

# Purification of a yeast protein that binds to origins of DNA replication and a transcriptional silencer

(autonomously replicating sequence-binding factor/*Saccharomyces cerevisiae*/mating type regulation/site-specific DNA binding)

JOHN F. X. DIFFLEY AND BRUCE STILLMAN

Cold Spring Harbor Laboratory, P.O. Box 100, Cold Spring Harbor, NY 11724

Communicated by James D. Watson, December 14, 1987

**ABSTRACT** Two factors that interact specifically with the chromosomal replicator, autonomously replicating sequence 1 (ARS1), were identified in whole-cell yeast extracts. One of these proteins, ARS binding factor I (ABF-I), was purified to homogeneity as a polypeptide of 135 kDa. ABF-I binds within a region previously shown to be essential for the function of ARS1 when yeast are grown under certain nutritional conditions, and deletion analysis presented here suggests that the ARS1 domain containing the ABF-I-binding site, domain B, is a complex element composed of at least two subdomains. ABF-I also binds within an ARS at the *HMR* silent mating locus. This ARS functions as a cis-acting transcriptional silencer, and the ABF-I-binding site within this ARS is important for both ARS and silencer function.

Eukaryotic DNA replication initiates from multiple origins once and only once during a discrete part of the cell cycle S phase. Both the number of origins used and the temporal activation of origins during S phase can vary enormously in response to conditions such as developmental state (1, 2). The molecular mechanism by which eukaryotic chromosomal DNA replication initiates is at present not known; however, the direct demonstration that autonomously replicating sequences (ARS) in the yeast *Saccharomyces cerevisiae* act *in vivo* as origins of replication (3–5) provides an experimental system to study chromosomal DNA replication.

Sequence requirements for ARS function suggest that eukaryotic origins of replication are complex (6–10). All ARSs contain and absolutely require the ARS core consensus sequence (WTTTATRTTTW; where W = A or T and R = purine), which alone is insufficient for efficient ARS activity. Sequences flanking the core are important for ARS function, and functional redundancy often appears in these flanking sequences. ARS1 is located at the 3' end of the *TRP1* gene and has been shown by deletion analysis to consist of three functional domains designated A, B, and C (6). Domain A contains the ARS core sequence and is essential for ARS1 function. Domain B is an 84-base-pair (bp) region located 29 bp 3' to the ARS core sequence and is extremely important for efficient ARS activity. Recently, this domain was shown to contain a binding site for a protein identified in crude extracts (11), which overlaps both a region of bent DNA (12) and a sequence conserved among many ARSs (13). Domain C is located further than 100 bp 5' to the ARS consensus sequence and has a smaller effect on ARS1 function. Domains B and C are functionally redundant because domain A will function with either domain B or C, although domain B appears to be more efficient in this capacity (6). Furthermore, deletion analysis within domain B indicates that it may contain distinct functional elements.

For example, a sequence within domain B that contains the binding site for the protein here described was essential for ARS function when galactose was the primary carbon source, but this sequence appeared less important when glucose was the primary carbon source (12).

Origin-specific DNA binding proteins are important components of most prokaryotic and eukaryotic viral replication systems (14–24). Interestingly, in addition to being required for DNA replication, they can often exert either positive or negative effects on transcription (24–29). Because specific DNA sequences are required for ARS function, proteins that bind specifically to these sequences probably have a role in eukaryotic DNA replication. In this paper we describe two DNA-binding factors that interact specifically with ARS1.

## MATERIALS AND METHODS

**Strains and DNAs.** The yeast strain used for the identification and purification of ARS binding factors I and II (ABF-I and ABF-II) was a protease-deficient strain BJ 1991 (*Mata leu2 ura3 trp1 prb1-1122 pep4B*) from R. Kostriken (Cold Spring Harbor Laboratory).

The *Rsa* I [nucleotide (nt) 927]–*Hind*III (nt 616), the *Rsa* I (nt 927)–*Pst* I (nt 827), and the *Rsa* I (nt 927)–*Hinf*I (nt 734) fragments of ARS1 (see Fig. 5) were subcloned into pUC19 (30) to yield pARS1.2, pARS1.3, and pARS1.4.1, respectively. A pARS1.4.1 fragment containing ARS1 sequences was excised with *Eco*RI and *Hind*III for use in DNA-binding assays. *Bal*-31 deletions were generated in domain B from either the *Hind*III site in the plasmid vector polylinker ( $\Delta$ H series) or the *Bgl* II site at nt 854 ( $\Delta$ B series) of pARS1.4.1. The *Hind*III site in the  $\Delta$ H series and the *Bgl* II site in the  $\Delta$ B series were replaced with *Hind*III or *Bgl* II linkers, respectively. These domain-B deletions contained on a *Hind*III–*Bgl* II fragment were then used to replace wild-type domain B in pARS1.4.1. To invert domain B with respect to domain A (pARS1.4.1), the *Bgl* II (nt 854)–*Bam*HI (pUC polylinker) fragment was cut out of pARS1.4.1, religated, and recut with *Bgl* II and *Bam*HI to eliminate religation in the wild-type orientation before transformation. The pARS1.7 series was constructed by ligating random, total *Sau*3A-digested pBR322 into the *Bgl* II site of pARS1.4.1. The *Eco*RI–*Hind*III fragment containing wild-type domain A and mutated domain B was then inserted into yeast plasmid YRp14/CEN4 between the unique *Eco*RI and *Hind*III sites for use in the colony color assay described by Hieter *et al.* (31).

**DNA-Binding Assay.** DNA-binding reactions were formulated as described using 10–20 ng of labeled DNA probe (32) and subjected to electrophoresis in low-ionic-strength poly-

acrylamide gels essentially as described (33). DNase I "foot-printing" (34) was done as described (32) without added competitor DNA. Recovery and renaturation of ABF-I from NaDodSO<sub>4</sub>/polyacrylamide gels (35) was essentially by the method of Hager and Burgess (36). Silver staining of Na-DodSO<sub>4</sub>/polyacrylamide gels was by the method of Oakley *et al.* (37).

**Preparation of Extracts and Purification of ABF-I.** Cells were grown to late logarithmic phase at 30°C in yeast extract/peptone/dextrose (YPD) medium, harvested by centrifugation, resuspended in cold phosphate-buffered saline, harvested again, and stored at -70°C. Cells from 36 liters of yeast were thawed in 2-3 volumes of buffer X [50 mM Pipes (piperazinediethanesulfonate)/10 mM sodium metabisulfite/1 mM phenylmethylsulfonyl fluoride/10 mM dithiothreitol/1 mM EDTA/20% (vol/vol) glycerol] and disrupted in a bead beater (Biospec Products, Bartlesville, OK) with an equal volume of acid-washed glass beads in 10 30-sec bursts. Extracts were diluted to 0.2 M (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> with 4 M stock. After 30 min on ice, cell debris was removed by centrifugation at 10,000 × *g* for 20 min. Conductivity was adjusted to 160 mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> with buffer X and loaded onto a column of heparin-agarose (38) (4 × 18 cm) in buffer X containing 160 mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>. The column was washed with 200 ml of buffer X containing 160 mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> and developed with a 1.5-liter linear gradient from 160 to 500 mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> in buffer X. ABF-I and ABF-II DNA-binding activities eluted at ≈300 mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>. Active fractions were combined, adjusted to 300 mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, and loaded onto a column of Blue Sepharose CL-6B, Pharmacia (2.2 × 6 cm) in buffer X containing 300 mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>. The column was washed with 50 ml of buffer X containing 300 mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>. These fractions contain all the ABF-I activity. The column was subsequently washed with 50 ml of buffer X/1.0 M NaCl, and ABF-II was eluted with 50 ml of buffer X/2.5 M NaCl/5% Triton X-100. The ABF-I fraction was adjusted to 1.0 M (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> and loaded onto a phenyl-Sepharose CL-4B column (3 × 8 cm; Pharmacia) in buffer X containing 1 M (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>. The column was washed with 50 ml of buffer X containing 0.5 M (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, and ABF-I was eluted with a 500-ml linear gradient from 0.5 to 0.0 M (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> in buffer X. ABF-I elutes in a broad peak centered at 400 mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>. Active fractions were combined and dialyzed against buffer X containing 50 mM NaCl. ABF-I was loaded onto a denatured calf thymus DNA cellulose column (39) (2.2 × 5 cm) equilibrated in buffer X containing 50 mM NaCl. The column was washed with 20 ml of buffer X containing 50 mM NaCl, and ABF-I was eluted at 160 mM NaCl with an 80-ml linear gradient from 50 to 350 mM NaCl in buffer X. Active fractions were dialyzed overnight against buffer X containing 500 mM NaCl and 30% (wt/vol) sucrose and loaded (5 ml) onto a Sephacryl S-300 column (2 × 90 cm; Pharmacia) in buffer X containing 500 mM NaCl; the column was run at 10 ml/hr, and 2.5-ml fractions were collected. Active fractions were dialyzed overnight against buffer X and loaded onto a Mono Q 5/5 column (Pharmacia) in buffer X, washed with 3 ml of buffer X containing 80 mM NaCl, and eluted with a 10-ml linear gradient from 80 to 350 mM NaCl in buffer X. Active fractions were combined, aliquoted, and stored at -70°C.

**RESULTS**

**ARS1-Specific DNA-Binding Activities.** ARS1 specific DNA-protein complexes were identified after electrophoresis through low-ionic strength polyacrylamide gels (33) using a modification of the DNA-binding reactions previously described (32). In Fig. 1, a crude whole-cell extract from late logarithmic phase yeast was titrated into reactions containing constant amounts of unlabeled competitor DNA and

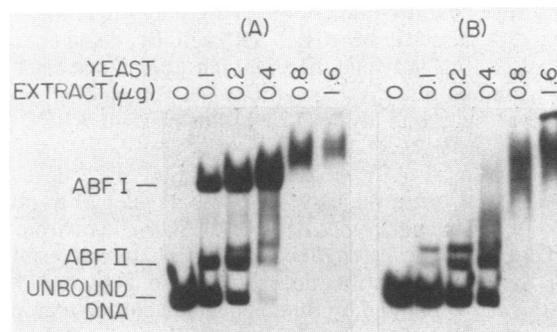


FIG. 1. Detection of ARS1-specific DNA-protein interactions with a crude whole-cell extract by gel retention analysis. Whole-cell yeast extract was titrated into DNA-binding reactions formulated and processed as described. Labeled DNA was either the EcoRI-HindIII fragment from pARS1.4.1 that contains domains A and B of ARS1 (A) or the BamHI-Sal I fragment from pBR322 (B).

labeled probe—either a fragment containing domains A and B from ARS1 (Fig. 1A) or an equal-sized fragment from pBR322 (Fig. 1B). Two DNA-protein complexes formed with ARS1 DNA and absent from equivalent positions in the pBR322 lanes were evident and were designated accordingly ABF-I and -II. Purification and characterization of ABF-I is presented here. Purification and characterization of ABF-II, which interacts specifically with ARS1 DNA, will be reported elsewhere.

**Interaction of ABF-I with Other ARSs.** A number of well-characterized ARSs were screened for ABF-I binding sites by testing the ability of linear plasmids containing these ARSs to compete with labeled ARS1 DNA for binding of partially purified ABF-I. Fig. 2 summarizes these results. Of the six ARSs tested (Fig. 2) and ARS2 and HML silent

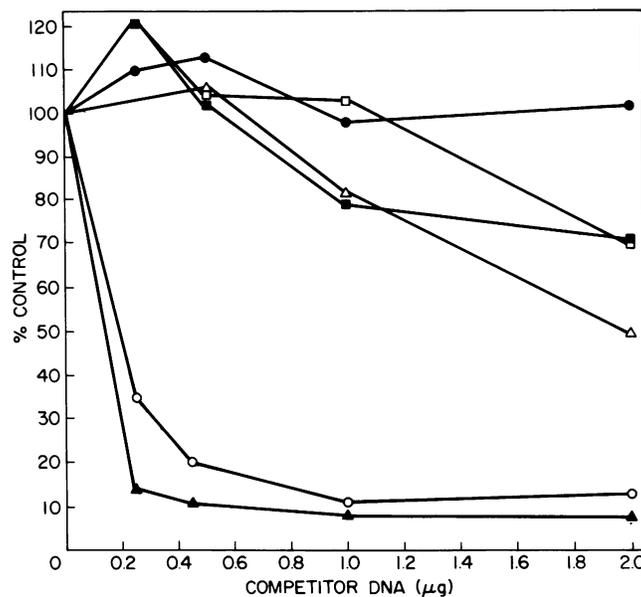


FIG. 2. Competition of the ABF-I-ARS1 complex by other ARSs. A partially purified fraction of ABF-I equivalent to the denatured DNA cellulose fraction was added to reactions formulated as described containing additional varying amounts of linearized plasmids (from 3 to 8.5 kilobases in size); pARS1.2 (○), pBR322 (●), YRp14/CEN4/histone H4 ARS (□), YRp14/CEN4/histone H2B ARS (■), the HindIII fragment containing HMR in pBR322 (▲), and YEp213 (2-μm origin of replication) (△). Bands corresponding to the ABF-I-ARS1 complex were excised from gels after autoradiography, and amounts of radioactivity in the complex were quantitated by liquid scintillation counting. Results are plotted as % of ABF-I-ARS1 complex without plasmid competitor.

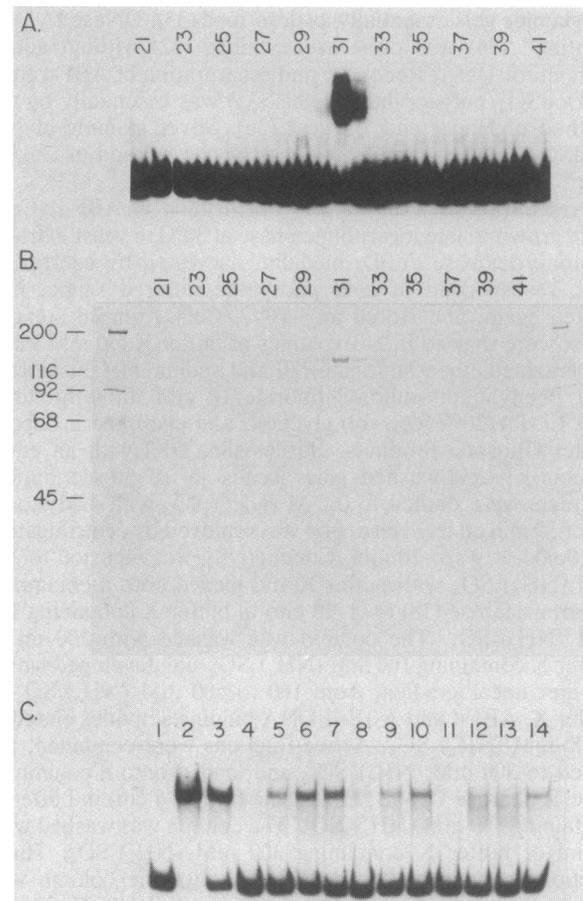
mating type cassette (data not shown), only the *HMR* silent mating type cassette, which has *ARS1* activity, could compete with *ARS1* efficiently and therefore must contain at least one ABF-I binding site. A high-affinity ABF-I binding site at *HMR* was subsequently localized with purified ABF-I (see below).

**Purification of ABF-I.** The purification procedure described in *Materials and Methods* was developed to purify ABF-I to homogeneity. ABF-I and ABF-II cochromatograph during the first step, heparin-agarose, which results in a 50- to 100-fold purification, and these activities were subsequently separated by Blue Sepharose chromatography. ABF-I was further purified as described above to yield  $\approx 5 \mu\text{g}$  of homogeneous protein from 36 liters [ $\approx 600 \text{ g}$  (wet weight) of cells] of yeast in late-logarithmic phase. Although accurate measurement of DNA-binding activity in the relatively crude fractions is difficult, we estimate roughly a 30,000- to 50,000-fold purification based on specific activity.

In Fig. 3 *A* and *B* a Mono Q column profile is shown. The elution of a 135-kDa polypeptide as judged by silver-stained NaDodSO<sub>4</sub>/PAGE (Fig. 3*B*) corresponds exactly with the elution of *ARS1*-specific DNA-binding activity (Fig. 3*A*), arguing strongly that the 135-kDa polypeptide is the active ABF-I protein. To demonstrate this point unambiguously, the 135-kDa polypeptide was recovered from a preparative NaDodSO<sub>4</sub>/polyacrylamide gel and renatured. The gel-purified and renatured 135-kDa polypeptide formed a complex with *ARS1* DNA that migrated through low-ionic strength nondenaturing gels at an identical position to authentic ABF-I activity from the Mono Q fraction (Fig. 3*C*). Approximately 30% of the ABF-I activity could be recovered in pilot denaturation-renaturation experiments without gel isolation (Fig. 3*C*, lane 3), and at least half of that could be recovered after gel isolation of the 135-kDa polypeptide. Furthermore, plasmids containing *ARS1* and *HMR* (Fig. 3*C*, lanes 8 and 11) but not vector (pBR322) or plasmids containing *ARS2*, *HML*, histone H4 *ARS*, histone H2B *ARS* or the 2- $\mu\text{m}$  origin of replication (lanes 7, 9, 10, 12–14) could compete with labeled *ARS1* DNA for binding of the renatured protein. Therefore, ABF-I-binding activity resides in the 135-kDa polypeptide.

**ABF-I-Binding Sites at *ARS1* and *HMR*.** DNase I footprinting with purified ABF-I was used to locate and define the ABF-I-binding sites at *ARS1* and *HMR* (Fig. 4). This analysis demonstrates that ABF-I binds to a single site in *ARS1* at the distal end of domain B and also at a single site in the *ARS* associated with *HMR* E region (*HMRE*). In Fig. 4*B*, the location and nucleotide sequence of the ABF-I binding sites at *ARS1* and *HMRE* is depicted.

**Functional Analysis of Domain B.** The ABF-I-binding site at *ARS1* is essential when yeast were grown in medium containing galactose (12). We have examined the effect of mutations within domain B on *ARS1* activity on medium containing glucose. Plasmid stability was measured as percent of plasmid lost per generation using the colony color assay described by Hieter *et al.* (31) used to indicate *ARS* strength. These results are summarized in Fig. 5. Domains A and B in the absence of domain C (pARS1.4.1) are lost at a rate of  $\approx 10\%$  per generation compared with 5% loss per generation for the wild-type *ARS1* (data not shown). Deletion of domain B (pARS 1.3) results in a plasmid that exhibits an extremely high degree of instability, confirming the crucial role of domain B in *ARS1* function (6). The  $\Delta\text{H5}$  mutation, which deletes to nt 754 and is unable to bind ABF-I (data not shown), exhibited a small, but significantly decreased stability, when compared with wild-type domains A and B. Because this decrease in stability is considerably less than the decrease exhibited by the complete deletion of domain B, this domain appears to have at least two definable elements. One element contains the ABF-I-binding site and under these conditions has a small role in *ARS* function,



**FIG. 3.** Fast protein liquid chromatography Mono Q chromatography of ABF-I. (A) One-tenth microliter of the Mono Q fractions indicated was assayed for *ARS1*-specific DNA-binding activity as described. (B) Ten microliters of the fractions indicated were run on a 10% polyacrylamide gel in NaDodSO<sub>4</sub> and stained with silver. *M<sub>r</sub>* markers are myosin (200 kDa),  $\beta$ -galactosidase (116 kDa), phosphorylase *b* kinase (92 kDa), bovine serum albumin (68 kDa), and ovalbumin (45 kDa). (C) Approximately 0.5  $\mu\text{g}$  of the Mono Q fraction of ABF-I was fractionated by NaDodSO<sub>4</sub>/PAGE as above and stained with 0.25 M KCl (39). The 135-kDa polypeptide was cut from the gel, eluted, and renatured as described. Fractions were assayed for DNA-binding activity as described. Lanes: 1, no added protein; 2, 0.5  $\mu\text{l}$  of the Mono Q fraction of ABF-I; 3, 1  $\mu\text{l}$  of this fraction denatured and renatured without PAGE fractionation; 4–6, 1, 3, and 5  $\mu\text{l}$  of gel-isolated renatured 135-kDa polypeptide, respectively; 7–14, 5  $\mu\text{l}$  of gel-isolated renatured 135-kDa polypeptide with 0.1  $\mu\text{g}$  of linearized pBR322, pARS1.2, YRp14/CEN4/ARS2, *HML*, *HMRE*, YRp14/CEN4/histone H4 *ARS*, YRp14/CEN4/histone H2B *ARS*, and YE213, respectively.

whereas the other element containing sequences between the ABF-I-binding site and domain A has a dominant role in *ARS* function. The internal deletions  $\Delta\text{B3}$  and  $\Delta\text{B5}$  that remove nucleotides 818–852 and 783–852, respectively, also have severe effects on *ARS* function. Although insertion or deletion of 4 bp at the *Bgl* II site separating domains A and B had no effect on *ARS* activity, either insertion of any random pBR322 DNA from 195 to 1080 bp into this site or inversion of domain B with respect to domain A at this site completely inactivated domain B. Domain B function is, therefore, both orientation- and position-dependent with respect to domain A.

## DISCUSSION

Two *ARS1*-specific DNA-binding factors (ABF-I and ABF-II) have been identified in crude extracts. These proteins are

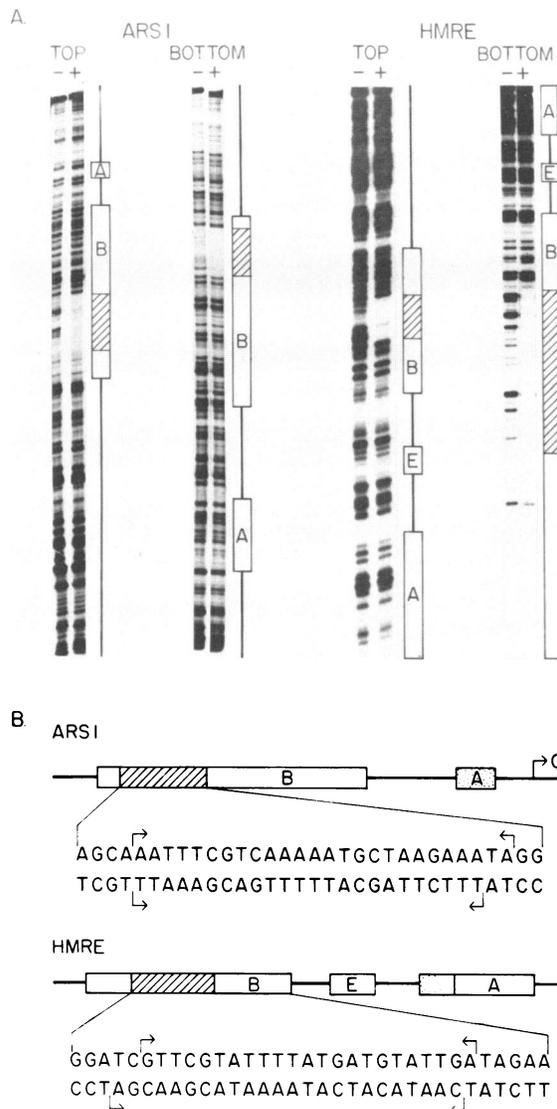


FIG. 4. DNase I footprinting of ABF-I at *ARS1* and *HMR*. (A) DNA fragments for footprinting were 5' end-labeled with polynucleotide kinase by standard procedures, and footprinting reactions were done as described with 5 ng of the Mono Q fraction of ABF-I. For footprinting *ARS1*, the *EcoRI-HindIII* fragment of pARS1.2 was used, 5' end-labeled at either the *HindIII* (*ARS1* top) or *EcoRI* (*ARS1* bottom) site. For footprinting at *HMR*, either pJA82.6Δ173 (*HMRE* top) or pJA82.6Δ224 (*HMRE* bottom) linker scanning mutants labeled at the unique *Xho I* linker insertion site were used (35). Alongside each footprint is a diagram of each region with sequence features aligned as on sequencing gel. Hatched region represents ABF-I-binding site. The three groups of gels at left are 5%, and the *HMRE* bottom gel (at right) is 12.5% 8 M urea sequencing gel. (B) Positions and base-pair sequences of the ABF-I-binding sites (hatched regions) at *ARS1* and *HMRE* with sequence features of these two regions (depicted to scale). The functional A and B domains at *ARS1*, and the A, E, and B elements at *HMR* were previously described (20, 37). Arrows, extent of the ABF-I footprint. For optimal comparison to the *ARS1-ABF-I* binding site, *HMRE* site should be inverted.

*ARS1*-specific by virtue of the fact that they form complexes with *ARS1* DNA, whereas equivalent complexes are not formed with pBR322 DNA. One of these proteins, ABF-I, has been purified to homogeneity by conventional chromatography as a polypeptide of 135 kDa. The other protein, ABF-II, has been purified as a 21-kDa protein that interacts

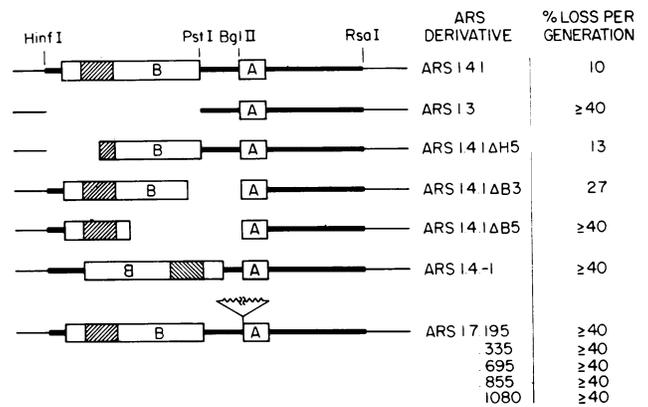


FIG. 5. Analysis of domain B function. *ARS1* derivatives were constructed as described and are depicted. The percent plasmid loss per generation was determined by the half-sector method described by Hieter *et al.* (31). Regions deleted are indicated by gaps, vector sequences are indicated by thin lines, functionally important domains of *ARS1* (A and B) (6, 7) are shown as boxes, and other yeast sequences in *ARS1* are denoted by thick lines. The ABF-I-binding site in domain B is indicated by a hatched box, and the position of inserts in the pARS1.7 series is denoted by the wavy line. Final number in plasmid names of pARS1.7 series indicates size of inserted DNA.

specifically at multiple sites in *ARS1* and will be reported elsewhere.

Competition analysis with seven of the best-characterized ARSs revealed that ABF-I is not a universal ARS-binding factor. However, this protein binds specifically to both *ARS1* and an ARS at the *HMR* silent mating type locus (*HMRE*) at sites that are important for the efficient function of these ARSs and suggests that *ARS1* and *HMRE* are members of a functionally related subset of all ARSs. The ABF-I-binding site at *ARS1* lies within domain B, an 84-bp region previously shown to be important for *ARS1* function. Snyder *et al.* (12) showed by deletion analysis that the ABF-I-binding site, which overlaps a region of bent DNA, is essential for ARS function when the primary carbon source for growth is galactose. In agreement, we demonstrate that a similar deletion exhibits only a small effect on *ARS1* function when the primary carbon source for growth is glucose, whereas deletion of the rest of domain B still has a profound effect on *ARS1* function. Taken together, these results suggest that domain B is a complex sequence element, the function of which is both position and orientation dependent with respect to domain A and which is composed of at least two subdomains. Although a function of the ABF-I-binding site could be to regulate specifically *ARS1* function under certain growth conditions, more probably all ARSs function less well on poor carbon sources, where the differences between "weak" and "strong" ARSs are more pronounced. The role of the ABF-I-binding site in function of the *HMRE* ARS is more clear. Small insertion/deletions that remove the ABF-I binding site from *HMRE* profoundly decrease mitotic stability of plasmids containing *HMRE*, perhaps by reducing efficient replication of these plasmids (40).

*HMRE*, besides being an ARS element, is also required for repression of transcription of the mating type information contained at this locus (41, 42). *HMRE* can silence transcription in a position- and orientation-independent manner, and the silencer can function on heterologous promoters (42). The *HMRE* region itself has been further divided into three functional elements, A, E, and B (40) and it is within one of these, element B, that ABF-I binds. Importantly, there is functional redundancy among these elements because deletion of any one has little or no effect on silencer function, whereas deletion of any two elements completely inactivates

the silencer (40). These three elements alone are sufficient for ARS activity, and the functional overlap between elements required for silencing and ARS activity is striking. Element A contains the ARS consensus sequence and is essential for ARS activity on small cloned fragments containing these three elements (40). Recently, Shore *et al.* (11) identified two proteins in crude extracts designated silencer binding factors B and E (SBF-B and SBF-E) that interact with elements B and E, respectively. SBF-B, in addition to binding at *HMRE*, also binds to *ARS1* within domain B, and the binding sites for both ABF-I and SBF-B at these two ARSs appear to be equivalent, although the potential identity of ABF-I and SBF-B awaits further characterization of SBF-B. In addition to importance for *HMRE* ARS function (see above), the ABF-I/SBF-B binding site becomes essential for silencer function in the absence of either the A or E elements, suggesting that ABF-I may have a role in both DNA replication and repression of transcription. Although the molecular mechanism of silencing is not understood, it is intriguing that silencer activity is so closely correlated with ARS function, particularly since reestablishment of the repressed transcriptional state at the silent mating type loci depends upon a round of DNA replication (43).

Because the *HMRE* silencer can function at either the 5' or 3' end of genes on any promoter (including the *TRP1* promoter) (42) and with the A and B elements alone (40), it is tempting to speculate that *ARS1*, by virtue of its similarity to *HMRE*, may have a role in repressing *TRP1* transcription. As for other replication origins (21, 24, 40), the relationship between transcription and replication at *TRP1-ARS1* may prove quite interesting.

We wish to thank Winship Herr and Amar Klar for critical reading of this manuscript, Phil Hieter for several useful plasmids, and Rich Kostriken for many helpful discussions, yeast strains, and plasmids. This work was supported by grants from the National Institutes of Health (AI20460) and the Rita Allen Foundation.

- Callan, H. G. (1973) *Cold Spring Harbor Symp. Quant. Biol.* **38**, 195–203.
- Blumenthal, A. B., Kriegstein, H. J. & Hogness, D. S. (1973) *Cold Spring Harbor Symp. Quant. Biol.* **38**, 205–223.
- Saffer, L. D. & Miller, O. L., Jr. (1986) *Mol. Cell. Biol.* **6**, 1148–1157.
- Brewer, B. J. & Fangman, W. L. (1987) *Cell* **51**, 463–471.
- Huberman, J. A., Spotila, L. D., Nawotka, K. A., El-Assoull, S. & Davis, L. R. (1987) *Cell* **51**, 473–481.
- Celniker, S. E., Sweder, K., Srien, F., Bailey, J. E. & Campbell, J. L. (1984) *Mol. Cell. Biol.* **4**, 2455–2466.
- Srien, F., Bailey, J. E. & Campbell, J. L. (1985) *Mol. Cell. Biol.* **5**, 1676–1684.
- Kearsey, S. (1984) *Cell* **37**, 299–307.
- Bouton, A. H. & Smith, M. M. (1986) *Mol. Cell. Biol.* **6**, 2354–2363.
- Montiel, J. F., Norbury, C. J., Dobson, M. J., Mills, J. S., Kingsman, A. J. & Kingsman, S. M. (1984) *Nucleic Acids Res.* **12**, 1049–1068.
- Shore, D., Stillman, D. J., Brand, A. H. & Nasmyth, K. A. (1987) *EMBO J.* **6**, 461–467.
- Snyder, M., Buchman, A. R. & Davis, R. W. (1986) *Nature (London)* **324**, 87–89.
- Palzkill, T. G., Oliver, S. G. & Newlon, C. S. (1986) *Nucleic Acids Res.* **14**, 6247–6263.
- Fuller, R. S., Funnell, B. E. & Kornberg, A. (1984) *Cell* **38**, 889–900.
- Tsurimoto, T. & Matsubara, K. (1981) *Nucleic Acids Res.* **9**, 1789–1799.
- Vocke, K. & Bastia, D. (1983) *Cell* **35**, 495–502.
- Abeles, A. L. (1986) *J. Biol. Chem.* **261**, 3548–3555.
- Tokino, T., Murotsu, T. & Matsubara, K. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 4109–4113.
- Masai, H. & Arai, K.-I. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 4781–4785.
- Germino, J. & Bastia, D. (1983) *Cell* **34**, 125–134.
- Rigby, P. W. J. & Lane, D. P. (1983) *Adv. Viral Oncol.* **3**, 31–57.
- Rawlins, D. R., Rosenfeld, P. J., Kelly, T. J., Jr., Milman, G. R., Jeang, K.-T., Hayward, S. D. & Hayward, G. S. (1986) *Cancer Cells* **4**, 525–542.
- Nagata, K., Guggenheimer, R. A. & Hurwitz, J. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 6177–6181.
- Stenlund, A., Bream, G. L. & Botchan, M. R. (1987) *Science* **236**, 1666–1671.
- Hansen, F. G. & Rasmussen, K. V. (1977) *Mol. Gen. Genet.* **155**, 219–225.
- Søgaard-Andersen, L., Rokeach, L. A. & Molin, S. (1984) *EMBO J.* **3**, 257–262.
- Chattoraj, D. K., Snyder, K. M. & Abeles, A. L. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 2588–2592.
- Kelley, W. & Bastia, D. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 2574–2578.
- Jones, K. A., Kadonaga, J. T., Rosenfeld, P. J., Kelly, T. J. & Tjian, R. (1987) *Cell* **48**, 79–89.
- Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).
- Hieter, P., Mann, C., Snyder, M. & Davis, R. W. (1985) *Cell* **40**, 381–392.
- Diffley, J. F. X. & Stillman, B. (1986) *Mol. Cell. Biol.* **6**, 1363–1373.
- Fried, M. & Crothers, D. M. (1981) *Nucleic Acids Res.* **9**, 6505–6525.
- Galas, D. J. & Schmitz, A. (1978) *Nucleic Acids Res.* **5**, 3157–3169.
- Laemmli, U. K. (1970) *Nature (London)* **227**, 680–685.
- Hager, D. A. & Burgess, R. R. (1980) *Anal. Biochem.* **109**, 76–86.
- Oakley, B. R., Kirsch, D. R. & Morris, N. R. (1980) *Anal. Biochem.* **105**, 361–363.
- Iverius, P. H. (1971) *Biochem. J.* **124**, 677–683.
- Alberts, B. & Herrick, G. (1971) *Methods Enzymol.* **21**, 198–217.
- Brand, A. H., Micklem, G. & Nasmyth, K. (1987) *Cell* **51**, 709–719.
- Abraham, J., Nasmyth, K. A., Strathern, J. N., Klar, A. J. S. & Hicks, J. B. (1984) *J. Mol. Biol.* **176**, 307–331.
- Brand, A. H., Breeden, L., Abraham, J., Sternglanz, R. & Nasmyth, K. (1985) *Cell* **41**, 41–48.
- Miller, A. M. & Nasmyth, K. A. (1984) *Nature (London)* **312**, 247–251.