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# The fission yeast homologue of CENP-B, Abp1, regulates directionality of mating-type switching

**Lorena Aguilar-Arnal, Francesc-Xavier Marsellach and Fernando Azorín***

Institute of Molecular Biology of Barcelona, CSIC, and Institute for Research in Biomedicine (IRB Barcelona), Parc Científic de Barcelona, Barcelona, Spain

**In fission yeast, mating-type switching involves replacing genetic information contained at the expressed *mat1* locus by that of either the *mat2P* or *mat3M* donor loci. Donor selection is nonrandom, as *mat1P* cells preferentially use *mat3M* for switching, whereas *mat1M* cells use *mat2P*. Switching directionality is determined by the cell-type-specific distribution of the Swi2–Swi5 complex that, in *mat1P* cells, localises to *mat3M* and, only in *mat1M* cells, spreads to *mat2P* in a heterochromatin-dependent manner. Mechanisms regulating spreading of Swi2–Swi5 across heterochromatin are not fully understood. Here, we show that the fission yeast homologue of CENP-B, Abp1, binds to the silent domain of the mating-type locus and regulates directionality of switching. Deletion of *abp1* prevents utilisation of *mat2P*, as when heterochromatin is disrupted and spreading of Swi2–Swi5 is impaired. Our results show that, indeed, deletion of *abp1* abolishes spreading of Swi2–Swi5 to *mat2P*. However, in *abp1*Δ cells, heterochromatin organisation at the mating-type locus is preserved, indicating that Abp1 is actually required for efficient spreading of Swi2–Swi5 through heterochromatin. Cbh1 and Cbh2, which are also homologous to CENP-B, have only a minor contribution to the regulation of directionality of switching, which is in contrast with the strong effects observed for Abp1.**

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## Introduction

In the fission yeast *Schizosaccharomyces pombe*, haploid cells switch mating type by means of a tightly regulated gene conversion event that involves long distance interactions between an expressed locus (*mat1*) with either of two silent donor loci (*mat2* and *mat3*), which are located 17 and 29 kb away from *mat1*, respectively (reviewed in Arcangioli and Thon, 2004). Mating-type information is contained in the silent *mat2P* (plus) and *mat3M* (minus) loci but it is expressed only after translocation to the *mat1* locus giving rise to *mat1P* or *mat1M* cells, depending on whether *mat2* or *mat3* information is expressed at *mat1*. Silencing at the *mat2* and *mat3* loci is mediated by heterochromatin that, in the mating-type region, extends for a 20-kb long domain (Thon and Klar, 1993; Grewal and Klar, 1997; Noma *et al*, 2001). This silenced domain is flanked by two inverted repeats (IR-L and IR-R) and, in addition to *mat2* and *mat3*, contains a repeated element (*cenH*) that is homologous to the centromeric dg/dh repeats and nucleates the formation of heterochromatin in an RNAi-dependent manner (Hall *et al*, 2002). At the mating-type region, heterochromatin formation is also induced through an additional RNAi-independent mechanism that involves binding of the transcription factors Atf1 and Pcr1 (Jia *et al*, 2004a).

Mating-type switching initiates during DNA replication with the introduction of a strand-specific single-strand break (SSB) imprint at *mat1*, which was proposed to result from the incorporation of two ribonucleotides (Vengrova and Dalgaard, 2004, 2006), and that, in the next round of DNA replication, is converted into a double-strand break (DSB) (reviewed in Arcangioli and Thon, 2004). This DSB is, then, healed by gene conversion using *mat2* or *mat3* as donors. Donor selection is, however, not random. In contrast, *mat1P* cells preferentially use *mat3* as a donor, whereas *mat1M* cells use *mat2* (reviewed in Arcangioli and Thon, 2004). Directionality of switching, therefore, ensures that cells switch to the opposite mating type with a very high frequency. Directionality of switching is determined by the cell-type specific distribution of the Swi2–Swi5 complex that promotes recombination (Jia *et al*, 2004b). In *mat1P* cells, Swi2–Swi5 localisation is restricted to a recombination-enhancer element (SRE) located adjacently to *mat3* so that, under these circumstances, only *mat3* is efficiently used as a donor. On the other hand, in *mat1M* cells, Swi2–Swi5 spreads across the entire mating-type region reaching the *mat2* locus that becomes the preferred donor site due to the structural constraints imposed by heterochromatin. Spreading of Swi2–Swi5 in *mat1M* cells relies on heterochromatin, as it is abolished in a *swi6* mutant without affecting its binding to SRE (Jia *et al*, 2004b). As a consequence, in a *swi6* mutant background, *mat3* is used as a donor at a much higher frequency than *mat2*, so that *swi6* mutant cells are predominantly of the *mat1M* type. Mutations in several other heterochromatin assembly factors (i.e. *crl4*, *rik1* and *sir2*) were also shown to affect directionality of switching (Ivanova *et al*, 1998; Nakayama *et al*, 2001; Shankaranarayana *et al*, 2003; Tuzon *et al*, 2004). These observations unveil the essential contribution of heterochromatin to spreading of Swi2–Swi5. Heterochromatin-dependent spreading was also reported for other multi-protein complexes such as SHREC (Sugiyama

*Corresponding author. Institut de Biologia Molecular de Barcelona-CSIC, Parc Científic de Barcelona, Josep Samitier, 1-5, Barcelona 08028, Spain. Tel.: +34 93 4034958; Fax: +34 93 4034979;
E-mail: fambmc@ibmb.csic.es

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*et al*, 2007). The mechanisms that regulate spreading of Swi2–Swi5 across heterochromatin are, however, not fully understood. Direct interaction with Swi6 likely contributes to binding of Swi2–Swi5 to heterochromatin, as Swi2 selectively colocalises with Swi6 and the two proteins interact strongly *in vitro* (Akamatsu *et al*, 2003; Jia *et al*, 2004b). However, *in vivo*, only a small proportion of Swi2 is associated to Swi6 (Jia *et al*, 2004b), indicating that additional factors must contribute to binding of Swi2–Swi5 to heterochromatin.

In this paper, we show that Abp1 binds at the mating-type locus and regulates directionality of switching. In *abp1*Δ cells, *mat3* is preferentially used as a donor as when, in *swi6*Δ or *crl4*Δ cells, spreading of Swi2–Swi5 to *mat2* is abolished due to disruption of heterochromatin. Our results show that deletion of *abp1* impairs spreading of Swi2–Swi5 to *mat2* without disrupting heterochromatin organisation at the mating-type region, indicating that Abp1 is actually required for efficient spreading of Swi2–Swi5 across heterochromatin.

Abp1 is homologous to CENP-B, an evolutionarily conserved sequence-specific DNA-binding protein that associates to centromeric heterochromatin (Murakami *et al*, 1996; Halverson *et al*, 1997). In *S. pombe*, Cbh1 and Cbh2 are also homologous to CENP-B and, together with Abp1, have redundant functions in the regulation of various nuclear processes (Halverson *et al*, 1997; Lee *et al*, 1997; Baum and Clarke, 2000; Ireland *et al*, 2001; Nakagawa *et al*, 2002). However, the contribution to the regulation of directionality of mating-type switching is specific to Abp1, as deletion of *cbh1* or *cbh2* shows no effects on switching. Interestingly, Abp1 also appears to have an important function in DNA replication (Murakami *et al*, 1996; Locovei *et al*, 2006), suggesting a possible link between spreading of Swi2–Swi5 and DNA replication.

## Results

### *Abp1 regulates directionality of mating-type switching*

Efficiency of mating-type switching can be monitored by staining with iodine vapours (Bresch *et al*, 1968; Thon and Klar, 1993; Jia *et al*, 2004b). Efficient switching results into colonies containing an homogeneous mixture of cells of opposite mating types that, when grown in sporulation medium, can readily mate and sporulate giving rise to colonies staining dark with iodine vapours due to the accumulation of starch products occurring during sporulation. In contrast, cells showing poor mating-type switching form light-staining colonies. Deletion of *abp1* in a switching-efficient homothallic $h^{90}$ strain (Figure 1A) results in a strong decrease in its ability to mate and sporulate. *abp1*Δ cells form colonies staining much lighter with iodine than wild-type $h^{90}$ colonies (Figure 1B). Actually, the frequency of spores observed in *abp1*Δ colonies is as low as 0.4% of total cells, whereas in wild-type colonies is approximately 81%. These results indicate that mating-type switching is very inefficient in *abp1*Δ cells.

As discussed above, a first step in mating-type switching is the generation of a strand-specific SSB imprint that, latter, is converted into a DSB. A number of factors (*swi1*, *swi3* and *swi7*) are known to participate in the formation of this SSB/DSB imprint (reviewed in Arcangioli and Thon, 2004). Abp1 could also participate in this process, as it contains a DDE domain with significant homology to the catalytic domains of the *pogo* family of transposases (Ireland *et al*, 2001). To test this possibility, we performed Southern blotting analysis to determine the presence of the SSB/DSB imprint in *abp1*Δ cells (Figure 1C). In these experiments, genomic DNA was digested with *Hin*dIII, which generates a 10.4-kb fragment spanning the *mat1* locus (Figure 1A). Presence of the SSB/DSB imprint results in cleavage of this *Hin*dIII fragment into two fragments of 5.4 and 5.0 kb, respectively. It must be noticed that, though many cells contain only a SSB at this position, it is converted into a DSB during DNA isolation due to sharing forces (Arcangioli, 1998). Bands reflecting formation of the SSB/DBS imprint are readily detected in *abp1*Δ (Figure 1C, lanes 2–5) as well as in wild-type $h^{90}$ cells (Figure 1C, lane 1), indicating that deletion of *abp1* does not affect generation of the imprint.

Next, we analysed whether inefficient switching of *abp1*Δ cells is the consequence of a deregulation on directionality of switching, so that they are unable to use either *mat2* or *mat3* as a donor locus. To test this possibility, we carried out quantitative multiplex PCR analysis using primers that specifically determine the presence at the expressed *mat1* locus of either *mat2* (*mat1P*) or *mat3* (*mat1M*) information (Jia *et al*, 2004b) (Figure 1D). In wild-type $h^{90}$ cells, efficient mating-type switching results in equal utilisation of *mat2* or *mat3* as a donor, so that bands representing *mat1P* and *mat1M* cells are roughly of the same intensity (M/P ratio of $0.95 \pm 0.06$) (Figure 1D, lanes 1–3). In contrast, *abp1*Δ cells are predominantly of the *mat1M* type, which contains *mat3* information at *mat1* ($M/P = 3.03 \pm 0.06$) (Figure 1D, lanes 4–6). Ectopic expression of Abp1 from an episomal vector reverts the switching defect of *abp1*Δ cells ($M/P = 1.24 \pm 0.01$) (Figure 1D, lanes 7 and 8). These results indicate that Abp1 regulates directionality of switching, so that *abp1*Δ cells preferentially use *mat3* as a donor.

To further confirm the contribution of Abp1 to the regulation of directionality of mating-type switching, we analysed the effects of deleting *abp1* in an $h^{09}$ background. In $h^{09}$ cells, mating-type information contained at the donor *mat2* and *mat3* loci is swapped, so that *mat3* contains plus (*P*) information (*mat3P*), whereas *mat2* contains minus (*M*) information (*mat2M*) (Figure 2A) (Thon and Klar, 1993). Switching is determined by the chromosomal position of the donor loci with respect to *mat1* rather than by the actual genetic information contained at *mat1* (Thon and Klar, 1993). As a consequence, in $h^{09}$ cells, switching does not replace mating information at *mat1*, as *mat1M* cells preferentially use *mat2M* as a donor and, vice versa, *mat1P* cells use *mat3P*, resulting in non-productive switching. Consistent with that, $h^{09}$ colonies stain lightly with iodine and show a low frequency of sporulation (Figure 2B). As shown in Figure 2C, the $h^{09}$ strain used in these experiments is predominantly of the *mat1M* type ($M/P = 2.43 \pm 0.17$) (Figure 2C, lanes 1–3). In this $h^{09}$ strain, deletion of *abp1* results also in a preferred utilisation of *mat3P*, so that *abp1*Δ cells become predominantly *mat1P* ($M/P = 0.82 \pm 0.12$) (Figure 2C, lanes 4–6). Concomitantly, *abp1*Δ cells stain darker with iodine than wild-type $h^{09}$ cells and sporulation is partially rescued, from 7 to 15% (Figure 2B).

Next, we asked whether Abp1 actually binds to the mating-type locus. To address this question, we performed chromatin immunoprecipitation (ChIP) experiments from cells carrying

 

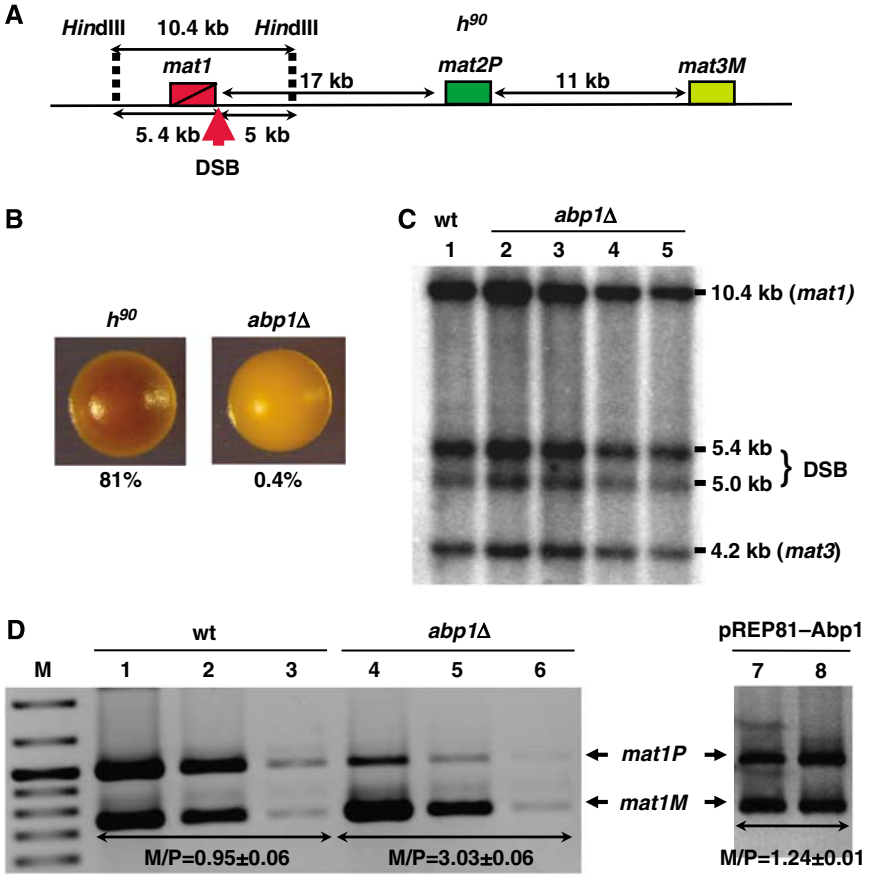


**Figure 1** Analysis of the effects on mating-type switching of deleting *abp1* in a homothallic $h^{90}$ strain. (**A**) Schematic representation of the structural organisation of the mating-type region in an $h^{90}$ strain. The positions of *mat1*, *mat2P* and *mat3M* loci are indicated. The position of the SSB/DSB imprint, contained within the 10.4-kb *Hin*dIII fragment spanning the *mat1* locus, is also indicated. (**B**) Iodine staining of wild-type ($h^{90}$) and *abp1*Δ colonies. Before staining, colonies were grown in sporulation medium at 25°C for 3 days. Numbers below each panel indicate the frequencies of sporulation. Similar results were obtained with several independent *abp1*Δ colonies. (**C**) Presence of the SSB/DSB imprint was determined by Southern blot analysis of a wild-type (lane 1) and four independent *abp1*Δ strains (lanes 2–5). Genomic DNA was digested with *Hin*dIII and probed with a 9-kb DNA fragment, which spans the *mat1* locus and contains *mat3M* information at *mat1*, so that it also detects a 4.2-kb *Hin*dIII band corresponding to the *mat3M* locus. Bands arising from cleavage at the SSB/DBS imprint of the 10.4-kb *Hin*dIII fragment are indicated (DSB). (**D**) Quantitative multiplex PCR determination of the predominant mating type adopted by wild-type (wt, lanes 1–3) and *abp1*Δ cells (lanes 4–6). PCR reactions were performed using appropriate primers to amplify *mat1M* and *mat1P* sequences simultaneously. For each strain, three 10-fold dilutions of the PCR products were analysed. Similar results were obtained with several independent *abp1*Δ colonies. Lanes 7 and 8, correspond to *abp1*Δ cells transformed with plasmid pREP81–Abp1 to express Abp1. In this case, single dilutions of the PCR products obtained from two independent isolates are shown. The positions of the bands corresponding to *mat1P* and *mat1M* are indicated. The M/P ratios (±s.d.) are indicated below the corresponding lanes.

HA-tagged Abp1 (Figure 3). In these experiments, the presence of Abp1 was determined at several regions spanning the entire silent domain of the mating-type locus (Figure 3A). Crosslinked material was immunoprecipitated with αHA antibodies and analysed by PCR using appropriate primers to amplify specific bands of the indicated mating-type regions (Figure 3A, bands ir, r2, c1, c2, l1 and l2), and, in the same reaction, with a second set of primers to amplify a control DNA fragment of similar length corresponding to the *act1* gene (Figure 3A, bands act). As shown in Figure 3A, only one of the regions analysed (l1) was found significantly enriched in the immunoprecipitated material, indicating binding of Abp1 to this region. Region l1, which spans approximately 500 bp, maps immediately upstream to the *cenH* element. To confirm binding of Abp1 to this region, and to narrow down its binding site, we analysed presence of Abp1 at different regions flanking l1 (Figure 3B, regions p1, p2 and p4), as well as within it (Figure 3B, region p3). As shown in Figure 3B, immunoprecipitation with αHA antibodies results in enrichment of region p3, but not the rest. Altogether, these results indicate that Abp1 binds to the silent domain of the mating-type locus, with a major binding-site centred around region l1, close to the *cenH* element. Similar results have been recently reported by others (Cam *et al*, 2007).

### *Directionality of mating-type switching is not compromised by deletion of cbh1 and/or cbh2*

In *S. pombe*, Cbh1 and Cbh2 are closely related to Abp1 having redundant functions in the regulation of several cellular processes (Halverson *et al*, 1997; Lee *et al*, 1997; Baum and Clarke, 2000; Ireland *et al*, 2001; Nakagawa *et al*, 2002). In particular, all three proteins show synergistic effects on the regulation of centromeric silencing and chromosome segregation (Nakagawa *et al*, 2002). Therefore, it is possible that Cbh1 and Cbh2 would also contribute to the regulation of directionality of mating-type switching. To address this

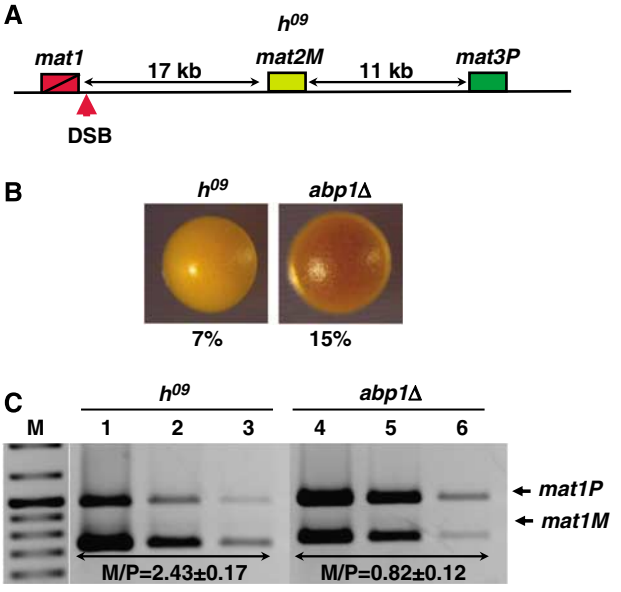


**Figure 2** Analysis of the effects on mating-type switching of deleting *abp1* in $h^{09}$ cells. (**A**) Schematic representation of the structural organisation of the mating-type region in an $h^{09}$ strain. The positions of *mat1*, *mat2M* and *mat3P* loci are indicated. The position of the SSB/DSB imprint is also indicated. (**B**) Iodine staining of wild-type ($h^{09}$) and *abp1*Δ colonies. Numbers below each panel correspond to the frequencies of sporulation. (**C**) Quantitative multiplex PCR determination of the predominant mating type adopted by wild-type $h^{09}$ (lanes 1–3) and *abp1*Δ cells (lanes 4–6). PCR reactions were performed as in Figure 1D. The M/P ratios (±s.d.) are indicated below the corresponding lanes.

possibility, we examined the effects on mating-type switching of deleting *cbh1* and *cbh2* in $h^{90}$ cells (Figure 4). *cbh1*Δ and *cbh2*Δ cells stain dark with iodine (not shown) and show high frequencies of sporulation (~80%), indicating that they do not have major defects in directionality of mating-type switching. Quantitative multiplex PCR analysis confirmed these results (Figure 4A). In *cbh1*Δ and *cbh2*Δ cells, the bands corresponding to *mat1P* and *mat1M* are of almost equal intensity (M/P = 0.95 ± 0.05 and 0.99 ± 0.03) (Figure 4A, lanes 1–6), indicating that, in these mutant backgrounds, both *mat2* and *mat3* are efficiently used as donor locus during mating-type switching, which is in contrast to the strong donor preference observed in *abp1*Δ cells (M/P = 3.03 ± 0.06). Similarly, *cbh1*Δ*cbh2*Δ double mutants show no significant defects on directionality of switching (M/P = 0.98 ± 0.01) (Figure 4A, lanes 7–9). On the other hand, in *abp1*Δ*cbh1*Δ and *abp1*Δ*cbh2*Δ double mutants, *mat3* is also the preferred donor locus, as in *abp1*Δ cells (Figure 4B). It must be noticed, however, that *abp1*Δ*cbh1*Δ and *abp1*Δ*cbh2*Δ double mutants show significantly higher M/P ratios than *abp1*Δ cells, suggesting a synergistic cooperation to regulate switching directionality. Actually, it was recently shown that Abp1 interacts both with Cbh1 and Cbh2 and that, in the mating-type locus, Cbh1 colocalises with Abp1 (Cam *et al*, 2007). Moreover, binding of Cbh1 to chromatin appears to depend on Abp1 but not vice versa (Cam *et al*, 2007). Altogether, these observations indicate that Abp1 has a major function in the regulation of mating-type switching, whereas Cbh1 and Cbh2 have only minor contributions that, most likely, depend on Abp1.

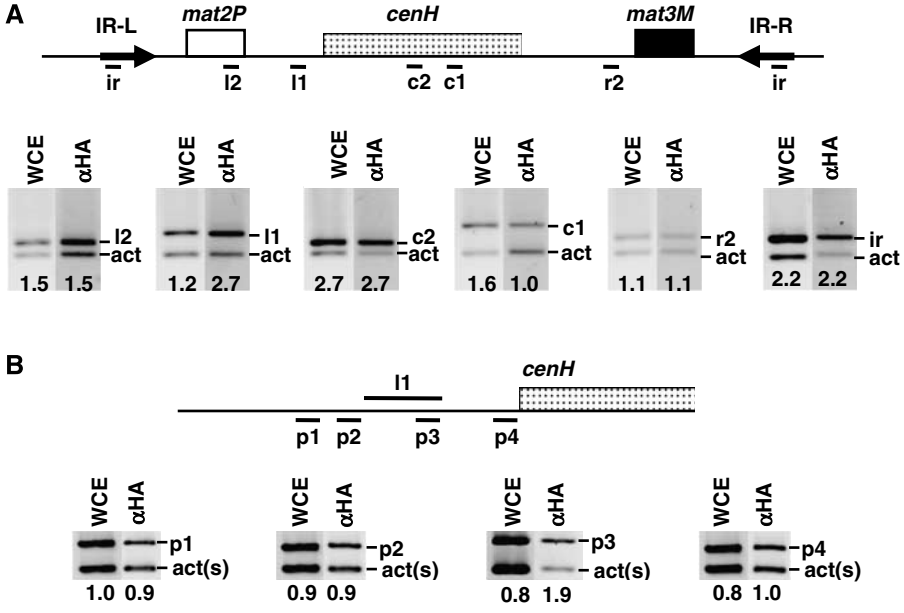


**Figure 3** Abp1 localises to the silent domain of the mating-type locus. (**A**) Binding of Abp1–HA at the silent domain of the mating-type locus was determined by ChIP-analysis using αHA antibodies. Immunoprecipitated material was analysed by multiplex PCR using primers specific for the indicated regions of the mating-type locus (bands ir, r2, c1, c2, l1 and l2) (see Supplementary Table SII for a description of the primers used) and, in the same PCR reaction, primers that amplify a fragment of similar length of the *act1* locus (bands act), used as control. (**B**) Similar experiments as those described in (A) but with primers designed to amplify shorter DNA fragments flanking region l1 (bands p1, p2 and p4) or contained within it (bands p3). In this case, a shorter fragment from the *act1* gene was used as control (bands act(s)). Lanes WCE correspond to PCR products obtained from the input material before immunoprecipitation. Lanes αHA correspond to the products obtained from the immunoprecipitated material. Numbers below each lane correspond to the ratio of the corresponding mating-type-specific band with respect to the control *act1* band.

 

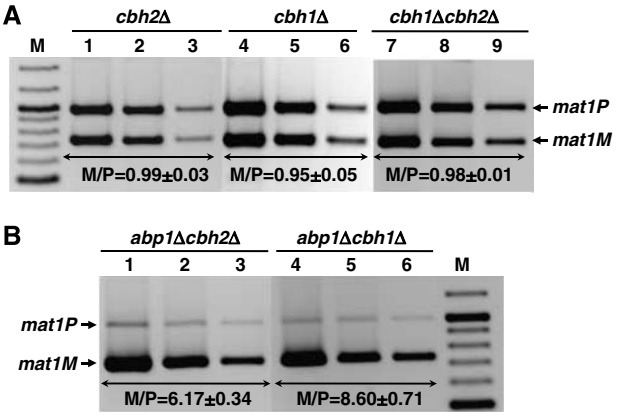


**Figure 4** Analysis of the effects on mating-type switching of deleting *cbh1* and *cbh2* in $h^{90}$ cells. (**A**) Quantitative multiplex PCR determination of the predominant mating type adopted by *cbh2*Δ (lanes 1–3), *cbh1*Δ (lanes 4–6) and *cbh1*Δ*cbh2*Δ cells (lanes 7–9). (**B**) Quantitative multiplex PCR determination of the predominant mating type adopted by *abp1*Δ*cbh2*Δ (lanes 1–3) and *abp1*Δ*cbh1*Δ cells (lanes 4–6). PCR reactions were performed as in Figure 1D. The M/P ratios (±s.d.) are indicated below the corresponding lanes.

### *Heterochromatin organisation of the mating-type region is preserved in abp1*Δ *cells*

Heterochromatin organisation of the mating-type region is essential for the efficient utilisation of *mat2* as a donor (Jia *et al*, 2004b). On the one hand, in *mat1M* cells, heterochromatin mediates spreading of the Swi2–Swi5 complex to *mat2*, so that it can be used as template for gene conversion. In addition, although in *mat1M* cells Swi2–Swi5 is also present at *mat3*, heterochromatin provides a higher order structure that makes *mat2* the preferred donor loci. As a consequence, mutations that disrupt heterochromatin organisation prevent efficient utilisation of *mat2* as a donor and, therefore, alter directionality of switching in a similar way as deletion of *abp1* does (Figure 5A) (Jia *et al*, 2004b).

These observations point out the possibility that the switching defects observed in *abp1*Δ cells could reflect a contribution of Abp1 to heterochromatin assembly at the mating-type locus. Actually, Abp1 was reported to have a modest contribution to heterochromatin assembly at centromeres, as centromeric silencing was found to be slightly reduced in *abp1*Δ cells (Nakagawa *et al*, 2002). To test this hypothesis, we analysed the effects of an *abp1*Δ deletion on

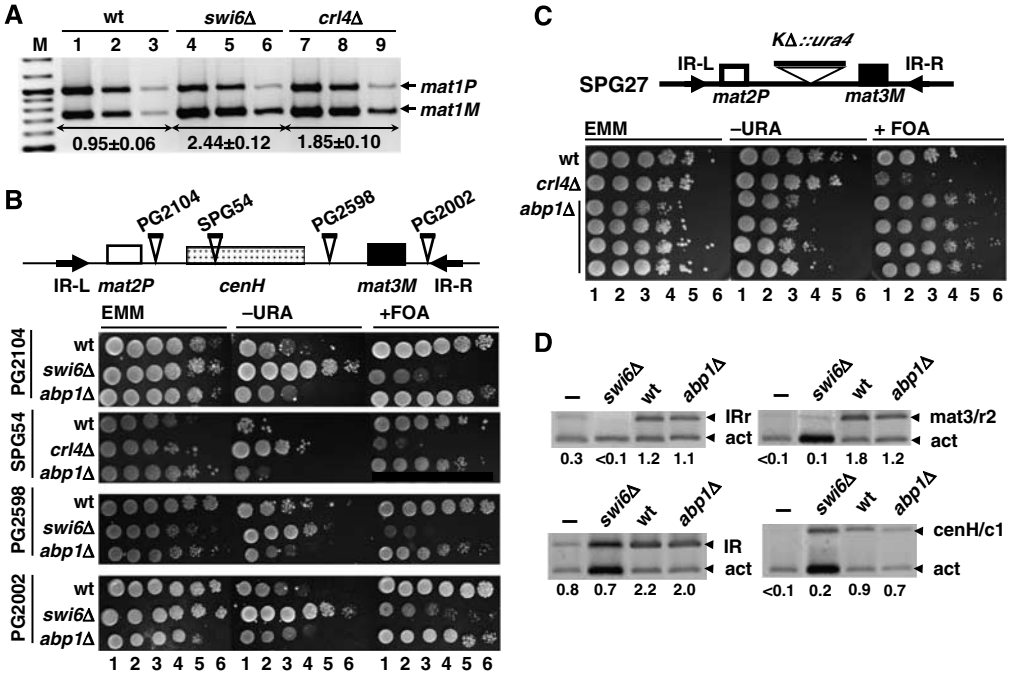


**Figure 5** Heterochromatin organisation of the mating-type region is not disrupted in *abp1*Δ cells. (**A**) Quantitative multiplex PCR determination of the predominant mating type adopted by wild-type (lanes 1–3), *swi6*Δ (lanes 4–6) and *crl4*Δ cells (lanes 7–9). PCR reactions were performed as in Figure 1D. The M/P ratios (±s.d.) are indicated below the corresponding lanes. (**B**) The effect of deleting *abp1* on heterochromatin-mediated silencing was determined in several strains carrying *ura4*$^+$ insertions at different positions of the silent domain of the mating-type locus. Sites of insertion are schematically indicated on top. Exponentially growing cells were plated as serial 10-fold dilutions (lanes 1–6) onto selective media with (panels EMM) or without uracil (panels −URA), or in the presence of FOA (panels +FOA). For each strain, several independent *abp1*Δ isolates were analysed and they all showed very similar results. (**C**) Similar results as in (B) but performed with strain SPG27, where the entire *cenH* element was replaced by *ura4*$^+$. In this case, the results obtained with four independent *abp1*Δ isolates are presented. (**D**) Swi6 deposition at the silent domain of the mating-type region was determined in wild-type (lanes wt) and *abp1*Δ cells (lanes *abp1*Δ) by ChIP analysis using αSwi6 antibodies. Immunoprecipitated material was analysed as in Figure 3 with primers to amplify specific bands of the IR-R and IR-L regions (bands IRr and IR), the *cenH* element (bands cenH/c1) and a region just left of *mat3* (bands mat3/r2) (see Supplementary Table SII for a description of the primers used). Lanes *swi6*Δ correspond to immunoprecipitation experiments performed with αSwi6 antibodies in a *swi6*Δ strain, missing Swi6. Lanes – correspond to mock immunoprecipitation experiments in which no antibodies were added. Numbers below each lane correspond to the ratio of the corresponding mating-type-specific band with respect to the control act band.

heterochromatin-mediated silencing at the mating-type locus. In these experiments, we used several reporter strains carrying an *ura4*$^+$ gene inserted at different locations across the silent heterochromatic domain of the mating-type locus (Figure 5B, top). In all these strains, the reporter *ura4*$^+$ gene is strongly silenced (Grewal and Klar, 1997; Thon *et al*, 2002), so that they grow poorly in the absence of uracil and strongly in the presence of fluoroorotic acid monohydrate (FOA) (Figure 5B, rows wt). In all these cases, silencing is relieved in *swi6*Δ or *crl4*Δ mutants (Figure 5B, rows *swi6*Δ and *crl4*Δ), which show extremely poor growth in the presence of FOA. Deletion of *abp1*, however, does not result in any significant relief of silencing, as indicated by the robust growth observed in the presence of FOA (Figure 5B, rows *abp1*Δ). Moreover, in the absence of uracil, both wild-type and *abp1*Δ cells show similar growth. The effect of deleting *abp1* was also analysed in strain SPG27, where the entire *cenH* element was replaced by *ura4*$^+$ (Figure 5C). In this strain, silencing is less strong as it relies mainly on the Atf1–Pcr1 pathway (Grewal and Klar, 1996). As a consequence, SPG27 cells show significant growth both in the absence of uracil and in the presence of FOA (Figure 5C, row wt), being more sensitive to slight changes in the levels of heterochromatin assembly factors. As shown in Figure 5C, in this strain, silencing is slightly reinforced in *abp1*Δ cells, as shown by the reduced growth observed in the absence of uracil (Figure 5C, rows *abp1*Δ). Concomitantly, growth in the presence of FOA increases. In these experiments, several independent *abp1*Δ isolates were analysed for each reporter strain and they all showed very similar effects.

ChIP analysis confirmed these results (Figure 5D). In these experiments, Swi6 distribution at the silent chromatin domain of the mating-type locus was determined in wild-type (wt) and *abp1*Δ cells. Swi6 deposition was analysed at the IR repeats, as well as at a region of the *cenH* element (c1) and at a region just left from *mat3* (r2) (Figure 5D, bands IRr, IR, cenH/c1 and mat3/r2). Both in wild-type and *abp1*Δ cells, immunoprecipitation with αSwi6 antibodies results in a similar enrichment of the four mating-type-specific bands indicated above (Figure 5D, lanes wt and *abp1*Δ), when compared to similar immunoprecipitation experiments performed in a *swi6*Δ strain, missing Swi6 (Figure 5D, lanes *swi6*Δ), or when no antibodies were added (Figure 5D, lanes –). These results show that, in *abp1*Δ cells, Swi6 deposition at the mating-type region is not decreased to any significant extent.

Altogether, these observations indicate that deletion of *abp1* does not disrupt heterochromatin organisation at the mating-type region. In contrast, in the SPG27 strain, silencing appears to increase in *abp1*Δ cells, suggesting that deletion of *abp1* actually reinforces heterochromatin-dependent silencing at the mating-type locus.

### *The switching defect of abp1*Δ *cells is suppressed in swi2*Δ *and swi5*Δ *mutants*

In *abp1*Δ cells, *mat3* is the preferred donor locus during mating-type switching (Figure 1). It is known that donor selection depends on the functionality of the Swi2–Swi5 complex (Jia *et al*, 2004b). Swi5 is a general DNA recombination/repair factor that forms a complex with Rhp51/Rad51, a DNA recombination factor that is also required for mating-type switching (Akamatsu *et al*, 2003; Grishchuk *et al*, 2004). Localisation of Swi5 to the mating-type locus depends on Swi2 that binds to the SRE located adjacent to *mat3* and, only in *mat1M* cells, spreads to *mat2* (Jia *et al*, 2004b). Therefore, these two factors cooperate to promote recombination and to regulate directionality of mating-type switching.

To analyse whether the switching defect of *abp1*Δ cells depends on Swi2–Swi5, we determined the effects of deleting *abp1* in *swi2*Δ and *swi5*Δ mutants. As reported earlier (Jia *et al*, 2004b), *mat2* is the preferred donor locus both in *swi2*Δ (M/P = 0.56 ± 0.13) and *swi5*Δ cells (M/P = 0.64 ± 0.12) (Figure 6B and D). In these mutant backgrounds, deletion of *abp1* does not alter donor selection as a similar preference for *mat2* is observed in *swi2*Δ*abp1*Δ (M/P = 0.56 ± 0.14) (Figure 6B) and *swi5*Δ*abp1*Δ double mutants (M/P = 0.77 ± 0.16) (Figure 6D), which also show low sporulation frequencies (Figure 6A and C). These results indicate that preferred utilisation of *mat3* in *abp1*Δ cells depends on the functionality of the Swi2–Swi5 complex.

### *Deletion of abp1 abolishes spreading of Swi2 across heterochromatin*

Efficient utilisation of *mat2* as a donor depends on spreading of the Swi2–Swi5 complex across the entire silent heterochromatic domain of the mating-type locus so as to reach *mat2*

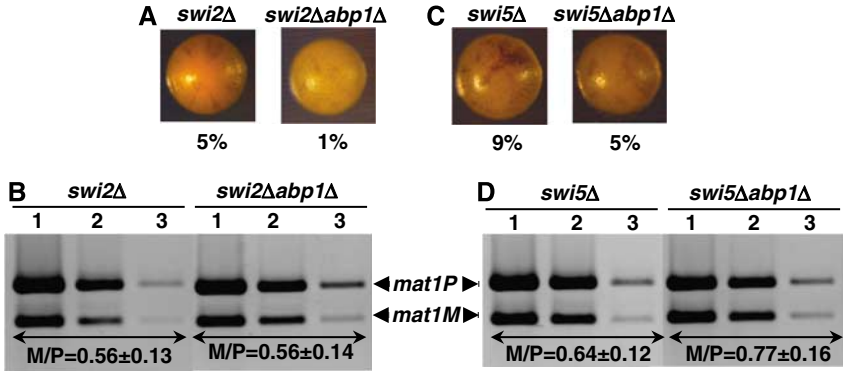


**Figure 6** Analysis of the effects on mating-type switching of deleting *abp1* in *swi2*Δ (**A**, **B**) and *swi5*Δ (**C**, **D**) cells. (A, C) Iodine staining of the indicated strains. Numbers below each panel correspond to the frequencies of sporulation. (B, D) Quantitative multiplex PCR determination of the predominant mating type adopted by each of the indicated strains. PCR reactions were performed as in Figure 1D. The M/P ratios (±s.d.) are indicated below the corresponding lanes.

 

(Jia *et al*, 2004b). Therefore, it is possible that deletion of *abp1* affects spreading of Swi2–Swi5 across heterochromatin. To address this question, we performed ChIP experiments to determine the effects of deleting *abp1* on the distribution of Swi2 across the silent domain of the mating-type region (Figure 7). For this purpose, Swi2 was myc tagged in both a stable M-strain (*mat1smto*) (Engelke *et al*, 1987) and a stable P-strain (*mat1PΔ17*) (Arcangioli and Klar, 1991). It was reported earlier (Jia *et al*, 2004b) that, in a P-strain, Swi2 distribution is constrained to *mat3*, whereas in an M-strain, it spreads to *mat2*. In these experiments, we determined the presence of Swi2 at two different sites located within *mat2* (l2 and l3), as well as at two locations surrounding *mat3* (r1 and r2) and at the region corresponding to the major Abp1-binding site (l1), located between *mat2* and *cenH* (Figure 7A). Consistent with previously reported results by others (Jia *et al*, 2004b), in the M-strain, Swi2 associates with all these five locations (Figure 7B, lanes wt in M-strain, and Figure 7C), whereas in the P-strain, it is found enriched only at the *mat3* region, especially at region r1 that spans the SRE (Figure 7B, lanes wt in P-strain, and Figure 7C). In the M-strain, deletion of *abp1* strongly alters the distribution of Swi2, so that, in *abp1*Δ cells, Swi2 is detected at *mat3* locations (r1 and r2), but not at *mat2* (l3 and l2) or at region l1 (Figure 7B, lanes *abp1*Δ in M-strain, and Figure 7C). In contrast, in the P-strain, deletion of *abp1* does not significantly alter the distribution of Swi2, so that, in this case, it is detected only at *mat3* locations, both in wild-type and *abp1*Δ cells (Figure 7B, lanes wt and *abp1*Δ in P-strain, and Figure 7C). Swi5 distribution is also likely to be affected in *abp1*Δ cells, as binding of Swi5 to the mating-type region, as well as its spreading through heterochromatin, is known to depend on the presence of Swi2 (Jia *et al*, 2004b). Altogether, these results indicate that, in the mating-type locus, Abp1 is required for efficient spreading of the Swi2–Swi5 complex across heterochromatin.

## Discussion

Here, we have shown that, in the fission yeast *S. pombe*, Abp1 contributes to the regulation of directionality of mating-type switching. A model to account for the results reported here is summarised in Figure 8. Directionality of switching depends on the cell-type-specific distribution of the Swi2–Swi5 complex that binds to the SRE located adjacent to *mat3* and, only in *mat1M* cells, spreads to *mat2* (Jia *et al*, 2004b). As a consequence, *mat1P* cells preferentially use *mat3* as a donor (Figure 8A) and, vice versa, *mat1M* cells use *mat2* (Figure 8B), thus ensuring efficient switching. Here, we have shown that deletion of *abp1* abolishes spreading of

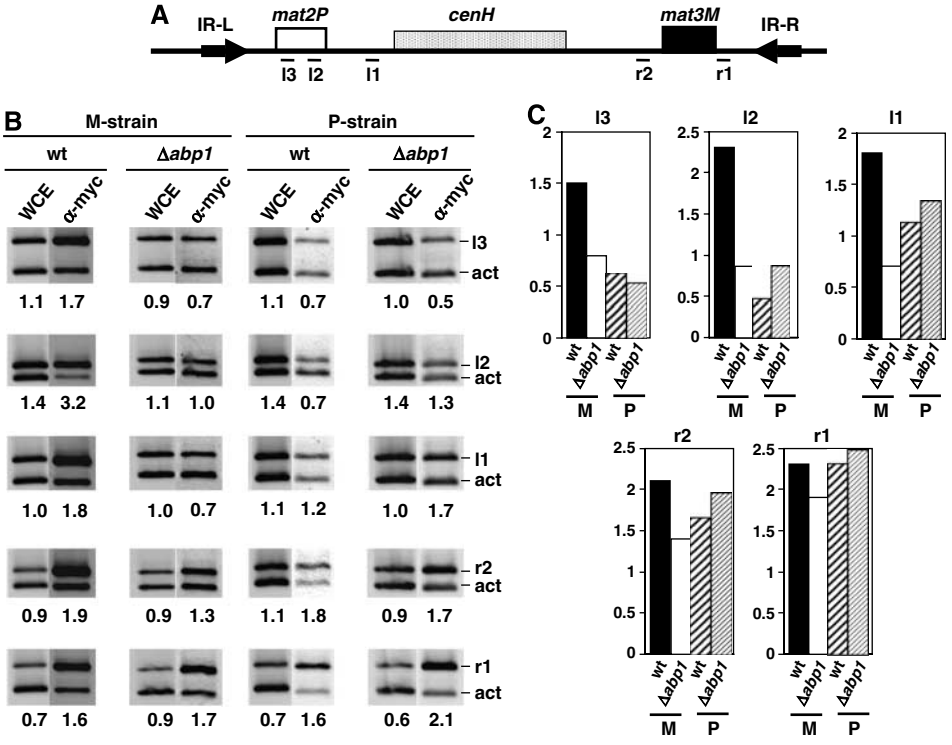


**Figure 7** Deletion of *abp1* impairs spreading of Swi2–Swi5 to *mat2*. The effects of deleting *abp1* on the distribution of Swi2–myc along the silent domain of the mating-type locus were determined in both a stable M-strain (*mat1smto*) and a stable P-strain (*mat1PΔ17*) by ChIP analysis using α-myc antibodies. (**A**) Regions of the silent domain of the mating-type locus where the presence of Swi2–myc was analysed (see Supplementary Table SII for a description of the primers used). (**B**) ChIP analysis corresponding to wild-type (wt) and *abp1*Δ cells derived from either a stable M-strain (panel M-strain) or a stable P-strain (panel P-strain). Material obtained after immunoprecipitation with α-myc antibodies was analysed as described in Figure 3 using primers specific for the indicated regions (bands r1, r2, l1, l2 and l3). Lanes WCE correspond to PCR products obtained from the input material before immunoprecipitation. Lanes α-myc correspond to the products obtained from the immunoprecipitated material. Numbers below each lane correspond to the ratio of the corresponding mating-type-specific band with respect to the control act band. (**C**) Quantitative analysis of the results shown in (B). For each of the mating-type-specific regions analysed, the relative fold of enrichment of the corresponding band in the immunoprecipitated material with respect to the input material (WCE) is presented for both wild type (wt) and *abp1*Δ cells in a stable M or P background.

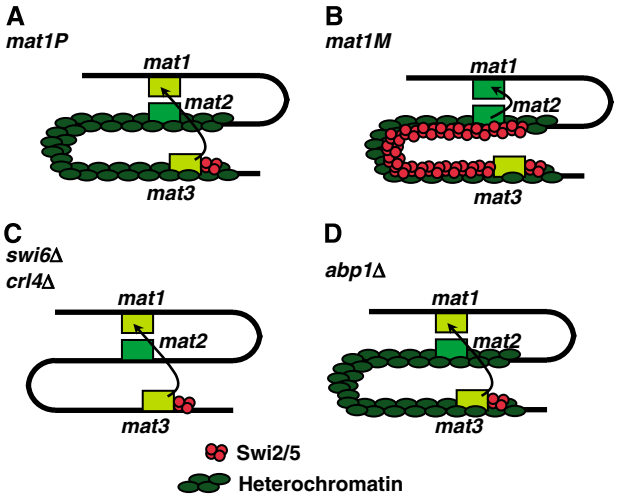


**Figure 8** Model to account for the contribution of Abp1 to the regulation of directionality of mating-type switching. In *mat1P* cells, the Swi2–Swi5 complex localises to *mat3* (**A**) and, only in *mat1M* cells, spreads to *mat2* (**B**), allowing its use as a donor during switching. Spreading of Swi2–Swi5 to *mat2* is mediated by heterochromatin being abolished by mutations in heterochromatin assembly factors (i.e. *swi6*Δ and *crl4*Δ), which prevent use of *mat2* as a donor (**C**). In *abp1*Δ cells, heterochromatin organisation of the mating-type locus is preserved but spreading of Swi2–Swi5 to *mat2* is impaired and, as a consequence, *mat2* is not efficiently used as a donor during switching (**D**).

Swi2–Swi5 to *mat2* and, therefore, *abp1*Δ cells are unable to use *mat2* as a donor locus during switching. It was shown that spreading of Swi2–Swi5 to *mat2* is mediated by the heterochromatin organisation of the mating-type locus (Jia *et al*, 2004b), so that mutants of heterochromatin assembly factors (i.e. *swi6*Δ and *crl4*Δ) are also unable to use *mat2* as a donor (Figure 8C). However, we have shown here that heterochromatin organisation at the mating-type locus is preserved in *abp1*Δ cells. Moreover, our results also show that, in *abp1*Δ cells, utilisation of *mat3* as a donor depends on *swi2* and *swi5*, indicating that deletion of *abp1* does not affect the functionality of the Swi2–Swi5 complex. Altogether, these results indicate that Abp1 is actually required for efficient spreading of Swi2–Swi5 across heterochromatin at the mating-type locus (Figure 8D).

Further work is required to determine the precise molecular mechanism(s) underlying the contribution of Abp1 to heterochromatin-mediated spreading of the Swi2–Swi5 complex. However, our results and those recently reported by others (Cam *et al*, 2007) indicate that Abp1 binds to the mating-type locus, where a major Abp1-binding site maps close to the *cenH* element. In addition to the mating-type locus, Abp1 also localises to multiple other sites, including *Tf2* retrotransposons, centromeric *dh* repeats and some autonomously replicating sequences (ARS) (Cam *et al*, 2007). Binding at the mating-type locus strongly suggests a direct contribution to spreading of Swi2–Swi5. In this context, Abp1, which is a sequence-specific DNA-binding protein that recognises AT-rich DNA (Murakami *et al*, 1996; Halverson *et al*, 1997; Kikuchi *et al*, 2002), is likely to be involved in recruitment of factors that facilitate such spreading. Actually, in the mating-type locus, Abp1 was shown to contribute to recruitment of the HDAC Crl3 (Cam *et al*, 2007), a component of SHREC (Sugiyama *et al*, 2007), that is required for heterochromatin assembly at the mating-type locus (Ekwall and Ruusala, 1994; Yamada *et al*, 2005). However, Abp1–Crl3 interaction does not appear to have a major contribution to heterochromatin assembly at the mating-type locus as, on the one hand, our results indicate that deletion of *abp1* does not impair silencing and/or Swi6 deposition at the mating-type locus and, moreover, in *abp1*Δ cells, high levels of Crl3 are detected at the silent domain of the mating-type region (Cam *et al*, 2007). Whether recruitment of Crl3 accounts to any extent for the contribution of Abp1 to the regulation of mating-type switching remains, however, to be determined. On the other hand, the possibility that Abp1 would directly mediate binding of the Swi2–Swi5 complex appears unlikely, as no specific enrichment of Swi2 is detected at the major Abp1-binding site of the mating-type locus. We cannot, however, exclude the possibility that Abp1 might bind additional sites within the mating-type locus, as both our binding studies and those performed by others (Cam *et al*, 2007) do not cover the entire locus. Actually, several potential Abp1-binding sites are found within the mating-type locus (Murakami *et al*, 1996; Halverson *et al*, 1997; Thon *et al*, 1999; Baum and Clarke, 2000).

Abp1 is known to participate in the regulation of multiple processes. In particular, Abp1 was shown to contribute to silencing of *Tf2* retrotransposons through the recruitment of Crl3 and a second HDAC, Crl6 (Cam *et al*, 2007). Interestingly, silencing of *Tf2* retrotransposons does not depend on Swi6, nor it does require other heterochromatin factors such as Crl4, Rik1 and Chp1 (Greenall *et al*, 2006), indicating that, also in this case, Abp1 is not involved in heterochromatin assembly.

At centromeres, it was proposed that Abp1 contributes to heterochromatin assembly, as it binds to several sites located within centromeric heterochromatin (Baum and Clarke, 2000; Cam *et al*, 2007), and deletion of *abp1* results in a modest relief of centromeric silencing, which is enhanced in *abp1*Δ*cbh1*Δ cells (Nakagawa *et al*, 2002). These observations are in contrast with our results showing that, at the mating-type locus, Abp1 is not required for heterochromatin assembly and silencing. In contrast, silencing at the mating-type locus appears to be slightly reinforced in *abp1*Δ cells. Most likely, this apparent contradiction is the consequence of disruption of centromeric heterochromatin, which could increase intracellular levels of silencing factors favouring their deposition at other heterochromatic sites, such as the mating-type locus. Similar effects were reported in *Saccharomyces cerevisiae* for *sir4* mutants, where telomeric silencing is reduced and, concomitantly, silencing at the rDNA locus increases (Smith *et al*, 1998). The molecular basis of the contribution of Abp1, as well as of Cbh1 and Cbh2, to heterochromatin assembly at centromeres is, however, not fully understood.

Finally, Abp1 is also likely to participate in DNA replication, as it was found to interact physically with Cdc23 (MCM10), a protein implicated in the initiation of DNA replication (Locovei *et al*, 2006). Consistent with a role in DNA replication, *abp1*Δ cells grow slowly, due to a delay in S-phase, and *abp1* interacts genetically with a number of genes involved in DNA replication, including *cdc23*, *orc1* and *orc2*. Actually, Abp1 was first identified on the basis of its specific binding to ARS (Murakami *et al*, 1996) and it associates with some ARS *in vivo* (Cam *et al*, 2007). All these observations

strongly support a contribution of Abp1 to DNA replication. Therefore, it is possible that the contribution of Abp1 to the regulation of mating-type switching is linked to DNA replication. At the mating-type locus, DNA replication has an essential function in the establishment of the SSB/DSB imprint that triggers recombination and gene conversion. After switching, the pattern of localisation of Swi2–Swi5 must be modified accordingly to the new mating information located at *mat1*. It is tempting to speculate that relocalisation of Swi2–Swi5 might take place during the next round of DNA replication, where Abp1 could exert an effect as a licensing factor that facilitates spreading of the complex in *mat1M* cells but not in *mat1P* cells.

Abp1, as well as the closely related Cbh1 and Cbh2 proteins, show significant homology to human CENP-B (Murakami *et al*, 1996; Halverson *et al*, 1997; Lee *et al*, 1997; Ireland *et al*, 2001). Homology is high at the N-terminal half, which contains the DNA-binding domain and mediates sequence-specific DNA recognition, and the DDE domain, which has putative endonuclease activity being similar to the catalytic domain of *pogo* transposases (Ireland *et al*, 2001). However, the C-terminal domain, which mediates protein–protein interactions, is less well conserved. Abp1, Cbh1 and Cbh2 have redundant functions in the regulation of several processes (Halverson *et al*, 1997; Baum and Clarke, 2000; Ireland *et al*, 2001; Nakagawa *et al*, 2002; Cam *et al*, 2007). In most cases, however, single mutants show only weak phenotypes that are strongly enhanced in double mutants. In contrast, the contribution of Abp1 to the regulation of directionality of mating-type switching is strong and specific, indicating that it constitutes a major function of Abp1.

## Materials and methods

### *Media, genetic procedures and cytological procedures*

*S. pombe* cells were grown in complete medium (YES) or Edinburgh minimal medium (EMM), according to standard procedures.

To estimate the efficiency of switching, individual colonies grown on sporulation medium were exposed to iodine vapours according to Thon and Klar (1993). The frequency of sporulation was determined from a 3-day-old culture by phase-contrast microscopy visualisation.

The effect of deleting *abp1* on silencing at the mating-type was determined by plating 10 μl of serial 10-fold dilutions of fresh growing cultures in EMM plates lacking uracil (−URA) or supplemented with 1 μg/μl of 5-FOA.

### *Strains and plasmids*

Strains used in these experiments are summarised in Supplementary Table SI. *abp1*Δ::*KAN*, *abp1*Δ::*NAT*, *abp1*Δ::*HPH*, *cbh1*Δ::*NAT*, *cbh2*Δ::*KAN*, *swi2*Δ::*KAN* and *swi5*Δ::*KAN* deletions were obtained by replacing the entire ORF of the corresponding gene with PCR products carrying KanMX6, NatMX6 or HphMX6 markers obtained from pFA6a plasmids (Bähler *et al*, 1998; Hentges *et al*, 2005; Van Driessche *et al*, 2005), using appropriate primers containing a region of approximately 80 nt of homology to either side of the insertion site. Abp1 was tagged with 3HA and Swi2 with 13myc using plasmids pFA6a–3HA and pFA6a–13myc, respectively (Bähler *et al*, 1998; Van Driessche *et al*, 2005). To express Abp1 in *abp1*Δ cells, plasmid pREP81 was used (Basi *et al*, 1993).

### *Southern analysis*

For Southern analysis, 20 μg of genomic DNA was digested with *Hin*dIII, run on a 0.6% agarose-TBE gel and blotted onto a nylon membrane (Hybond-N$^+$; Amersham Biosciences). A 9-kb *Sal*I–*Sph*I *mat1M* fragment, obtained from plasmid pON177 (Styrkársdóttir *et al*, 1993), was $^{32}$P-labelled with Ready-To-Go™ DNA Labeling Beads (Amersham Biosciences) and used as a probe. This fragment spans the region corresponding to the *mat1* locus and contains *mat3M* information at *mat1*, so that, in addition to the 10.4-kb *Hin*dIII fragment of the *mat1* locus, it also recognises a 4.2-kb fragment corresponding to the *mat3M* locus.

### *Quantitative multiplex PCR analysis*

Switching efficiency was analysed by quantitative multiplex PCR analysis of genomic DNA to determine the genetic content at the *mat1* locus (Jia *et al*, 2004b). Primers used in these experiments were MT1 (common to *mat1P* and *mat1M*) 5′-AGAAGAGAGAGT AGTTGAAG-3′; MP (*mat1P* specific) 5′-ACGGTAGTCATCG GTCTTCC-3′ and MM (*mat1M* specific) 5′-TACGTTCAGTAGACGTA GTG-3′. Serial dilutions (10-fold) of the products obtained after PCR amplification were run on a 1.5% agarose-TBE gel, stained with 0.5 μg/ml ethidium bromide and captured using a Syngene GeneGenius System (Syngene, Cambridge, UK) equipped with GeneSnap version 6 gel documentation software. GeneTools version 3 software (Syngene) was used for quantitative analysis.

### *ChIP experiments*

ChIP experiments were performed as described elsewhere (Pidoux *et al*, 2004). When the distribution of Swi6 was determined, IP was carried out with rabbit polyclonal αSwi6 antibodies (Abcam no. 14898) and ProteinA-Sepharose™ CL-4B beads (GE Healthcare no. 17-0780-01). In the case of Abp1–HA, rabbit polyclonal αHA antibodies (Abcam no. 9110) and ProteinA-agarose beads (Upstate no. 16–157) were used and, for Swi2, we used goat polyclonal α-myc antibodies (Abcam no. 9132) and ProteinG-PLUS-Agarose beads (Santa Cruz Biotechnology; SC-2002). PCR products were run on 2% agarose-TBE gels, visualised with 0.5 μg/ml ethidium bromide and captured using a Syngene GeneGenius System (Syngene) equipped with GeneSnap version 6 gel documentation software. GeneTools version 3 software (Syngene) was used for quantitative analysis. Primers used are summarised in Supplementary Table SII.

### *Supplementary data*

Supplementary data are available at *The EMBO Journal* Online (http://www.embojournal.org).

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