

# A possible role for the yeast TATA-element-binding protein in DNA replication

(*Saccharomyces cerevisiae*/autonomously replicating sequence elements/RNA polymerase II promoters/DNase I protection/mitotic stability)

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**ABSTRACT** The TATA-element-binding protein (TBP) is involved in the initiation of transcription by all three eukaryotic RNA polymerases. The following observations implicate TBP in the initiation of DNA replication at yeast chromosomal origins as well: (i) Recombinant yeast TBP binds specifically to functionally important regions of many yeast replication origins *in vitro*. (ii) TBP-binding sites from RNA polymerase II promoters can activate defective replication origins *in vivo*. (iii) Point mutations in TBP-binding sites that diminish their affinity for TBP *in vitro* reduce their ability to support replication *in vivo*.

Cellular origins of DNA replication have been identified in the yeast *Saccharomyces cerevisiae* (1, 2) as autonomously replicating sequences (ARSs), based on their ability to promote plasmid maintenance (3–5). ARSs are generally bipartite structures, consisting of an 11-bp core consensus sequence (domain A), absolutely required for ARS activity *in vivo*, and a region 3' to the thymidine-rich strand of this sequence (domain B), which greatly stimulates activity (6–10). Recently, a multiprotein complex that specifically recognizes domain A was identified (11). The binding of this origin-recognition complex is ATP dependent and is sensitive to point mutations that abolish ARS activity *in vivo*. In addition, a transcription factor known as ABF1 or OBP1 has been shown to bind to domain B of several ARSs (9, 12, 13), and binding sites for this factor can function as replication enhancers in test constructs (10). ABF1 does not recognize all domain Bs, however, and is thus unlikely to be a general replication factor.

We have noticed that all domain B sequences so far reported contain a candidate recognition site(s) for the general transcription factor TFIID, which binds the TATA element of RNA polymerase II promoters (for sequences capable of interacting with TFIID, see refs. 14–16). Isolation of yeast TFIID identified a monomeric protein of 27 kDa (17), also known as TATA-binding protein (TBP). TFIID from higher organisms is an oligomer of TBP in association with several additional polypeptides (18–22). TBP is also a component of RNA polymerase I and III transcription factors (23–25), although the promoters for these enzymes usually lack TATA sequences. In this report, we verify the interaction of TBP with regions of ARS domain B *in vitro* (as well as instances of domain A interaction) and explore the functional consequences of this interaction *in vivo*. Our results raise the possibility that the role of TBP may be even more general than previously realized, extending beyond transcription to replication as well.

## EXPERIMENTAL PROCEDURES

**Plasmids.** Truncated forms of ARSs were incorporated in pCXAA (from A. Buchman, Pennsylvania State University),

which contains the HMRE silencer, yeast centromere CEN4, *LacZ*, pUC18, and *URA3* sequences. Digestion of pCXAA with *Bam*HI and recircularization to remove a *Bam*HI–*Bam*HI fragment containing the *CYC1* promoter yielded pCXAAΔC. For pARS307 plasmids, a 190-bp *Eco*RI–*Sal*I fragment from pVH402 (from C. Newton, New Jersey Medical School) was subcloned into pSP65. A 67-bp *Sau*3AI–*Hind*III fragment was then excised and used to replace the *Bam*HI–*Cla*I fragment (containing the silencer) of pCXAAΔC. TBP-binding oligonucleotides were inserted between the *Hind*III and *Bam*HI sites of the resulting plasmid. Essentially the same procedure was used to prepare pARSH4 plasmids, containing a truncated form of the H4 ARS, except that a *Bam*HI–*Hind*III fragment from pL71 (from M. Smith, University of Virginia) was used to replace the *Bam*HI–*Cla*I fragment of pCXAAΔC. TBP-binding oligonucleotides were synthesized in both orientations with linkers (GATCC and TCGAAGAATTC) for ligation with the *Bam*HI and *Hind*III sites of the vectors. An *Eco*RI site was included in the *Hind*III linker to facilitate cloning into other vectors but was not used in this study.

**DNase I Protection Analysis.** Purified TFIID (26) was mixed with 5–20 ng of end-labeled ARS-containing DNA fragment in 20 μl of 12 mM Tris-HCl, pH 8.0/40 mM Hepes-KOH, pH 8.4/60 mM KCl/5 mM MgCl<sub>2</sub>/12% (vol/vol) glycerol/poly(dG-dC) at 50 mg/ml. After incubation at room temperature for 20 min, the mixtures were digested with DNase I and processed for sequencing gel electrophoresis, as described (27).

**Mitotic Stability Measurement.** Yeast strain JCW15 (a *leu2Δ100 trp1Δ1 lys2-801 ura3-52 ade2-101*) was transformed with plasmids containing TBP-binding oligonucleotides replacing part of ARS domain B. Individual transformants were grown in minimal medium (0.67% yeast nitrogen base/0.5% ammonium sulfate/2% glucose) supplemented with adenine, leucine, tryptophan, and lysine but not with uracil (SD-ura). When the OD of the culture reached a value between 0.05 and 0.5, appropriate dilutions were spread on either SD-ura or SD+ura plates. Mitotic stability is reported as [(number of colonies on SD-ura plates)/(number of colonies on SD+ura plates)] × 100.

## RESULTS

**Interaction of TBP with ARS Domain B *in Vitro*.** Binding of purified TBP to candidate sites was investigated by DNase I protection (footprint) assays (Fig. 1). TBP protected a 30-bp region centered around nt 793 (see ref. 28 for numbering system) of ARS1 (Fig. 1a), which includes the B2 element shown previously to be important for ARS function (28).

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Abbreviations: ARS, autonomously replicating sequence; TBP, TATA-element-binding protein.

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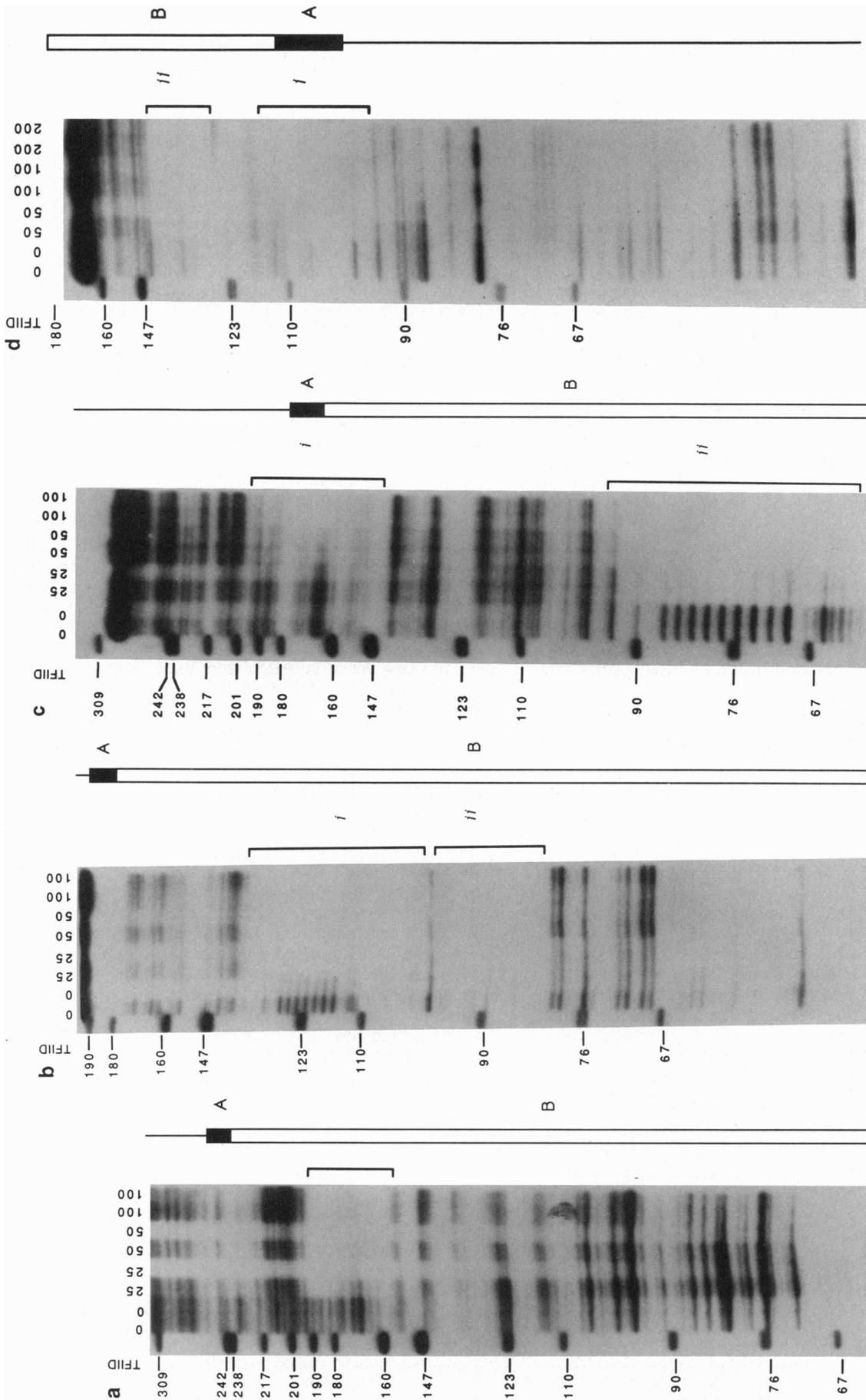


FIG. 1. DNase I-protection analysis of TBP-binding to ARS DNA. ARS-containing DNA fragments used for the analysis were as follows. (a) An 830-bp *Hind*III-*Eco*RI fragment (labeled at the *Hind*III site) from pVH7 spanning ARS1. (b) A 190-bp *Eco*RI-*Sal*I fragment (labeled at the *Eco*RI site) from pVH402 spanning ARS307. (c) A 265-bp *Eco*RI-*Hind*III fragment (labeled at the *Hind*III site) from pCX-E265 spanning the HMRE ARS. (d) A 166-bp *Hind*III-*Eco*RI fragment (labeled at the *Hind*III site) from pRL79 spanning the H4 ARS. Positions of the protected regions (brackets) and of ARS domains A (filled boxes) and B (open boxes) are indicated at right. Letters next to brackets correspond to designations in Table 2; sites closer to domain A are designated i. The amounts of TBP (ng) added are indicated at top; for each concentration of TBP, two amounts of DNase I were used to achieve comparable digestion levels.

Table 1. Effects on mitotic stability of TBP-binding oligonucleotides from RNA polymerase II promoters inserted in ARS domain B

Site	TBP Sequence	<i>In vivo</i> transcription, units of $\beta$ -galactosidase	<i>In vitro</i> binding, ng for 1/2 maximal competition	Mitotic stability, average ( $\pm$ SD)			
				pARS307 plasmids		pARSH4 plasmids	
				-	+	-	+
$\Delta$				1.2 (0.91)	1.2 (0.91)	4.1 (2.1)	4.1
R35	ATTATCATTTAATTAC	450	6.5	6.4 (2.1)	7.0 (1.1)	37 (9.2)	50
R35mt	ATTATCAGTGAATTAC	ND	25	1.9 (1.2)	3.1 (1.4)	8.8 (1.8)	25
HIS3	CTATAAAGTAATGTG	100	5.5	11 (5.4)	16 (1.8)	8.6 (0.33)	24
HIS3mt	CTTATAAAGTAATGTG	ND	15	1.0 (0.61)	1.4 (0.4)	6.4 (1.5)	14

Relative *in vivo* transcription activities are from ref. 16. Relative *in vitro* binding affinities were determined by gel-shift experiments (34) with 0.1 ng of TBP and the *EcoRI*-*HindIII* fragment containing HMRE ARS (Fig. 1) as probe. Amounts of oligonucleotides (ng in double-stranded form) required for half-maximal competition of TBP binding are presented. For each plasmid, three or four independent transformants were picked for mitotic stability determination, and the average and SD (in brackets) were reported. Exceptions are the pARSH4 plasmids with TBP sites in the + orientation (see below), where only one determination per plasmid was made. Orientations of TBP-binding oligonucleotides with respect to ARSs were with the oligonucleotide sequence on the same strand as the thymidine-rich sequence of domain A (+) or on the opposite strand (-). ND, not done.

Despite the generally A+T-rich nature of ARS1 domain B (68% from nt 739 to 856), element B2 is the only site protected from DNase I by TBP. High-resolution genomic footprinting analysis has indicated that element B2 is bound by a protein(s) *in vivo* (29, 30).

Two sites in domain B of ARS 307 (previously known as ARS C2G1) were protected from nuclease cutting in the presence of TBP: nt 85–95 and nt 102–135 (Fig. 1*b*; see ref. 31 for the nucleotide numbering system). Previous deletion analysis indicated that removal of parts of either TBP site reduced the stability of plasmids carrying this ARS (consider constructs 80:106 and 113:128 in ref. 8). Removal of both TBP sites accentuated the reduction in ARS activity (construct 80:133 in ref. 8).

At the HMRE ARS, located within a silencer (32), a 40-bp region (nt 430–470) containing a long stretch of alternating adenosine and thymidine residues was protected by TBP (Fig. 1*c*). Deletion of this region, however, does not much affect plasmid maintenance (A. R. Buchman, personal communication). The deletion may be compensated by the presence in domain A of an exact copy of the canonical TATA element (TATAAA); indeed, this element was protected by TBP (Fig. 1*c*; nt 335–370 in ref. 32). Alternatively, the requirement for the TBP site in domain B may only be apparent under certain culture conditions, as has been reported for the ABF1 site in ARS1 (33).

Finally, an ARS in the vicinity of the histone H4 gene contained two regions of protection by TBP (Fig. 1*d*; nt 75–95 and 107–123 in ref. 7), one spanning domain A and the other part of domain B. The latter lies at the boundary of fragments that support high-frequency transformation in certain vectors (consider plasmids rL55 and rL71 in ref. 7).

**Effect of TBP-Binding Sites on ARS Function *in Vivo*.** The affinity of TBP interaction with ARSs revealed by DNase I protection assays was comparable to that of interaction with TATA elements in promoters (N.F.L., unpublished work);

TBP binding was also specific because only discrete regions of domain B were protected and because the assays were done in the presence of a large excess of poly(dG-dC) DNA. Nevertheless, it might be argued, in light of the A+T-rich nature of TBP-binding sites, that the results of deletion mutagenesis and of DNase I protection assays reflect a requirement not for TBP but rather for a high A+T content of domain B. To address this question, we tested the ability of TBP sites from RNA polymerase II promoters and point mutant forms of these sites to restore ARS function to defective origins *in vivo*. The point mutations in the TBP sites reduced the binding affinity with little or no change in A+T content. Two defective ARSs were used, one derived from ARS307, with 73% of domain B deleted (ARS nt 134–201 retained, with domain A and the remainder of domain B located at positions 182–192 and 134–181, respectively) and the other derived from the H4 ARS, with 83% of domain B deleted (ARS nt 1–102 retained, with domain A and the remainder of domain B located at positions 81–91 and 92–102, respectively). Two TBP sites were used as well, a randomly selected sequence designated R35, shown previously to support a high level of basal and activated transcription (16) and a site based on the TATA element of the *HIS3* promoter. The TBP sites and point mutant derivatives were inserted in domain B of the defective ARSs, cloned in a vector containing a yeast centromere and a *URA3* selectable marker, and assessed for ARS function in mitotic stability assays (Table 1). Remarkably, the promoter elements enhanced ARS function by as much as 15-fold, to a level within an order of magnitude of that typically found with wild-type ARSs (e.g., 71–89% for the HMRE ARS in ref. 35). The enhancement of ARS function by TBP sites was bidirectional, consistent with a previous report that domain B is active in either orientation (36). Point mutations in the TBP sites that impair TBP binding reduced or abolished the enhancement of ARS function. The differences were particularly striking in the pARS307 series,

Table 2. TBP-binding sites in ARSs

ARS	TBP-binding sequence
ARS1	AGTAGTATTTATTTAAGTATTGTTTGCA
ARS307 (i)	TTATAATCTTTTAGCATATATATATATATTG
ARS307 (ii)	TTAAATAAGT
HMRE (i)	CAATTTAATACCTAAATATAAAAAATGTTATTATA
HMRE (ii)	TATAAATAGTATCAATATATATATATATATATATTTATTG
H4 (i)	ATACAAAACATAAAAAATAAT
H4 (ii)	CCTTCTTTATTTACTTT

Sites are designated i and ii depending on their proximity to domain A (see Fig. 1). The TBP-binding sequence on the adenosine-rich strand of domain A is displayed.

where neither mutant element had a significant effect on ARS function. In pARSH4, the mutant promoter elements retained some capacity to stimulate ARS function, possibly due to residual TBP-binding activity, although a contribution to ARS function by a mechanism distinct from TBP binding is not excluded. Similar results have been obtained with other TBP sites and ARS elements. For example, the R35 TBP site stimulated the activity of a deleted HMRE ARS, whereas the mutated R35 site did not (data not shown). Another randomly selected TBP site, designated R80 (16), enhanced function in both pARSH4 and pARS307 (data not shown).

## DISCUSSION

Our data raise the possibility of TBP involvement in ARS-dependent replication but do not constitute proof of such a role. The strongest evidence comes from the capacity of transcriptional TATA sequences to restore function to deleted ARS elements *in vivo* and the loss of this effect due to point mutations in the TATA sequences that abrogate TBP binding. A more stringent test might be done by using a mutant form of TBP with altered DNA-binding specificity (37), which should require the appropriately modified TATA sequence for action at an ARS element.

How might TBP participate in the enhancement of origin function by ARS domain B? One possibility is in the separation of DNA strands required for initiation of both replication and transcription. In *Escherichia coli* replication, melting of the DNA duplex is brought about by *dnaA* protein acting at specific sequences in the unique chromosomal origin (38). Under conditions that disfavor melting, replication becomes dependent on transcription of sequences flanking the origin. Transcription creates a region of strand separation that facilitates initiation, even when this region is located as much as 200 bp from the origin. A similar role of domain B in promoting duplex melting at domain A would be consistent with the important, but not essential, nature of domain B for ARS function (3). It has been suggested that domain B DNA possesses an inherent capacity to facilitate duplex melting (39, 40), and other sequences with this property have been described (41), but for reasons of efficiency and control, any such process would likely be catalyzed by proteins. Although there is no evidence that TBP plays this role in transcription,  $\sigma^{70}$ , which binds to a TATA-like sequence in *E. coli* promoters, is thought to be involved in duplex melting by *E. coli* RNA polymerase holoenzyme (42). TBP itself does not appear to unwind DNA (43), but it may participate with other general transcription factors in the process. The further possibility that TBP binding leads to transcription initiation in domain B also remains to be examined.

Replication and transcription have other features in common besides duplex melting that might account for the involvement of TBP in both processes, such as DNA bending and the involvement of multiple protein factors. ARS1 domain B DNA exhibits a persistent bend (33), and TBP has been shown to bind its recognition sequence in a bent state (34). TBP is associated with distinctive sets of additional proteins in transcription by the three nuclear RNA polymerases (18–25), and there might be a further oligomeric form for replication. It is perhaps noteworthy in this regard that the TBP recognition sites identified in ARS domain Bs generally do not contain the canonical TATAAA element prevalent in RNA polymerase II promoters (Table 2). TBP sites in domain Bs might interact preferentially with replication-specific TBP oligomers.

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