. Genomic DNA was prepared as in Figure 4A, was digested with *Pac* I, and analyzed by pulsed-field gel electrophoresis followed by Southern hybridization. The horizontal arrowhead and the asterisks indicate the position of *M26* (or *M375*) and the adjacent breakages, respectively.

Using similarity to the budding yeast Swi2/Snf2 protein, we identified three possible ADCR genes. One of them, referred as *snf22*$^+$ (SPACC 1620.14c), has a bromodomain and Swi2/Snf2-like conserved ATP-binding/helicase motifs (Figure 6A). The *snf22*$^+$ deletion mutants exhibited inefficient mating, but once they formed zygotes, they produced spores almost normally. Using the *snf22*Δ and the *gcn5*Δ *snf22*Δ double mutants, the meiotic chromatin and recombination frequency around *M26* were analyzed. In the *snf22*Δ and *snf22*Δ*gcn5*Δ strains, we detected no significant chromatin changes even

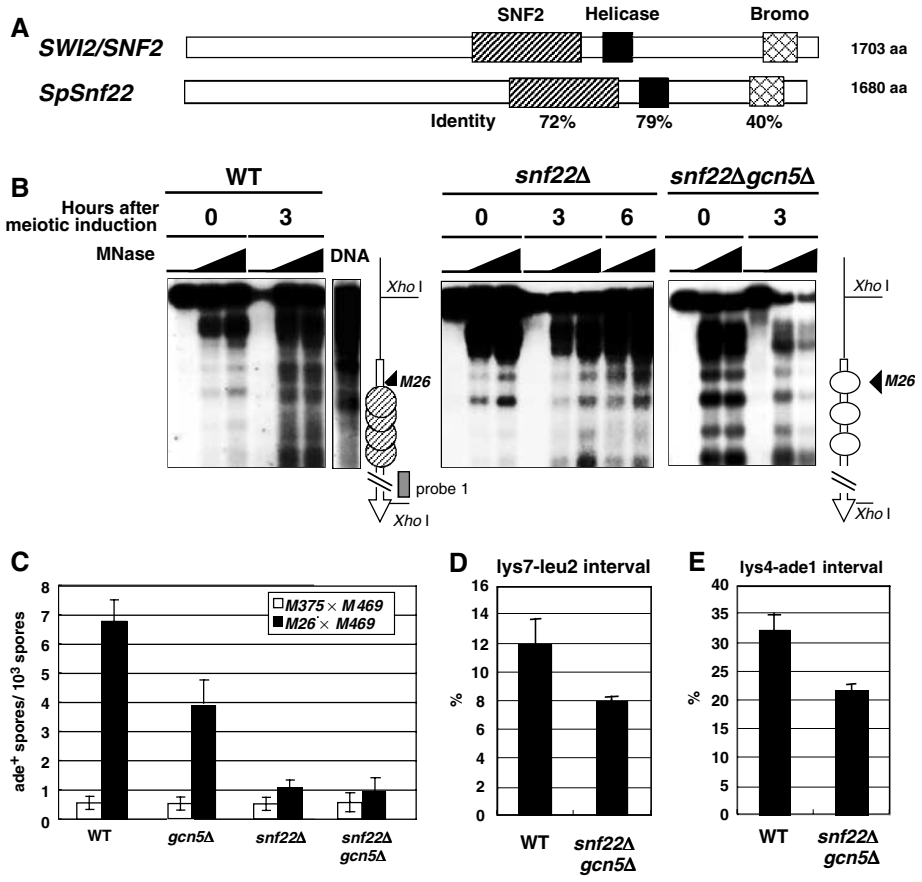

Figure 6 Effects of the *snf22*$^+$ deletion on meiotic chromatin remodeling and recombination at *M26*. (**A**) Primary structures of *S. cerevisiae* Swi2/Snf2 and *S. pombe* SpSnf22. Hatched, filled, and cross-hatched boxes represent SNF2, helicase domains, and bromodomains, respectively. Identity of amino-acid sequences of each domain was indicated by percentages. (**B**) Requirement for SpSnf22 in meiotic chromatin remodeling at *M26*. Meiotic chromatin remodeling at the *ade6* locus in DTY2 (*ade6-M26*, lanes WT), D13A1B1 (*ade6-M26*, *snf22*Δ, lanes *snf22*Δ), and D13G5A1B1 (*ade6-M26*, *snf22*Δ, *gcn5*Δ, lanes *snf22*Δ*gcn5*Δ) diploids was analyzed as in Figure 1, using 0, 20, and 30 units/ml of MNase. Note that the relative intensity of each band varies in different chromatin preparations, although the patterns and positions of the MNase-sensitive sites are reproducible in each experiment. Schematic drawings of nucleosome mapping are the same as in Figure 1. (**C**) Requirement for SpSnf22 in meiotic recombination at *ade6-M26*. Standard genetic crosses between the *ade6-M26* haploid strain and the *ade6-469* haploid strain were performed, and the spores were plated on nonselective medium to determine the viable cell titer (T) and on selective medium lacking adenine to determine the *ade6*$^+$ recombinant titer (R). The recombinant frequency in each experiment is R/T. Data are the recombinant frequencies from 2–3 independent experiments. (**D, E**) Effects of the *snf22*Δ mutation upon intergenic recombination. Standard genetic crosses and measurements of the recombination frequencies between lys7-leu2 (D) and lys4-ade1 (E) intervals were as described in Figure 4. Each recombination frequency represents average of two independent crosses.

after 3–6 h of meiotic induction (Figure 6B). Importantly, meiotic recombination frequencies at *M26* in these strains were greatly reduced ($\sim$1/6 of the normal *M26* levels) almost close to the negative control *M375* levels (Figure 6C). On the other hand, the *snf22*Δ*gcn5*Δ mutant only partially influenced genome-wide recombination of the *lys2-leu2* and *lys4-ade1* intervals (ca. 30% of the wild-type levels, Figure 6D and E). These results indicate an epistatic relationship between *gcn5*$^+$ and *snf22*$^+$ in meiotic chromatin regulation and recombination activation at *M26*.

Discussion

We studied the molecular mechanisms leading to activation of the *M26* meiotic recombination hotspot, especially with regard to chromatin remodeling. We report that: (1) the Atf1·Pcr1 complex, essential for *M26* hotspot activity, is indispensable for chromatin remodeling; (2) histones H3 and H4 are hyperacetylated around *M26* in an *M26*- and Atf1-dependent manner; (3) SpGcn5p is a HAT *in vitro*, and is involved in the hotspot-specific histone acetylation around *M26* and global histone acetylation; (4) Gcn5p is important for the chromatin remodeling and DSB formation around *M26*; and (4) Swi2/Snf2-like SpSnf22 is absolutely required for meiotic chromatin remodeling and recombination around *M26*. These results suggest a mechanism on activation of the *M26* recombination hotspot.

Role of Atf1·Pcr1 in M26 activation

Chromatin remodeling at the *M26* meiotic recombination hotspot fails to occur in mutants lacking any subunits of the Atf1·Pcr1 heterodimer and in strains harboring mutations in the DNA binding site for Atf1·Pcr1. In addition, the chromatin remodeling defects in *atf1*Δ or *pcr1*Δ mutants cannot be complemented by the inactivation of the Pat1 kinase or by the overproduction of Ste11, situations that alleviate the lack of transcription activation of the downstream genes in the absence of the Atf1·Pcr1 complex. These

results provide compelling evidence that the Atf1·Pcr1 complex bound to the *M26* DNA site directly mediates chromatin remodeling *in cis*, rather than by some indirect mechanism, such as transcriptional activation of the downstream genes.

Then, how does Atf1·Pcr1 induce chromatin remodeling? One can consider a possibility that Atf1·Pcr1 itself might mediate chromatin remodeling through histone acetylation, since *S. pombe* Atf1 is similar to human ATF2 with intrinsic HAT activity (Kawasaki *et al*, 2000). However, this may not be the case, because no HAT activity was detected in the purified recombinant Atf1 protein (data not shown). Furthermore, neither Atf1 nor Pcr1 share the conserved amino-acid residues that are important for HAT activity. An alternative possibility is that the binding of Atf1·Pcr1 to *M26* may simply interfere with the local nucleosome positioning. This possibility is also ruled out, because Atf1·Pcr1 is expressed (Wahls and Smith, 1994; Kon *et al*, 1998) and binds to the *M26* site under culture conditions in which *M26* chromatin remodeling cannot be detected at all (Wahls and Smith, 1994; Kon *et al*, 1998).

From these, it is likely that the Atf1·Pcr1·*M26* complex acts to recruit chromatin-modifying machinery such as HATs and ADCRs to the *M26* site. Similar mechanisms are often found in transcription regulation sites. For example, the loading of the *Drosophila* NURF (ADCR) is achieved indirectly, via the binding to the GAGA-binding factor occupying its target sites on the DNA (Tsukiyama *et al*, 1994). In addition, the ATF/CREB family transcription factors in higher eucaryotes recruit coactivator proteins (e.g., CBP) with HAT activity (Ogryzko *et al*, 1996) to transcription promoters with CRE-like sequences.

Roles of SpGcn5 HATs and SpSnf22 in M26 activation

The present results indicate that the histones around the *M26* site are hyperacetylated, and that the elements required for acetylation are identical to those required for chromatin remodeling at *M26*. The histone acetylation levels at *ade6-M26* are significantly higher than those at *ade6-M375* (Figure 2A–C), demonstrating a requirement for the *M26* DNA site. This hotspot-specific histone hyperacetylation requires the Atf1·Pcr1 heterodimer (Figure 2D and E) and the SpGcn5 HAT (Figure 3C and D). These results suggest that the Atf1·Pcr1·*M26* complex may recruit SpGcn5 to the *M26* site (Figure 7). However, preliminary *in vitro* experiments failed to reveal direct interactions between purified Atf1 and purified SpGcn5 (data not shown). Thus, as proposed in Figure 7, it is likely that Atf1 recruits SpGcn5 via adaptor proteins, like the Tra1p subunit of the SAGA complex and the NuA4 complex in *S. cerevisiae* (Brown *et al*, 2001).

The loss of *gcn5*$^+$ causes a delay and impairment of meiotic chromatin remodeling and DSB formation at the *M26* site (Figure 5). This indicates that the SpGcn5-mediated histone acetylation facilitates chromatin remodeling and DSB formation at *M26*, but is not absolutely required for them. It is suggested that the function of SpGcn5 is to promote the rate of remodeling by marking histones with acetylation to be interacted with bromodomain-containing ADCRs, as suggested in the case of budding yeast (Hassan *et al*, 2001). This idea is also consistent with the previous observation that the deletion of budding yeast *GCN5* caused a delay of chromatin remodeling at the *PHO8* promoter (Barbaric *et al*, 2001). Finally, regions with chromatin structure altered by

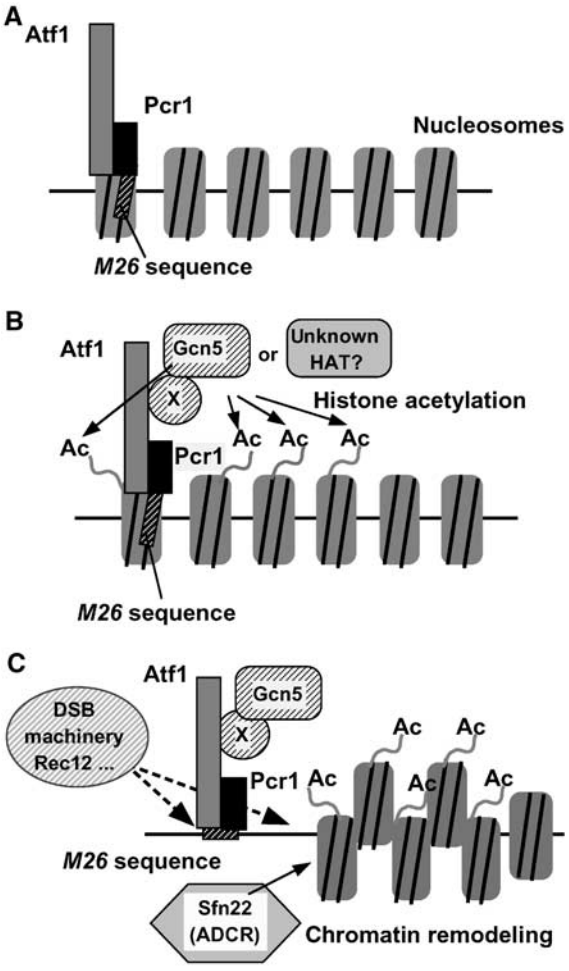

Figure 7 A regulatory model of meiotic recombination at *M26* by HAT-mediated chromatin remodeling. (**A**) In cells lacking Gcn5 HAT, the histones around *M26* are supposed to be hypoacetylated. In this situation, chromatin remodeling and subsequent DSB breaks occur very slowly. (**B**) Histones around *M26* are hyperacetylated by Gcn5 HAT and possibly other HATs, which are introduced to the *M26* site via the interaction with the Atf1·Pcr1 protein complex and a putative adaptor protein (shown as protein X). (**C**) Acetylated histones may be preferential targets of other ATP-dependent chromatin remodeling proteins (ADCR), such SpSnf22. DSB machinery, such as Rec12, is recruited to the open chromatin region created around *M26*, thereby activating meiotic recombination locally.

ADCRs may provide preferential loading sites of the meiotic DSB machinery such as Rec12; thereby DSB formation may be accelerated.

The *gcn5*$^+$ deletion has only partial effects on *M26*-dependent meiotic recombination. It should be noticed that the deletion of the ADCR-like factor *snf22*$^+$ causes a drastic reduction of meiotic recombination frequency at *M26*, and the *snf22*Δ *gcn5*Δ double mutant generally exhibits a deficiency very similar to the *snf22*Δ single mutant. Thus, *snf22*$^+$ and *gcn5*$^+$ have an epistatic relationship, possibly existing on the same pathway for chromatin remodeling. Therefore, the partial effects of the *gcn5*$^+$ deletion are very likely explained by the presence of redundant HAT pathways prior to the action of ADCRs. For example, the acetylation of histone H4, which still occurs at significant levels in *gcn5*Δ mutants, may function redundantly with acetylation of

histone H3 for the activation of recombination. In the budding yeast, histone H4 is preferentially acetylated by HAT called Esa1p (Clarke *et al*, 1999). Although Esa1p is supposed to be important in later DSB repair processes (Bird *et al*, 2002), it will be interesting to study the roles of Esa1p.

Interestingly, the genome-wide acetylation levels of histones H3 and H4 are decreased to similar, very low levels in *atf1*Δ as well as *gcn5*Δ cells (Figures 2F and G and 3G and H). One possibility is that Atf1 activates transcription of the *gcn5*$^+$ gene. This seems unlikely, since there are no Atf1·Pcr1 binding sites upstream of *gcn5*$^+$ and the *atf1*Δ mutation has no detectable effects on *gcn5*$^+$ transcription (data not shown). It thus seems likely that Atf1 participates in the majority of the SpGcn5-mediated histone acetylation. However, the number of Atf1 binding sites is limited in the *S. pombe* genome to explain such global effects, implicating additional indirect functions of Atf1 in the SpGcn5-mediated histone acetylation.

In contrast to the partial effects of *gcn5*$^+$ deletion, the deletion of the *snf22*$^+$ confers very severe defects on meiotic chromatin remodeling and recombination. This indicates the vital role of the SpSnf22 in chromatin remodeling, and suggests that chromatin remodeling is a prerequisite to the *M26* hotspot activation. It is most likely that SpSnf22 functions as an ADCR for chromatin remodeling at *M26*, considering conserved ADCR domains in SpSnf22. It is interesting to examine the biochemical activity of SpSnf22 using an *in vitro* reconstitution system in future experiments.

Possible molecular mechanisms of M26 activation and diverse roles of transcription factors in chromatin

The present results provide important insights into the molecular process of the *M26* recombination activation. As reported previously, the Atf1·Pcr1 heterodimer occupies the *M26* sites already in mitotic cells (Wahls and Smith, 1994; Kon *et al*, 1998). Maximal histone acetylation occurs subsequently, at the very beginning of the meiotic induction (0–1 h, see Figure 2). Chromatin remodeling appears by 2–3 h and meiotic DSBs accumulate at around 4 h, followed by completion of the later recombination processes prior to meiosis I. These differences in the timing of the events reveal a temporal sequence of the processes for the *M26* hotspot activation (see Figure 7): (1) Atf1·Pcr1 binding to *M26*, (2) HAT recruitment and histone acetylation, (3) ADCR recruitment and chromatin remodeling, and (4) loading of recombination initiating proteins.

In this notion, Atf1·Pcr1 may be better thought as a 'chromatin landmark' for the entry of HATs and ADCRs, rather than a canonical 'transcription factor'. Atf1·Pcr1 functions to activate recombination at *M26*, but might act to stimulate transcription at other loci, depending on the local patterns of bound factors and cellular signals. This idea raises a question of unexpected diverse roles of transcription factors in various DNA-related reactions. Analyses on the versatility of transcription factors in chromatin would be an interesting aspect of a future study.

Materials and methods

Yeast strains, media, sporulation, and genetic analyses

S. pombe strains used in this study are listed in Supplementary data 2. Media and sporulation conditions were as described (Mizuno *et al*, 1997). Strain constructions, meiotic crosses, and analyses of recombination frequencies were as described (Ponticelli *et al*, 1988).

Identification and disruption of the gcn5$^+$ *and snf22*$^+$ *gene*

After BLAST searches using the amino-acid sequences of *S. cerevisiae* Swi2/Snf2 helicase domain, and HAT domain and bromodomain of the *S. cerevisiae* and human Gcn5p, directed against all translated open reading frames (ORFs) in the *S. pombe* genome, we identified several ORFs encoding proteins with significant homology. Among these, we focused on *SPAC1952.05* and SPACC 1620.14c genes, which exhibit the highest homology to *GCN5* and Swi2/Snf2, respectively. *SPAC1952.05* is identical to the recently reported *S. pombe gcn5*$^+$ (Mitsuzawa *et al*, 2001). Gene disruptions of *gcn5*$^+$ and *snf22*$^+$ were performed by a one-step gene disruption, which inserted *ura4*$^+$ and *kanMX* into the open reading frame of *gcn5*$^+$ and *snf22*$^+$, and was confirmed by Southern hybridization.

HAT assay of SpGcn5

To prepare the recombinant 6× -histidine tagged version of wild-type SpGcn5 (His-SpGcn5), a DNA fragment (1366 bp) encoding the *gcn5*$^+$ gene was cloned into pET-15b and expressed in *Escherichia coli* BL21. The His-SpGcn5 protein was purified with TALON metal affinity resin (Clontech) according to the manufacturer's instructions. A mutated version of His-SpGcn5 (His-SpGcn5-G205A) was prepared as follows. A plasmid encoding SpGcn5-G205A was constructed by mutation of the *gcn5*$^+$ cDNA using the QuikChange Site-Directed Mutagenesis Kit (Stratagene), followed by subcloning into the pET-15b vector. The His-SpGcn5-G205A protein was expressed and purified as described above. The HAT activities of His-SpGcn5 and His-SpGcn5-G205A were assayed using purified human core histones, as described elsewhere (Ogryzko *et al*, 1996).

Analyses of chromatin structure and mRNA

Analysis of chromatin structure was as described (Mizuno *et al*, 1997). Total RNA from *S. pombe* cells was prepared and analyzed by Northern blotting, as described (Kon *et al*, 1998).

Ch-IP using antiacetylated histones

Ch-IP was performed as described elsewhere (Rundlett *et al*, 1998) with slight modifications. Briefly, cell extracts prepared from crosslinked cells were incubated with antiacetylated histone H3 (AcLys(9/14)) and antiacetylated histone H4 (AcLys(5/8/12/16)) (Upstate). The immune complexes were collected by Dynabeads-ProteinA (Dynal) and washed thoroughly. The DNAs present in the cell extracts and in the immunoprecipitates were purified and applied to a nylon membrane by using a slot blot with a vacuum manifold. The membranes were hybridized to radioactively labeled probes for total genomic DNA or for the *ade6-M26* region (probe M26: corresponds to nucleotides126–692 relative to the first A of the *ade6* coding region). Hybridization signals were quantified using a Fuji BAS2500 Image Analyzer. All Ch-IP data presented were averages of three independent experiments.

Detection of meiotic DSB

Meiosis-specific DSBs around *ade6-M26* were detected by the following two methods, as described (Steiner *et al*, 2002; Young *et al*, 2002). Genomic DNA embedded in agarose plugs was digested with the restriction endonuclease *Pac I*. The DNA was subjected to pulse field gel electrophoresis followed by Southern blotting. Hybridization probes were amplified by PCR using *S. pombe* genomic DNA as a template with the primer pairs (FW 5′-AAACGACTCACACTTTATAGGAGCAAC-3′; RV 5′-AGGCAGCCT CAAAAGCCC-3′), gel purified, and labeled with ^{32}P. Alternatively, plugs digested with *Afl* II were analyzed by standard agarose gel electrophoresis followed by Southern blotting. Hybridization probes were amplified by PCR using *S. pombe* genomic DNA as a template with primer pairs (FW 5′-GCTCCAAGGCAAAATATGTC-3′; RV 5′-AACATACAGGTTGGATCTTAAG-3′), gel purified, and labeled with ^{32}P. To monitor meiotic breaks of the entire chromosome, the DNA embedded in plugs was analyzed by pulsed-field gel electrophoresis, as described (Cervantes *et al*, 2000).

Supplementary data

Supplementary data are available at *The EMBO Journal* Online.

Note added in proof
Accession numbers for gcn5$^+$ is AB162439 and Snf22$^+$ is AB162438.

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References

Agalioti T, Lomvardas S, Parekh B, Yie J, Maniatis T, Thanos D (2000) Ordered recruitment of chromatin modifying and general transcription factors to the IFN-β promoter. *Cell* **103:** 667–678

Barbaric S, Walker J, Schmid A, Svejstrup JQ, Hörz W (2001) Increasing the rate of chromatin remodeling and gene activation-a novel role for the histone acetyltransferase Gcn5. *EMBO J* **20:** 4944–4951

Bird AW, Yu DY, Pray-Grant G, Qiu Q, Harmon KE, Megee PC, Grant PA, Smith MM, Christman MF (2002) Acetylation of histone H4 by Esa1 is required for DNA double-strand break repair. *Nature* **419:** 411–415

Brown CE, Howe L, Sousa K, Alley SC, Carrozza MJ, Tan S, Workman JL (2001) Recruitment of HAT complexes by direct activator interactions with the ATM-related Tra1 subunit. *Science* **292:** 2333–2337

Brown CE, Lechner T, Howe L, Workman JL (2000) The many HATs of transcription coactivators. *Trends Biochem Sci* **25:** 15–19

Burgess SM, Ajimura M, Kleckner N (1999) GCN5-dependent histone H3 acetylation and RPD3-dependent histone H4 deacetylation have distinct, opposing effects on *IME2* transcription, during meiosis and during vegetative growth, in budding yeast. *Proc Natl Acad Sci USA* **96:** 6835–6840

Cervantes MD, Farah JA, Smith GR (2000) Meiotic DNA breaks associated with recombination in *S. pombe*. *Mol Cell* **5:** 883–888

Clarke AS, Lowell JE, Jacobson SJ, Pillus L (1999) Esa1p is an essential acetyltransferase required for cell cycle progression. *Mol Cell Biol* **19:** 2515–2526

Cosma MP, Tanaka T, Nasmyth K (1999) Ordered recruitment of transcription and chromatin remodeling factors to a cell cycle- and developmentally regulated promoter. *Cell* **97:** 299–311

Gutz H (1971) Site specific induction of gene conversion in *Schizosaccharomyces pombe*. *Genetics* **69:** 317–337

Hassan AH, Neely KE, Workman JL (2001) Histone acetyltransferase complexes stabilize Swi/Snf binding to promoter nucleosomes. *Cell* **104:** 817–827

Hirota K, Hoffman CS, Shibata T, Ohta K (2003) Fission yeast Tup1-like repressors repress chromatin remodeling at the *fbp1*$^+$ promoter and the *ade6-M26* recombination hotspot. *Genetics* **165:** 505–515

Iino Y, Yamamoto M (1985) Mutants of *Schizosaccharomyces pombe* which sporulate in the haploid state. *Mol Gen Genet* **198:** 416–421

Kanoh J, Watanabe Y, Ohsugi M, Iino Y, Yamamoto M (1996) *Schizosaccharomyces pombe gad7*$^+$ encodes a phosphoprotein with a bZIP domain, which is required for proper G1 arrest and gene expression under nitrogen starvation. *Genes Cells* **1:** 391–408

Kawasaki H, Schiltz L, Chiu R, Itakura K, Taira K, Nakatani Y, Yokoyama KK (2000) ATF-2 has intrinsic histone acetyltransferase activity which is modulated by phosphorylation. *Nature* **405:** 195–200

Kon N, Krawchuk MD, Warren BG, Smith GR, Wahls WP (1997) Transcription factor Mts1/Mts2 (Atf1/Pcr1, Gad7/Pcr1) activates the *M26* meiotic recombination hotspot in *Schizosaccharomyces pombe*. *Proc Natl Acad Sci USA* **94:** 13765–13770

Kon N, Schroeder SC, Krawchuk MD, Wahls WP (1998) Regulation of the Mts1-Mts2-dependent *ade6-M26* meiotic recombination hot spot and developmental decisions by the Spc1 mitogen-activated protein kinase of fission yeast. *Mol Cell Biol* **18:** 7575–7583

Krebs JE, Kuo MH, Allis CD, Peterson CL (1999) Cell cycle-regulated histone acetylation required for expression of the yeast *HO* gene. *Genes Dev* **13:** 1412–1421

Kuo MH, Brownell JE, Sobel RE, Ranalli TA, Cook RG, Edmondson DG, Roth SY, Allis CD (1996) Transcription-linked acetylation by Gcn5p of histones H3 and H4 at specific lysines. *Nature* **383:** 269–272

Kuo MH, Zhou J, Jambeck P, Churchill ME, Allis CD (1998) Histone acetyltransferase activity of yeast Gcn5p is required for the activation of target genes *in vivo*. *Genes Dev* **12:** 627–639

McBlane JF, van Gent DC, Ramsden DA, Romeo C, Cuomo CA, Gellert M, Oettinger MA (1995) Cleavage at a V(D)J recombination signal requires only RAG1 and RAG2 proteins and occurs in two steps. *Cell* **83:** 387–395

McMurry MT, Krangel MS (2000) A role for histone acetylation in the developmental regulation of V(D)J recombination. *Science* **287:** 495–498

Mitsuzawa H, Seino H, Yamao F, Ishihama A (2001) Two WD repeat-containing TATA-binding protein-associated factors in fission yeast that suppress defects in the anaphase-promoting complex. *J Biol Chem* **276:** 17117–17124

Mizuno K, Emura Y, Baur M, Kohli J, Ohta K, 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

Mizuno K, Hasemi T, Ubukata T, Yamada T, Lehmann E, Kohli J, Watanabe Y, Iino Y, Yamamoto M, Fox ME, Smith GR, Murofushi H, Shibata T, Ohta K (2001) Counteracting regulation of chromatin remodeling at a fission yeast CRE-related recombination hotspot by SAPK, cAMP-dependent kinase, and meiosis regulators. *Genetics* **159:** 1467–1478

Ogryzko VV, Schiltz RL, Russanova V, Howard BH, Nakatani Y (1996) The transcriptional coactivators p300 and CBP are histone acetyltransferases. *Cell* **87:** 953–959

Ohta K, Shibata T, Nicolas A (1994) Changes in chromatin structure at recombination initiation sites during yeast meiosis. *EMBO J* **13:** 5754–5763

Petes TD (2001) Meiotic recombination hot spots and cold spots. *Nat Rev Genet* **2:** 360–369

Ponticelli AS, Sena EP, Smith GR (1988) Genetic and physical analysis of the *M26* recombination hotspot of *Schizosaccharomyces pombe*. *Genetics* **119:** 491–497

Rundlett SE, Carmen AA, Suka N, Turner BM, Grunstein M (1998) Transcriptional repression by UME6 involves deacetylation of lysine 5 of histone H4 by RPD3. *Nature* **392:** 831–835

Schuchert P, Langsford M, Kaslin E, Kohli J (1991) A specific DNA sequence is required for high frequency of recombination in the *ade6* gene of fission yeast. *EMBO J* **10:** 2157–2163

Steiner WW, Schreckhise RW, Smith GR (2002) Meiotic DNA breaks at the *S. pombe* recombination hot spot *M26*. *Mol Cell* **9:** 847–855

Sugimoto A, Iino Y, Maeda T, Watanabe Y, Yamamoto M (1991) *Schizosaccharomyces pombe ste11*$^+$ encodes a transcription factor with an HMG motif that is a critical regulator of sexual development. *Genes Dev* **5:** 1990–1999

Szankasi P, Heyer WD, Schuchert P, Kohli J (1988) DNA sequence analysis of the *ade6* gene of *Schizosaccharomyces pombe*. Wild-type and mutant alleles including the recombination hot spot allele *ade6-M26*. *J Mol Biol* **204:** 917–925

Takeda T, Toda T, Kominami K, Kohnosu A, Yanagida M, Jones N (1995) *Schizosaccharomyces pombe atf1*$^+$ encodes a transcription factor required for sexual development and entry into stationary phase. *EMBO J* **14:** 6193–6208

Tsukiyama T, Becker PB, Wu C (1994) ATP-dependent nucleosome disruption at a heat-shock promoter mediated by binding of GAGA transcription factor. *Nature* **367:** 525–532

Wahls WP, Smith GR (1994) A heteromeric protein that binds to a meiotic homologous recombination hot spot: correlation of binding and hot spot activity. *Genes Dev* **8:** 1693–1702

Watanabe Y, Yamamoto M (1996) *Schizosaccharomyces pombe pcr1*$^{+}$ encodes a CREB/ATF protein involved in regulation of gene expression for sexual development. *Mol Cell Biol* **16:** 704–711

Wolffe AP (1997) *Chromatin: Structure and function*, 3rd edn San Diego, USA: Academic Press

Wu TC, Lichten M (1994) Meiosis-induced double-strand break sites determined by yeast chromatin structure. *Science* **263:** 515–518

Young JA, Schreckhise RW, Steiner WW, Smith GR (2002) Meiotic recombination remote from prominent DNA break sites in *S. pombe*. *Mol Cell* **9:** 253–263

Zenvirth D, Simchen G (2000) Meiotic double-strand breaks in *Schizosaccharomyces pombe*. *Curr Genet* **38:** 33–38