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A role for the yeast SWI/SNF complex in DNA replication

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ABSTRACT

The yeast SWI/SNF complex is required for expression of many genes and for the full functioning of several transcriptional activators. Genetic and biochemical studies indicate that SWI/SNF uses the energy of ATP hydrolysis to antagonize chromatin-mediated transcriptional repression. We have tested the possibility that SWI/SNF might also play a role in DNA replication. A mitotic minichromosome stability assay was used to investigate the replication efficiency of a variety of autonomous replication sequences (ARSs) in the presence and absence of SWI/SNF. The stability of minichromosomes that contain ARS1, ARS309 or ARS307 is not altered by lack of SWI/SNF, whereas the functioning of ARS121 is crippled when SWI/SNF is inactivated. The SWI/SNF dependence of ARS121 does not require the replication enhancer factor, ABF1, and thus, it appears to be a property of a minimal ARS121 origin. Likewise, a minimal derivative of ARS1 that lacks the ABF1 replication enhancer acquires SWI/SNF dependence. Replacing the ABF1 binding site at ARS1 with a binding site for the LexA–GAL4 chimeric activator also creates a SWI/SNF-dependent ARS. Our studies suggest that the SWI/SNF chromatin remodeling complex can play a role in both replication and transcription and, furthermore, that SWI/SNF dependence of ARS elements is a property of both an ARS-specific replication enhancer and the overall organization of ARS sequence elements.

INTRODUCTION

The organization of a eukaryotic genome into chromatin can lead to repression of cellular processes that require accessibility of DNA sequences to enzymatic machineries. Nucleosomes, the basic building blocks of chromatin, are general repressors of transcription *in vivo*, and *in vitro* nucleosome assembly can block the ability of transcription factors to access their binding sites (reviewed in 1). Likewise, nucleosomes can block the firing of a DNA replication origin *in vivo* (2), and assembly of DNA into chromatin leads to a general repression of DNA replication efficiency *in vitro* (3–6). Since bulk chromatin in an interphase cell is primarily composed of large, 100–200 nM condensed fibers, mechanisms must exist to rapidly and reversibly unfold or de-compact specific loci to facilitate DNA accessibility.

Replication of yeast chromosomes requires the activation of multiple *cis*-acting replication origins. Yeast origins were initially identified as sequence elements that allowed extrachromosomal maintenance of plasmids and thus they were called autonomously replicating sequences (ARSs) (reviewed in 7). ARS elements are modular, usually containing at least three distinct sequence elements (Fig. 1A). All ARS elements contain a match to an essential 11 bp ARS consensus sequence (ACS), WTTTAYRTTTW (where W is A or T, Y is T or C and R is A or G), which is part of a larger 17 bp extended consensus element (8). The ACS serves as a binding site for the conserved origin recognition complex (ORC) which plays a crucial role in origin function *in vivo* (9,10). In addition, each ARS contains sequences 3′ to the T-rich strand of the ACS, called the B element, which is composed of at least two, non-redundant sequence elements (e.g. B1 and B2). The B1 element is adjacent to the ACS and it contributes to ORC binding as well as an additional, uncharacterized function (11,12). In contrast, the B2 element does not appear to represent a protein binding site, but instead appears to function as a DNA unwinding element (DUE) (13,14).

In addition to the ACS, B1 and B2 elements, many ARSs contain a fourth sequence element that functions as a replication enhancer. This sequence element can be located 3′ of the T-rich strand of the ACS, as is the case for ARS1 and ARS305 (Fig. 1; 15,16), or the enhancer can be found at the 5′-side of the ACS, as is the case for ARS121 and ARS1501 (Fig. 1; 17). In the case of ARS1, the B3 enhancer element contains a binding site for the ABF1 transcription factor and the role of ABF1 in ARS1 function can be provided by a host of other transcriptional activators, including RAP1, GAL4, p53 and a LexA–VP16 chimeric activator (15,18). Several other ARS elements, such as ARS2, ARS120 and ARS121, also contain functionally important ABF1 binding sites, whereas other ARSs contain distinct sequence elements that may perform similar functions [i.e. B4 in ARS305 (16) and REN1501 in ARS1501 (17)]. Although the role of these elements in origin function is not clear, it has been proposed that one role for transcriptional activators in DNA replication may be to counteract nucleosomal repression (3–5). Consistent with this view, deletion of the ABF1 binding site within ARS1 (the B3 element) leads to an invasion of nucleosomes into the essential ACS element and a decrease in ARS1 function (19). Furthermore, *in vitro* studies have shown that many acidic transcriptional

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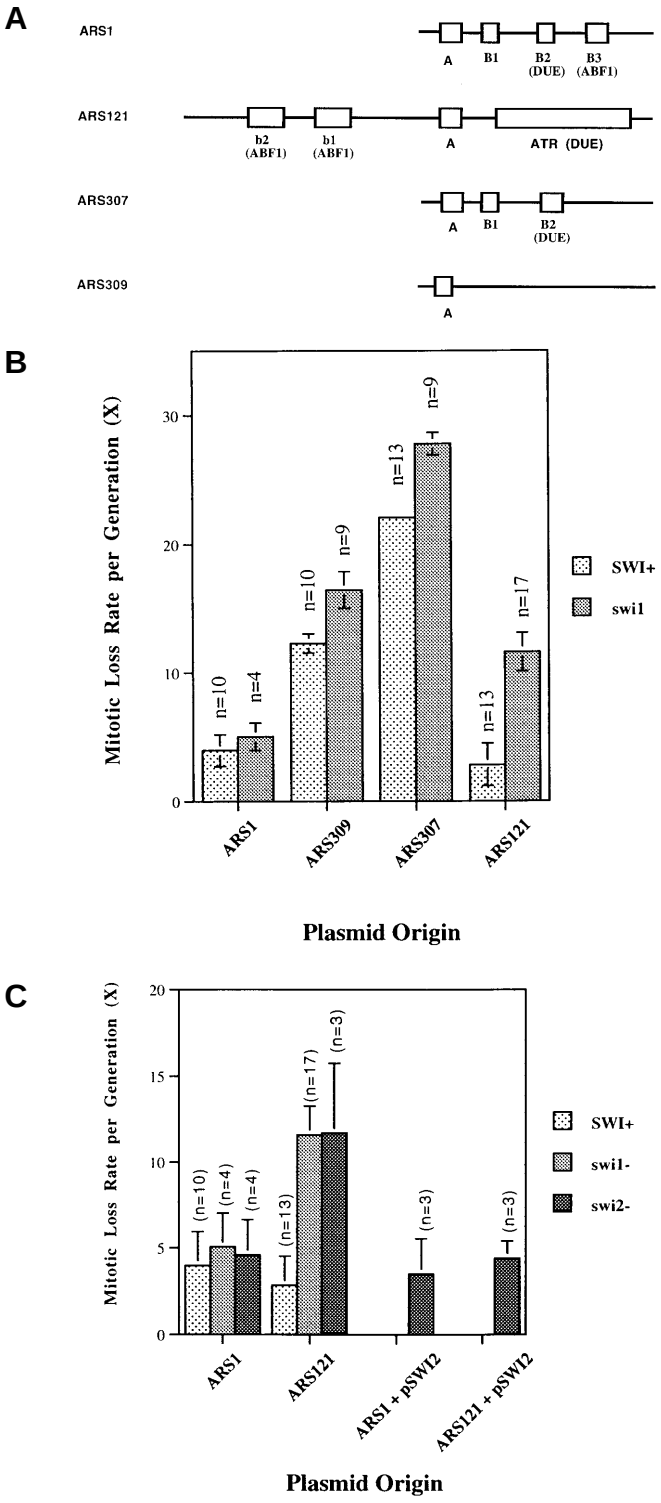

Figure 1. The SWI/SNF complex is required for efficient maintenance of a minichromosome containing ARS121. (**A**) Schematics of ARS1, ARS121, ARS307 and ARS309 are shown to depict their modular *cis*-acting sequence elements. (A indicates the ARS consensus sequence, ACS.) (**B**) Mitotic stability assays were performed in isogenic *SWI*$^+$ (CY296) and *swi1*Δ (CY298) strains carrying minichromosomes pARS/WTA (ARS1), p309-326 (ARS309), pC2G1A (ARS307) or yCp5AB121 (ARS121). (**C**) Mitotic stability assays in *SWI*$^+$ (CY296), *swi1*Δ (CY298) and *swi2*Δ (CY120) strains carrying pARS/WTA (ARS1) or yCp5AB121 (ARS121). Assays were also performed in the *swi2*Δ strain (CY120) harboring a plasmid that contains *SWI2* (pSWI2). The number of experiments (*n*) is shown above each column and the standard error is indicated by brackets.

activators can stimulate *in vitro* DNA replication by antagonizing the repressive effects of nucleosomes (3–5).

The SWI/SNF complex, a 2 MDa assembly of 11 different polypeptides (20–22), is required for many transcriptional activators to enhance transcription in yeast (reviewed in 23,24). Mutations that alter chromatin components partially alleviate the defects in transcription due to inactivation of the SWI/SNF complex (25–27) and SWI/SNF complex can use the energy of ATP hydrolysis to disrupt nucleosome structure *in vitro* (21). Thus it has been proposed that the primary role of this complex may be to facilitate the function of gene regulatory proteins in a chromatin environment by remodeling chromatin structure. Since nucleosomal structure in eukaryotes imposes an impediment to the initiation of replication as well as transcription, we have investigated whether the SWI/SNF complex might also play a role in DNA replication *in vivo* in yeast.

MATERIALS AND METHODS

Strains and plasmids

All strains are congenic to S288C and are isogenic derivatives of strain yPH274 (28). The *swi*1 and *swi2* deletion alleles are described in Peterson and Herskowitz (29).

ARS plasmids pARS/WTA (ARS1), pARS/835–842 (ARS1 B1 linker scanning mutant), pARS/756,758 (ARS1 B3 double point mutant), pARS/798–805 (ARS1 B2 linker scanning mutant) and pARS/LexA 798–805, as well as a high copy/*HIS3* plasmid that expresses either the LexA DNA binding domain (pLEX[1–82]) or a LexA–GAL4 fusion protein (pMA411) are described in Marahrens and Stillman (15). Plasmid p309–326 (ARS309) and pC2G1A (ARS307) are described in Theis and Newlon (30). Plasmid yCp5AB121 (ARS121) is described in Walker *et al*. (31) and plasmids AB121B1 (ARS121 b1 mutant), AB121B2 (ARS121 b2 mutant) and AB121B1B2 (ARS121 b1b2 double mutant) are described in Walker *et al*. (32). Plasmid CP337 [SWI2 in RS315 (28)] is described in Richmond and Peterson (33).

Mitotic plasmid stability assays

For the plasmid stability assay, 5 ml cultures were grown in SD medium [6.7 g/l yeast nitrogen base without amino acids (Difco), 2% glucose] and supplemented with all amino acids except uracil (34) (selective medium) and grown to saturation at 30°C. Aliquots of these cultures were then diluted and plated in triplicate on SD – uracil and YEPD [1% yeast extract (Difco), 2% bacto-peptone (Difco), 2% glucose] media. Colonies were counted from both sets of plates to determine the value, *A*, which is the percentage of cells that maintain the minichromosome under selective conditions. An additional aliquot of the original cultures was diluted into 3 ml of YEPD medium (non-selective medium) to a final OD_{600} value of 0.0003 or 0.05 for *SWI*$^+$ and *swi*$^-$ cultures, respectively. These cultures were grown to saturation at 30°C (12 generations for *SWI*$^+$ and five generations for *swi*$^-$ cells), diluted and plated in triplicate on SD – uracil and SD + uracil media. Colonies were counted from both sets of plates to determine the value, *B*, which is the percentage of cells that maintain the plasmid after non-selective growth (% URA$^+$). The rate of plasmid loss/generation was calculated using the equation $X = 1 - e^r$, where *X* is the rate of plasmid loss/generation, $r = \ln(A/B)/N$ and *N* is the number of generations in non-selective medium (35).

Site-directed mutagenesis of ARS121

Mutagenesis of ARS121 was performed by a two-step PCR-mediated mutagenesis procedure. Primer oligonucleotides (DNA International) were as follows: ARS121-ACON, 5′-GTTAAACA-TAAAATCTCACTTC-3′; PBR-TAG, 5′-GAGGATCCCCGG-GTACGTATCACGAGGCCCTTTCG-3′; ARS121.HINDIII.3′, 5′-GCCGAAGCTTAGAATTTTGGCTCTG-3′; HHF2.5SL, 5′-GAGGATCCCCGGGTAC-3′. Primers ARS121-ACON and PBR-TAG were used in a PCR reaction to generate the point mutations in ARS121 using yCp5AB121 (31) as a template. The PCR product, yCp5AB121, and primers ARS121.HINDIII.3′ and HHF2.5SL were then used in a subsequent PCR reaction. The resulting 0.4 kb PCR product and plasmid yCp5AB121 were digested with *Eco*RI and *Hin*dIII (New England Biolabs) and ligation yielded plasmid CP561. ARS121 mutations were confirmed by DNA sequence analysis (Sequenase; USB).

RESULTS

SWI/SNF complex is required for efficient function of the yeast replication origin, ARS121

Yeast ARSs are examples of cellular chromosomal sequences that can function as origins of DNA replication on plasmids and in a chromosomal context (7). To address the role of the yeast SWI/SNF complex in DNA replication, we measured the mitotic stability of plasmids that contain a yeast selectable marker (*URA3*), a cloned centromere (CEN3) and a replication origin (ARS). Isogenic *SWI*$^+$ or *swi1*$^-$ cells harboring such minichromosomes were grown to saturation in medium that selected for plasmid maintenance, diluted into non-selective medium and then allowed to grow for an additional 12 or five generations for *SWI*$^+$ or *swi1*$^-$ cells, respectively. The rate of plasmid loss/generation of growth in non-selective medium is indicative of the functioning of the replication origin (15,35–40). Figure 1B presents the results of mitotic stability assays for four minichromosomes that contain different ARS elements. The stability of minichromosomes that contain ARS1, ARS307 or ARS309 is not significantly altered by inactivation of the SWI/SNF complex (less than a 1.3-fold increase in rate of plasmid loss in the *swi1* versus *SWI*$^+$ strains). In contrast, the stability of a minichromosome that contains ARS121 is dramatically reduced in the *swi1* mutant (2.9 ± 1.7% in *SWI*$^+$ versus 11.6 ± 1.5% in *swi1*). This decrease in plasmid stability is similar in magnitude to defects due to a partial loss of function mutation in *CDC17* (41), which encodes a DNA polymerase. Thus, these plasmid stability assays indicate that SWI/SNF is required for efficient functioning of at least one yeast replication origin, ARS121.

SWI/SNF function in transcription requires the ATPase activity of the SWI2/SNF2 subunit. Mutations in this domain eliminate the ability of SWI/SNF to support transcriptional activation *in vivo* (33,42,43) and nucleosome disruption activity *in vitro* (21). To investigate whether full activity of ARS121 also requires the SWI2/SNF2 subunit of the SWI/SNF complex, the mitotic stability of an ARS1 or ARS121 minichromosome was determined in isogenic *swi2*Δ and *SWI*$^+$ cells (Fig. 1C). The stability of a minichromosome that contains ARS1 is not significantly altered in the *swi2* deletion mutant (3.5 ± 2 in *SWI2*$^+$ versus 4.6 ± 4 in *swi2*Δ, n = 4), however, the stability of a minichromosome that contains ARS121 is decreased 3.4-fold (4.4 ± 1 in *SWI*$^+$ versus 11.7 ± 4 in *swi2*Δ, n = 3). Thus, these results suggest that the SWI2/SNF2 subunit is required for the functioning of the SWI/SNF complex in DNA replication.

ARS121 is distinct from most ARS elements as it contains only four 9/11 partial matches to the ACS. Only one of these partial matches is essential for ARS121 function (44), whereas the three non-essential 9/11 matches are contained within a B2/DUE element (denoted NTR) which is located 3′ to the T-rich strand of the essential ACS (Fig. 1A). One possibility is that the SWI/SNF-dependence of ARS121 is due to its essential, partial match to the ACS. To test this possibility, PCR mutagenesis was used to change 2 bp within the essential ACS of ARS121 so that it matches the ARS consensus sequence and nearly matches the ACS contained within ARS1 (Fig. 2A). We then tested if this ARS121 derivative (ARS121-ACON) was still dependent on SWI/SNF function. The results shown in Figure 2B indicate that changing the essential ACS of ARS121 to a perfect 11/11 match has no effect on plasmid stability in wild-type cells nor does it change the SWI/SNF dependence of this origin. The stability of a minichromosome that contains ARS121-ACON is still decreased 4.6-fold in a *swi2* mutant (2.4 ± 0.4 in *SWI*$^+$ versus 10.9 ± 3.1 in *swi2*$^-$, n = 3). Thus these results suggest that the SWI/SNF dependence of ARS121 is not due to its essential, imperfect match to the ACS.

ARS121 contains a replication enhancer consisting of two binding sites (b1 and b2) for the ABF1 transcription factor located 5′ of the T-rich strand of the essential ACS (Fig. 1). Mutation of one or both binding sites leads to a 2- to 3-fold decrease in the stability of plasmids containing ARS121 (32). Since SWI/SNF plays an important role in the functioning of many transcriptional activators, we tested whether the SWI/SNF dependence of ARS121 involved ABF1. Plasmid stability assays were performed in *SWI*$^+$ and *swi1*$^-$ cells with ARS121 derivatives that harbor mutations in one (b1– or b2–) or both (b1–b2–) ABF1 binding sites (Fig. 3). Similar to previous studies (32), mutation of the ABF1 binding sites caused a 2- to 3-fold decrease in plasmid stability in *SWI*$^+$ cells. Surprisingly, full functioning of these ARS121 derivatives remained dependent on SWI/SNF function. For example, the stability of the ARS121 derivative that contains a mutation of both ABF1 binding sites (b1–b2–) is still decreased 3-fold in a *swi1* mutant (10% loss/generation in *SWI*$^+$ versus 29% in *swi1*$^-$, n = 3). Thus, SWI/SNF does not appear to facilitate the functioning of ABF1 at ARS121, since, if this was the case, removal of the ABF1 binding sites would be equivalent to inactivation of SWI/SNF.

Derivatives of ARS1 have increased dependence on SWI/SNF function

Our deletion studies suggest that the role of SWI/SNF at ARS121 is to facilitate the functioning of the central core (ACS, B1 and B2/DUE/NTR elements), rather than the replication enhancer (ABF1 binding sites). However, the ACS, B1 and B2-like elements appear to be functionally interchangeable among different ARS elements (17,45; Fig. 2) and thus it is not clear why only some ARS elements require SWI/SNF function. One possibility is that SWI/SNF-independent ARS elements contain distinct replication enhancers that are able to perform a function that is redundant with SWI/SNF. Some replication enhancer elements do appear to be ARS-specific; for instance, the B4 enhancer element from ARS305 cannot be replaced with an ABF1 binding site from ARS1 (16) and the ABF1 binding sites

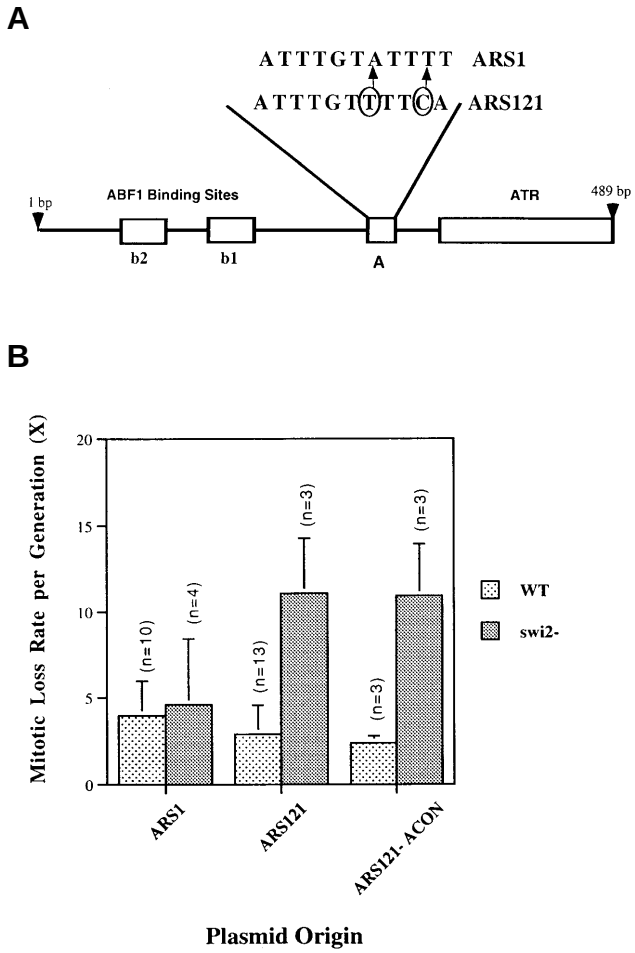

Figure 2. An ARS121 derivative that contains a consensus A element remains SWI/SNF dependent. (**A**) Sequence comparison of the A elements of ARS1 and ARS121 compared to the ARS consensus sequence. Mutations introduced to change the ARS121 A element to a perfect consensus are indicated by arrows. (**B**) Mitotic stability assays in *SWI*$^+$ (CY296) and *swi2*Δ (CY120) strains carrying either yCp5AB121 (ARS121) or pCP561 (ARS121-ACON).

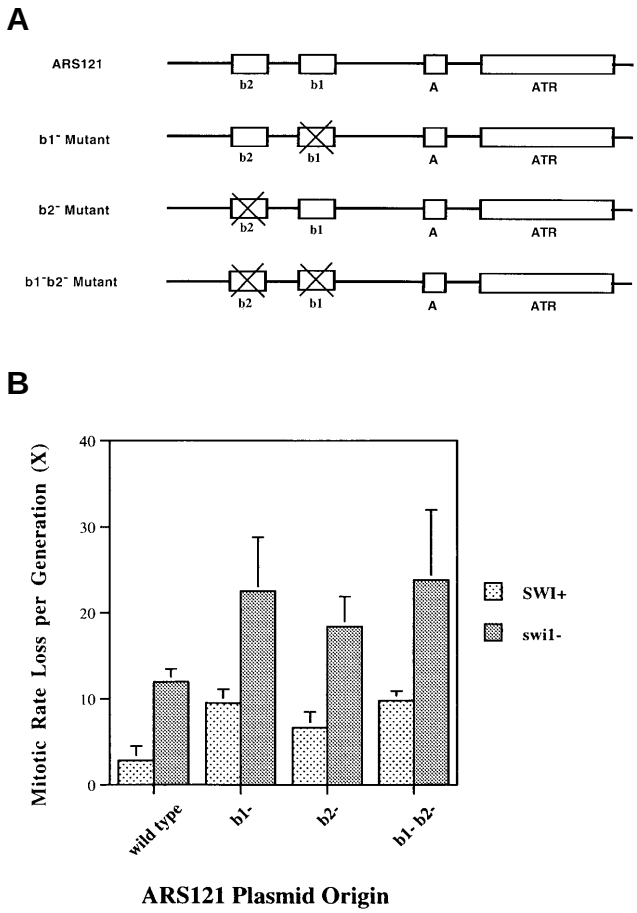

Figure 3. Derivatives of ARS121 that lack ABF1 binding sites remains SWI/SNF dependent. (**A**) Schematics of ARS121 derivatives. (**B**) Mitotic stability assays were performed in isogenic *SWI*$^+$ (CY296) and *swi1*Δ (CY298) strains carrying minichromosomes yCp5AB121 (ARS121), yCp5AB121B1 (ARS121 b1 mutant), yCpAB121B2 (ARS121 b2 mutant) and yCpAB121B1B2 (ARS121 b1b2 double mutant). Data shown are the averages of three independent experiments.

at ARS121 cannot be replaced with binding sites for a variety of other transcriptional activators (46). This hypothesis predicts that a SWI/SNF-independent ARS might become dependent on SWI/SNF function in the absence of its replication enhancer.

We analyzed the mitotic stability of minichromosomes that carry ARS1 derivatives with mutations in either the single ABF1 binding site (B3 element) or in the B1 or B2/DUE core elements (Fig. 4). The stability of a minichromosome that contains an intact ARS1 is not affected by inactivation of SWI/SNF (Figs 1B and 4B), however, an ARS1 derivative that contains a double point mutation in the single ABF1 binding site is ~3-fold less stable in the *swi1* mutant as compared to the *SWI*$^+$ strain (6.3 ± 0.5% in *SWI*$^+$ versus 17.5 ± 0.3% in *swi1* cells) (Fig. 4B). Thus, an ARS1 derivative that lacks a replication enhancer requires SWI/SNF function for full activity. Surprisingly, a minichromosome carrying a linker scanning mutation in the B1 element is also ~2-fold less stable in a *swi1* mutant (15.4 ± 0.3% in *SWI*$^+$ versus 33.2 ± 0.7% in *swi1* cells), even though this derivative still contains the ABF1 binding site. In contrast, the stability of a minichromosome that contains a mutation in the B2/DUE element was not affected by inactivation of SWI/SNF (34.7 ± 3.6 in *SWI*$^+$ versus 30.1 ± 1.7 in *swi1* cells). Thus, these results suggest that, in the case of ARS1, a single ABF1 binding site or the B1 element can perform a function that is redundant with SWI/SNF action.

Marahrens and Stillman (15) have demonstrated that a chimeric activator protein, LexA–GAL4, can functionally substitute for ABF1 when a consensus LexA binding site replaces the ABF1 site within ARS1. Since a single binding site for ABF1 was sufficient to make ARS1 independent of SWI/SNF function (Fig. 4), we tested whether the binding of LexA–GAL4 could also confer SWI/SNF independence. Mitotic stability assays were performed with cells that harbored a minichromosome with an ARS1 derivative that has a LexA binding site replacing the ABF1 site. When the LexA DNA binding domain was expressed, minichromosomes that contain this ARS1 derivative were lost from *SWI*$^+$ cells at high frequency (Fig. 5A; 19.7 ± 3.0%). If cells express a LexA fusion protein that contains the GAL4 transcriptional activation domain (LexA–GAL4), the mitotic stability of this

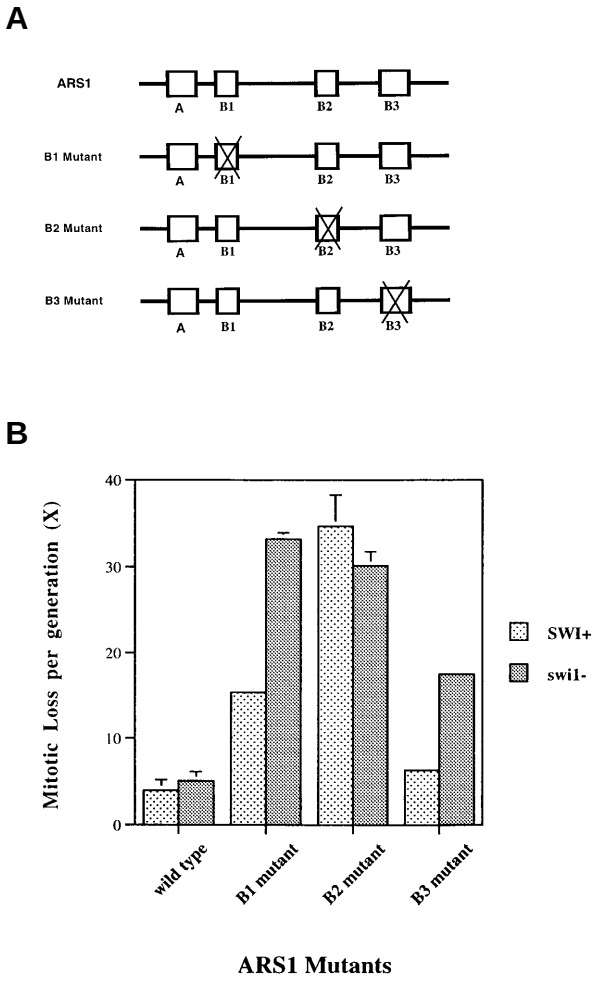

Figure 4. Derivatives of ARS1 have increased dependence on SWI/SNF function. (**A**) Schematics of ARS1 derivatives. (**B**) Mitotic stability assays were performed in isogenic *SWI*$^+$ (CY296) and *swi1*Δ (CY298) strains carrying minichromosomes pARS/WTA (ARS1), pARS/835–842 (ARS1 B1 linker scanning mutant), pARS/756,758 (ARS1 B3 double point mutant) or pARS/798–805 (ARS1 B2 linker scanning mutant).

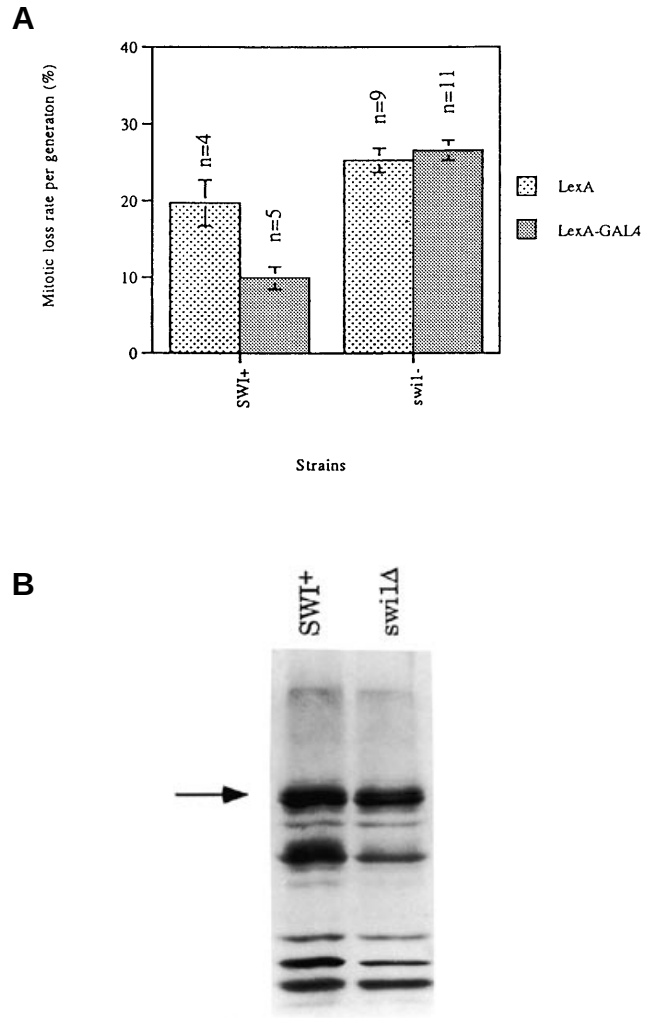

Figure 5. The SWI/SNF complex is required for the GAL4 transcriptional activation domain to enhance origin function. (**A**) Mitotic stability assays were performed in *SWI*$^+$ (CY296) or *swi1*Δ (CY298) strains harboring the minichromosome pARS/LexA 798–805. Strains also contained a plasmid that expressed either the LexA DNA binding domain (pLEX[1–82]) or a LexA–GAL4 fusion protein (pMA411). Assays were performed as described in Materials and Methods except that selective medium lacked both uracil and histidine and non-selective medium lacked only histidine. Data shown are the averages of three independent experiments. (**B**) Western analysis. Whole cell extracts (29) were prepared from equal numbers of *SWI*$^+$ (CY296) and *swi1*Δ (CY298) cells harboring plasmid pMA411 and expression of the LexA–GAL4 fusion protein (denoted by arrow) was identified by western blot using an antibody directed against the C-terminus of GAL4 (29).

minichromosome was enhanced (Fig. 5A; 9.9 ± 1.5%; see also 15). In the absence of an intact SWI/SNF complex, however, this minichromosome remains very unstable even if LexA–GAL4 is expressed (Fig. 5A; 25.3 ± 1.7% for LexA and 26.6 ± 1.3% for LexA–GAL4). Importantly, the LexA–GAL4 fusion protein is expressed at equivalent levels in the *SWI*$^+$ and *swi1* cells (Fig. 5B). Thus, LexA–GAL4 can only substitute for ABF1 in *SWI*$^+$ cells; in the absence of SWI/SNF this chimeric activator is unable to enhance origin function.

SWI/SNF is required for the full functioning of ARS121 and for ARS1 derivatives that lack a replication enhancer or contain a replication enhancer composed of a LexA–GAL4 binding site. The ability of SWI/SNF to enhance the functioning of these origins may represent a direct role for SWI/SNF or, alternatively, SWI/SNF may function via an indirect mechanism to stimulate replication. If SWI/SNF functions directly, then origin function might be enhanced by stably tethering SWI/SNF complex to an ARS element via a LexA DNA binding domain. Figure 6 shows mitotic stability assays for a minichromosome with an ARS1 derivative that contains a LexA binding site in place of the ABF1 site and with cells that express either the LexA DNA binding domain, LexA–GAL4 or a LexA–SWI2 fusion protein. In the presence of only a LexA DNA binding domain, this minichromosome is maintained in only 0.5% of the cells after growth in non-selective medium. However, expression of either the LexA–GAL4 or LexA–SWI2 fusion protein leads to a similar, 4-fold increase in mitotic stability. Thus, tethering the ATPase subunit of the SWI/SNF chromatin remodeling complex can enhance replication origin function *in vivo*.

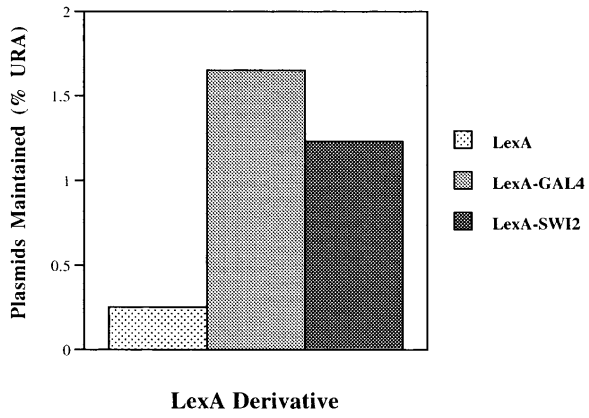

Figure 6. Tethering the ATPase subunit of SWI/SNF to ARS1 enhances ARS function. Mitotic stability assays were performed in a *SWI*$^+$ (CY296) strain harboring the minichromosome pARS/LexA 798–805. Strains also contained a plasmid that expressed the LexA DNA binding domain (pLEX[1–82]), LexA–GAL4 fusion protein (pMA411) or a LexA–SWI2 fusion protein (CP337). Data shown are the averages of four independent experiments; the standard error was <10%. Assays were performed as in Figure 5.

DISCUSSION

Here we have shown that SWI/SNF function is not restricted to transcription, but that it can also play a role in DNA replication. As is the case for transcription, most yeast replication origins (ARS elements) do not require SWI/SNF function. These SWI/SNF-independent origins include ARS1, ARS307, ARS309 and an ARS1 derivative lacking the B2 *cis*-acting element. Origins that require SWI/SNF for optimal activity include ARS121, derivatives of ARS121 that lack ABF1 binding sites, ARS1 derivatives lacking either the ABF1 binding site or the B1 element and an ARS1 derivative that requires a LexA–GAL4 chimeric activator. Genetic and biochemical studies have indicated that SWI/SNF complex may facilitate the function of transcriptional activators by contending with chromatin-mediated repression of transcription. By analogy, we propose that SWI/SNF complex may perform a similar role at replication origins in cases where chromatin structure impinges on their function.

Role of SWI/SNF in mitotic stability of minichromosomes

Mitotic stability of minichromosomes requires the efficient functioning of both a replication origin (ARS) and a centromere (CEN). If a minichromosome does not replicate efficiently, then too few copies will be available to segregate to the daughter cell. Likewise, if segregation is impaired, the daughter cell will not receive a copy of the minichromosome. Several results demonstrate that SWI/SNF influences the function of the replication origin and not the centromere. First, minichromosomes that contain wild-type ARS elements all contain a cloned CEN element, but only the plasmid that contains ARS121 requires SWI/SNF function. Second, each of the ARS1 derivatives are contained in the same plasmid backbone and have an identical CEN element. However, only ARS1 derivatives containing mutations in either the B1 or B3 element or an ARS1 derivative that is dependent upon LexA–GAL4 require SWI/SNF function. In contrast, wild-type ARS1 and an ARS1 derivative lacking the B2 element remain SWI/SNF independent. Thus, the SWI/SNF dependence of minichromosome stability is a property of the replication origin, not the CEN element. Such specificity for SWI/SNF function also indicates that SWI/SNF does not simply govern expression of a general replication factor, but that it plays a more direct role in origin function. Furthermore, SWI/SNF does not appear to govern replication by controlling expression or activity of ABF1, since the full functioning of ARS121 and ARS1 derivatives that lack ABF1 binding sites still require SWI/SNF.

Role of replication enhancers in determining SWI/SNF dependence of ARS function

Why does the full functioning of some ARSs require SWI/SNF action, whereas others do not? In the case of ARS1, our data suggest that the single ABF1 binding site allows ARS1 to function in the absence of SWI/SNF. A double point mutation in this ABF1 site reduces ARS1 function and increases the requirement for SWI/SNF. Replacing the ABF1 site with a binding site for the LexA–GAL4 chimeric activator allows more efficient function in *SWI*$^+$ cells, but LexA–GAL4 is unable to restore SWI/SNF independence to this ARS1 derivative. Although it is not known exactly how ABF1 enhances ARS1 function, it is known that the ABF1 binding site is required to keep nucleosomes from encompassing the essential ACS element (19). Since one transcriptional role for SWI/SNF is to facilitate the binding of factors to nucleosomal sites (47), it seems likely that a repositioning of nucleosomes over the ARS1 ACS is a likely cause for the increased dependence of this ARS1 derivative on the SWI/SNF chromatin remodeling complex. Mutations in the B1 binding site may also influence the chromatin context of ARS1 which then influences the SWI/SNF dependence of this derivative; likewise, ARS121 may have an inherent chromatin context which makes this origin SWI/SNF dependent. This chromatin context may depend on elements within the plasmid or be an inherent feature of the chromosomal copy of ARS121.

The full functioning of the ARS121 origin also relies on ABF1 binding sites, however, in this case the two ABF1 sites are not sufficient to provide a function that is redundant with SWI/SNF action. Previous studies have demonstrated that the two ABF1 sites at ARS121 are not functionally equivalent to the single site at ARS1 (46). Although the function of the single ABF1 site at ARS1 can be replaced by sites for RAP1, GAL4, p53 or LexA fusion activators (15,18), heterologous activators cannot substitute for the two ABF1 sites at ARS121 (46). These two different ABF1-dependent replication enhancers differ with respect to their orientation and distance from the essential ACS element. The two ABF1 binding sites at ARS121 are located 160 and 220 bp upstream of the essential ACS element and 260–320 bp upstream of the B2/DUE/NTR element. In contrast, the single ABF1 site at ARS1 is adjacent to the B2/DUE element and it is located only 80 bp 3′ of the T-rich strand of the ACS. Venditti and colleagues (19) have proposed that the binding of ABF1 can act as a boundary that establishes the nucleosome-free state of ARS1; this proposed boundary function is likely to contribute to the SWI/SNF independence of ARS1. Boundary elements, however, can only propagate their effects over short distances (~80 bp; 48) and thus ABF1 may not be able to influence nucleosome positioning at ARS121, leading perhaps to the increased dependence of this ARS on the SWI/SNF chromatin remodeling complex.

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