Proc. Natl. Acad. Sci. USA
Vol. 87, pp. 4665–4669, June 1990
Biochemistry

A DNA replication enhancer in *Saccharomyces cerevisiae*

(autonomously replicating sequence/OBF1 DNA-binding site)

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Communicated by Mary J. Osborn, April 13, 1990

ABSTRACT **We have dissected the autonomously replicating sequence ARS*121* using site-directed *in vitro* mutagenesis. Three domains important for origin function were identified; one of these is essential and contains an 11-base-pair sequence resembling the canonical ARS core consensus; the second region, deletion of which affects the efficiency of the origin, is located 3′ to the T-rich strand of the essential sequence and encompasses several elements with near matches to the ARS core consensus; the third region, containing two OBF1 DNA-binding sites and located 5′ to the essential sequence, also affects the efficiency of the ARS. Here we demonstrate that a synthetic OBF1 DNA-binding site can substitute for the entire third domain in origin function. A dimer of the synthetic binding site, fused to a truncated origin containing only domains one and two, restored the origin activity to the levels of the wild-type ARS. The stimulation of origin function by the synthetic binding site was relatively orientation independent and could occur at distances as far as 1 kilobase upstream to the essential domain. Based on these results we conclude that the OBF1 DNA-binding site is an enhancer of DNA replication. We suggest that the DNA-binding site and the OBF1 protein are involved in the regulation of the activation of nuclear origins of replication in *Saccharomyces cerevisiae*.**

Replication of eukaryotic nuclear DNA is initiated at the start of the S phase and proceeds by multiple initiations along a chromosome, generating multiple replicons. However, the regulation and the mechanism of genomic DNA replication are not understood. *Saccharomyces cerevisiae* provides a convenient model system for studying DNA replication in eukaryotic cells since origins of replication, amenable to biochemical and genetic manipulations, have been isolated. These origins, called autonomously replicating sequences (ARSs), were first identified as DNA fragments that confer on plasmids bearing them the ability to replicate autonomously in yeast (1). Compelling evidence has been obtained recently indicating that ARSs incorporated into plasmid DNA function as origins of replication *in vivo* (2, 3). Furthermore, functional DNA replication origin within several hundred base pairs of an ARS element was located in a 22.5-kilobase (kb) stretch of chromosome III and rRNA-encoding DNA (rDNA) on chromosome XII, strongly suggesting that ARSs are also the origins of chromosomal replicons (4–6). Most ARSs are present as single-copy DNA in the genome with the exception of two families of repetitive ARSs. They are the ARSs that are located on the tandemly repeating rDNA units on one arm of chromosome XII and those that are associated with the telomeres of yeast chromosomes (7–9). All ARSs share a common 11-base-pair (bp), [A/T]TTTAT[A/G]TTT-[A/T], core consensus sequence essential for origin function (10). ARS*121*, which is discussed in this paper, is an exception to this rule since it contains a sequence functionally homologous to the consensus that differs from the consensus by a mismatch of 2 bp. Recently, Palzkill and Newlon (11) proposed a central role for this sequence in origin function by demonstrating that synthetic copies of the consensus sequence in tandem could sustain autonomous replication *in vivo*. Others have reported, however, that in naturally occurring ARSs the core consensus sequence alone is insufficient for optimal ARS function. Nucleotide sequences on either side of the core consensus are required, although the amount and the sequence of the flanking DNA vary depending on the ARS (1, 12–14).

We have recently isolated a protein, OBF1, that binds specifically to a DNA sequence present in a broad spectrum of origins (15, 16). We have also shown, using *in vitro* site-directed mutagenesis, that the OBF1 DNA-binding site is important for optimal function of ARS*121* as an origin of replication (17). A similar binding site in ARS*1* and *HMRE*, recognized by OBF1 (16), is a target for the binding of the ABF I protein, identified and isolated by others (18–21). The role of the ABF I DNA-binding site in ARS*1* function is not clear. It appears to affect ARS*1* function significantly only when the plasmid-bearing yeast is grown in a medium containing galactose (22). In *HMRE*, it seems to have a role in ARS activity and transcriptional silencing of the mating type locus (14, 23). The ABF1 and OBF1 DNA-binding sites share a common DNA binding motif TCN_7ACG, first identified by Buchman *et al.* (18). This motif is not restricted to ARSs alone; it was also found at sites that regulate transcription. In fact, it has been shown that DNA-binding sites containing the above motif have a role in transcriptional activation of the genes adjacent to a ty2 element (24), the ribosomal gene TCM1 (25), the genes involved in mitochondrial biogenesis (26), and the YPT1 (ras-like GTP-binding protein) and TUB2 (β-tubulin) genes (27). Factors SUF, TAF, GF1, and BAF1, which bind to these transcriptional activating sites, respectively, have also been identified. Recent isolation of the genes encoding ABF I and BAF1 proteins established that the two are identical (28–30). Isolation of the OBF1 gene awaits further investigation. Because the recognition of a single DNA element by different proteins is not unprecedented—the Oct-1 and Oct-2 transcriptional activators are such an example (31–36)—the possibility that the ARS enhancer is recognized by more than one protein cannot be dismissed. Therefore the question of identity of the various factors remains to be resolved.

In this report we have dissected the ARS*121* and delineated the essential and auxiliary elements for origin function. In particular, we have elucidated the unique properties of the OBF1 DNA-binding site in the function of an ARS. Our results indicate that this site is an enhancer of DNA replication. Therefore this DNA element may have *in vivo* a role in the regulation of replication and transcription.

MATERIAL AND METHODS

Strains and Growth Conditions. The *Escherichia coli* strain used for transformation and plasmid propagation was HB101

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Abbreviation: ARS, autonomously replicating sequence.

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(37). *S. cerevisiae* strain 8534-8C (*MATα*; *leu2-3*; *-112his4Δ34 ura3-52*) was used for all plasmid transformation and maintenance studies (17, 38). Bacteria were grown in LB broth or on LB plates at 37°C. Media were supplemented with ampicillin (100 μg/ml) where appropriate. Yeast were grown at 30°C in YPD (rich) and SD (minimal) media, prepared as described (39). SD medium was always supplemented with histidine (20 μg/ml) and leucine (30 μg/ml). Uracil was added at 50 μg/ml where needed.

Plasmid Constructions and DNA Manipulations. Plasmids YCp5AB121, YCp5A121, and YCp5-3 (YIp5/CEN3) were constructed as described (17). Linker-substitution mutagenesis of ARS*121* (Table 1) was carried out as before (17, 40). Mutated ARS*121* DNA fragments were inserted into *Eco*RI/*Hin*dIII-cleaved YIp5 to test for ARS function and into YCp5-3 for determination of plasmid mitotic stability. The ARS*121* deletions, with the exception of Δs2, were produced by cleaving the plasmid DNA, containing a mutated site, with *Eco*RI and the restriction endonuclease (*Pst* I) whose cleavage site is encoded for in the substitution mutation (Table 1). Δs2 was produced as a *Sac* I fragment from the s2 parent and inserted into *Sac* I-cleaved pUC19. Δs3 was produced as an *Eco*RI/*Pst* I fragment from the parent s3 and inserted into *Eco*RI/*Pst* I-cleaved pUC19. Δs2 and Δs3 were then moved as *Eco*RI/*Hin*dIII fragments into YIp5 and YCp5-3. All other deletions, removed from parent constructions as *Eco*RI/*Pst* I fragments, were inserted directly into YIp5 and YCp5-3 (cleaved with *Eco*RI and *Hin*dIII) using a *Pst* I/*Hin*dIII single-stranded oligonucleotide bridge (AGCTTGCA). The substitution and deletion mutations in ARS*121*—s2, s3, s23, s123, and Δs2—do not contain the T at nucleotide 434 (Fig. 1). Plasmid constructs containing a synthetic OBF1 DNA-binding site (Fig. 3*B*) were created by first annealing the phosphorylated, complementary oligonucleotides (site I, Figs. 1 and 3*B*), followed by insertion of the double stranded DNA into the *Kpn* I site of p19A121, a plasmid containing the 224-bp *Rsa* I/*Hin*dIII fragment of ARS*121* (17) (Fig. 1; fragment A*121*). Dimerization of the synthetic OBF1 DNA-binding site was carried out as described (41), followed by inserting the dimer binding site, as an *Eco*RI/*Hin*dIII fragment, into p19A121 at the *Eco*RI and *Kpn* I sites via a *Kpn* I/*Hin*dIII single-stranded oligonucleotide bridge (AGCTGTAC). All synthetic DNA-binding site/A*121* chimeras were sequenced and moved into YCp5-3 as *Eco*RI/*Hin*dIII fragments. All oligonucleotides used to make synthetic OBF1 DNA-binding sites and *in vitro* generated mutations were produced using a Cyclone DNA synthesizer (Biosearch). Constructions containing φX174 fragments between the synthetic OBF1-binding site and the ARS were created by inserting either the 286-bp *Xmn* I, the 536-bp *Stu* I/*Hpa* I, or the 1079-bp *Hpa* I fragment of φX174 into the *Sna*BI site of Ch(−) in Fig. 3. YCp5A121 derivatives containing φX174 DNA were created by inserting the above fragments into the *Eco*RI site (filled in by T4 DNA polymerase) of the plasmid. Plasmid DNA was prepared from *E. coli* cultures by the standard alkaline lysis procedure. Plasmids were used to transform yeast to Ura prototrophy by the lithium acetate procedure (42).

DNA Sequencing. All DNA sequence analyses were performed as described (43).

Analysis of Plasmid Mitotic Stability. Plasmid mitotic stability analyses, under nonselective and selective conditions, were performed as described (17). Mitotic stability determinations were performed on at least four independent transformants for each plasmid construct.

RESULTS

Identification of the Essential ARS*121* Origin Core Sequence. ARS*121* was first isolated as a 6.6-kb DNA fragment and was subsequently shown to be contained within a 489-bp fragment (17). The DNA sequence and a schematic description of the ARS are shown in Fig. 1. The function of an ARS as an origin of replication can be assayed by mitotic stability of a plasmid bearing the ARS, provided that the plasmid also contains a centromeric sequence (9, 14, 17, 23). Using such an assay we demonstrated that a truncated ARS (A121), lacking the two OBF1 DNA-binding sites, is a less efficient origin than the wild-type ARS (AB121) (ref. 17; Figs. 1*B* and 2*A*). Under these conditions the mitotic stability was reduced from about 47% to 12% when the plasmid-bearing cells were grown in nonselective medium for 10 generations (Fig. 2*A*). This represents an increase in the plasmid loss rate per generation from about 5% (AB121) to about 12% (A121), which translates into a 30-min longer doubling time for cells bearing A121 when grown in selective medium (data not shown). To our surprise, the DNA sequence of the essential region (A121, Fig. 1) did not reveal an 11-bp sequence that perfectly matches the ARS core consensus sequence ([A/T]TTTAT[A/G]TTT[T/A]). Four DNA elements, each with two mismatches to the consensus, were found in the truncated A121 region (Figs. 1*B* and 2*A*, boxes 1–4). Box 3 contains a symmetrical sequence with a 2-bp mismatch to the ARS consensus in either orientation.

To determine which one of these elements is essential for origin function, the sites were modified by linker substitution mutagenesis (Table 1) and the plasmid constructs bearing these mutations were used to transform yeast. Unlike the

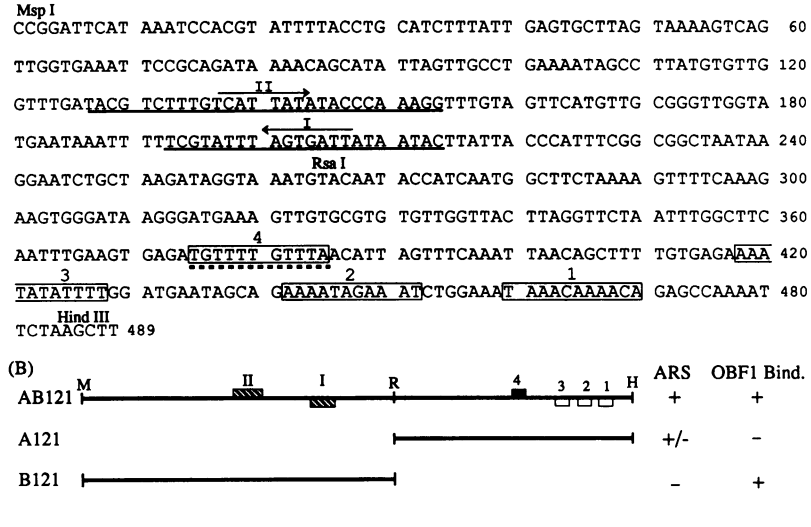

FIG. 1. (*A*) Nucleotide sequence of the 489-bp *Msp* I/*Hin*dIII restriction fragment containing ARS function and the two OBF1 binding sites. Boxed sequences are the partial matches to the ARS core consensus sequence (Table 1). Dashed underline indicates the essential partial match to the core consensus. Underlining denotes the two OBF1 binding sites [regions protected from DNase I by the OBF1 protein (17)]. Arrows indicate directionality of the consensus binding site sequence (TCN_7ACG). (*B*) Fragments were cloned and tested for ARS function and OBF1 binding (17). Hatched boxes denote position and relative orientation of binding site sequence TCN_7ACG. Open and filled boxes indicate position and relative orientations of the T-rich strand of the ARS core consensus sequence partial matches. Boxes 1–4 correspond to nucleotide sequences boxed in *A*. M, *Msp* I; R, *Rsa* I; H, *Hin*dIII. +, Functional in mitotic stability or OBF1 binding assay; −, inactive in either assay; +/−, functional at a lower efficiency than the wild-type ARS.

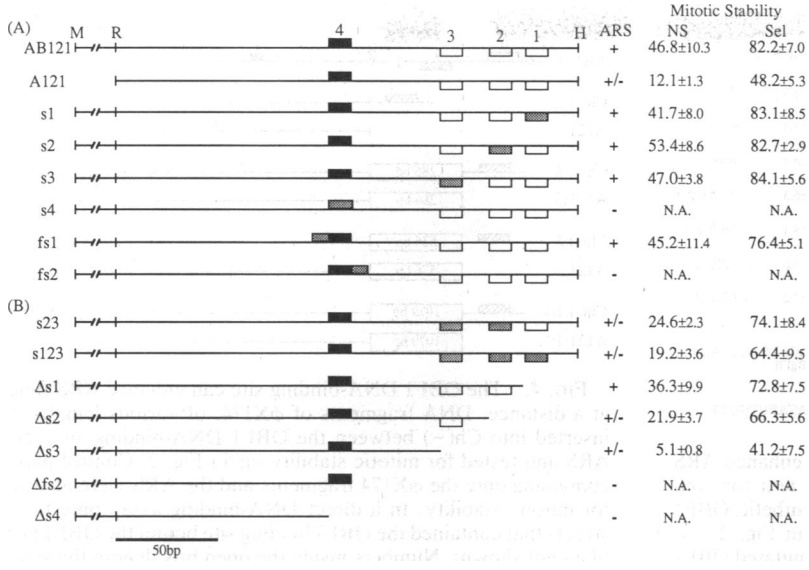

FIG. 2. Substitution and deletion analysis of the essential domain of ARS*121*. See text for preparation and analysis of mutagenized and deleted ARS*121* fragments. Mitotic stability is expressed as the percentage of the population bearing the plasmid. All constructions, except A*121*, contain the entire 489-bp ARS*121* fragment (both binding sites are present, see Fig. 1). Open and filled boxes, same as in Fig. 1. Stippled boxes denote sequences that have been mutagenized (sequences in Table 1). (*A*) Substitution mutagenesis of single sites in ARS*121*. (*B*) Multiple substitution and deletion mutations in ARS*121*. M, *Msp* I; R, *Rsa* I; H, *Hin*dIII; NS, nonselective; Sel, selective; +, −, and +/− are as in Fig. 1; N.A., not applicable since these constructs do not function as ARSs.

plasmids s1, s2, and s3 (mutated in boxes 1, 2, and 3, respectively), which still contained a functional ARS, s4 (mutated in box 4) was unable to transform yeast (Fig. 2*A*). Thus, the sequence TGTTTTGTTTA (box 4) is the element essential for ARS*121* function. Sequences (6 bp) on either side of the essential element were also mutagenized by linker substitution (Fig. 2*A*, Table 1). Only one of those, fs2, located immediately adjacent to the 3′ end of the essential sequence, obliterated origin activity as judged by the inability of the plasmid bearing this mutation to transform yeast. This indicates that the essential core sequence consists of a region larger than just the 11-bp ARS core consensus, in agreement with the analysis of other ARSs (44).

An ARS Auxiliary Domain Located 3′ to the Essential Element. DNA sequences flanking the core consensus, 3′ to the T-rich strand, are required for efficient ARS function of a number of origins, ARS*1*, *HO* ARS, the histone *H4* ARS, and others (44). Palzkill and Newlon (11) identified in this region multiple near matches to the ARS core consensus and ascribed to them an important role in ARS activity. They proposed that these sites may serve as recognition sequences for the binding of an initiation protein, analogous to the multiple tumor (T) antigen-binding sites at the simian virus 40 origin or the DnaA protein-binding sites at oriC, the *E. coli* origin of replication.

Table 1. Sequence alterations in partial matches to core consensus

Site Wt*	Site Mut	Sequence of partial matches and substitutions†
1		^{450}ATCTGGAAATAAACAAAACAGAGCCAAAA
	s1	GTCTGCAGCTT
2		^{432}GAATAGCAGAAAATAGAAATCTGGAAATA
	s2	GCTGAGCTCCA
3		^{408}TTTGTGAGAAAATATATTTTGGATGAATA
	s3	ACTCTGCAGAT
4		^{365}GAAGTGAGATGTTTTGTTTAACATTAGTT
	s4	GTCTGCAGCAT
	fs1	CTGCAG
	fs2	CTGCAG
ARS core consensus		$^{A}_{T}$TTTAT$^{A}_{G}$TTT$^{A}_{T}$

Wt, wild type; Mut, mutant.
*Numbers correspond to core consensus partial matches in Fig. 1.
†Superscript numbers indicate the position of the first base in the string according to numbering in Fig. 1. Solid underline indicates sequence altered in mutations s1–s4. Dashed underline indicates sequence altered in mutations fs1 and fs2.

We have also identified similar elements, with near matches to the ARS core consensus sequence, present in ARS*121* 3′ to the T-rich strand of the essential sequence (Fig. 2). To elucidate the possible role of these sequences in ARS function, single sites were mutagenized by linker substitution. The sequence alterations in plasmids s1, s2, and s3 did not affect origin activity as measured by the plasmid mitotic stability assay (Fig. 2*A*). However, a double mutation (s23) caused a 2-fold reduction in origin efficiency. This effect was further augmented in plasmid s123, containing mutated sequences in boxes 1, 2, and 3 (Fig. 2*B*). On the other hand, a deletion of the three boxes caused a considerably larger (10-fold) reduction in the efficiency of the ARS (Fig. 2*B*). Thus the region containing boxes 1, 2, and 3 is an auxiliary nonessential domain that affects the efficiency of the origin. Since deleting elements 1–3 was more deleterious than the triple linker-substitution mutation, not only the near matches but also the structural organization of the entire domain is important for origin function. Because these elements are not essential, the possibility that they serve as recognition sites for an initiation protein appears unlikely, although it cannot be excluded. Similar conclusions have been reached based on the analysis of the *H4* ARS (45).

The OBF1 DNA-Binding Site Functions as an Enhancer of DNA Replication in an Orientation-Independent Manner. We have previously described the presence in ARS*121* of two DNA-binding sites with different affinities for OBF1 (17). They are contained within the auxiliary *Msp* I–*Rsa* I region located 5′ to the T-rich strand of the essential sequence (box 4) and appear to be inverted with respect to each other (Fig. 1). Our previous analysis suggested that the two OBF1 DNA-binding sites are the only elements in the 5′-flanking domain important for origin function (17). To test this possibility, we constructed a chimeric origin in which a synthetic, high-affinity OBF1 DNA-binding site (site I) was fused to the truncated A*121* origin from which the naturally occurring OBF1 DNA-binding sites were deleted (Fig. 3). As shown in Fig. 3, the synthetic binding site enhanced origin function, about 3-fold, to a level intermediate between the AB*121* (wild type) and A*121* (truncated) origin. This enhancement was achieved irrespective of the orientation of the binding site (Fig. 3) and was similar to the activity of the origin mutated in site II, which is the low-affinity binding site for OBF1 found in the wild-type ARS*121* (17). When a tandem, head to tail dimer of the OBF1 DNA-binding site was fused to A*121*, the resulting origin was at least as efficient as the wild-type ARS (Fig. 3). On the other hand, a mutated DNA-binding site (Fig. 3*B*), which is not recognized by the

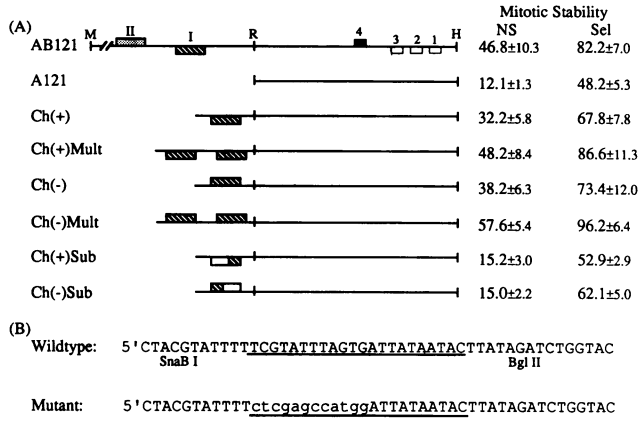

FIG. 3. A synthetic OBF1 DNA-binding site can enhance ARS function in an orientation independent manner. See text for construction and analysis of plasmids containing the synthetic OBF1 binding site(s). Mitotic stability was determined as in Fig. 2. (*A*) Analysis of the effect of one or two binding sites or a mutated OBF1 binding site on the function of ARS*121* (A121 fragment). Hatched box indicates the OBF1 DNA-binding site I (Fig. 1) and the stippled box denotes the binding site II (Fig. 1). Ch(+)Sub and Ch(−)Sub contain a mutation that removes the sequence TCN_7ACG. Constructs with the OBF1 binding site in the same orientation as site I in ARS121 (AB*121* fragment) are identified with a (+); those with a (−) are in the opposite orientation. (*B*) Sequence of the wild-type and mutated synthetic OBF1 binding sites used in *A*. Underlined sequence, in wild type, is the domain (site I, ARS*121*) protected by OBF1 from limited DNase I digestion (17). Lowercase letters in the mutated binding site sequence indicate substitutions. Abbreviations as in Fig. 2. All constructs contain the *Rsa* I/*Hin*dIII region of AB121.

purified OBF1 protein (data not shown), did not enhance origin activity. These results imply that the OBF1 DNA-binding site, perhaps in conjunction with the OBF1 protein, acts to enhance the initiation of DNA replication.

The ARS Enhancer Works at Long Distances. DNA sequences with high similarity to the ARS*121* enhancer were identified in many ARSs at various distances from the ARS core consensus sequence, ranging from about 70 bp in *HMRE* to 200 bp in the telomeric ARS, ARS*120*. We therefore decided to determine the effect of distance from the ARS essential core element on the replication-stimulating activity of the enhancer. In this experiment, which is shown in Fig. 4, a synthetic OBF1 binding site was inserted at various distances from the ARS*121* essential core, using ϕX174 restriction DNA fragments to link the enhancer to the truncated A*121* ARS. In the wild-type ARS*121* the T residue, the first nucleotide in the sequence TCN_7ACG of site I, is separated from the T residue found in the center of TGTTTTGTTTA, the essential element (Fig. 1, box 4), by 175 nucleotides. In the chimeric plasmids, Ch(−), Ch(−):3, Ch(−):5, and Ch(−):10 (Fig. 4), this distance was 144, 430, 680, and 1223 nucleotides, respectively. It is evident that the enhancer element stimulated origin activity even when present at distances as far as 1.2 kb from the essential core. The maximum permissible distance between the enhancer and the ARS core consensus remains to be determined.

DISCUSSION

Understanding the regulation of DNA replication in eukaryotic cells hinges upon our ability to elucidate the mechanism of initiation of replication at a single replicon. Thus insight into the structural organization of an origin is necessary, and the identification and characterization of the proteins responsible for initiation of DNA replication are crucial. In this paper, we have delineated the important DNA elements of ARS*121* for origin function, using site-directed *in vitro* mu-

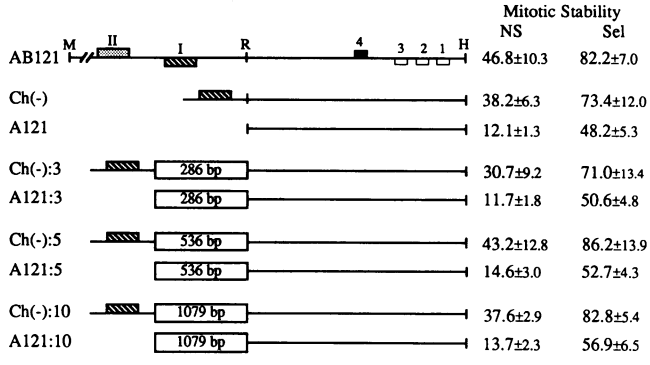

FIG. 4. The OBF1 DNA-binding site can enhance ARS function at a distance. DNA fragments of ϕX174, of various lengths, were inserted into Ch(−) between the OBF1 DNA-binding site and the ARS and tested for mitotic stability, as in Fig. 2. Control plasmids containing only the ϕX174 fragments and the ARS were also tested for mitotic stability. In a direct DNA-binding assay only the constructs that contained the OBF1 binding site bound the OBF1 protein (data not shown). Numbers inside the open box denote the size (bp) of the inserted ϕX174 restriction DNA fragment. Hatched box represents the OBF1 DNA-binding site as in Fig. 3. Abbreviations as in Fig. 2. All constructs contain the *Rsa* I/*Hin*dIII region of AB121.

tagenesis. We found that the essential origin core contains an element functionally homologous to the ARS core consensus, although it differs from the consensus motif by a 2-bp mismatch. ARS*121* also contains two auxiliary domains, flanking the essential core. They are not essential but affect the efficiency of the origin of replication. One of these, containing the sequences with near matches to the ARS core consensus, is located 3′ to the T-rich strand of the essential element. The other auxiliary domain, containing the two OBF1 binding sites, flanks the essential domain at the 5′ end.

The precise role of the essential core sequence in origin function is not known. However, because of its absolute requirement, it seems likely to be a part of a recognition site for an initiation protein. The function of the auxiliary 3′ domain that influences the efficiency of the origin is also not clear. Similar DNA sequences, shown to have a role in ARS function, were found in other ARSs as well (44). For example, in ARS*1* such sequences, present in domain B, were shown by deletion analysis to be important for optimal origin activity (20, 46, 47). Our analysis of ARS*121* demonstrates that deleting such elements was more detrimental to replication than their mutagenesis by linker substitution. One possibility is that the structural organization of this entire domain facilitates unwinding of the duplex during the initiation of DNA replication, as suggested for the *H4* ARS (48) and analogous to the 13-mer repeats of oriC, the *E. coli* origin of replication (49). Alternatively, this domain could provide a site for transcriptional activation of initiation of DNA synthesis or have a role in the nucleosomal organization at the origin.

The molecular dissection of ARS*121* enabled us to elucidate the functional properties of the cis-acting OBF1 DNA-binding site, which was found in a broad spectrum of yeast origins. Our results indicate that the OBF1 DNA-binding site is an enhancer of DNA replication, and we suggest that this site has a role in the regulation of activation of replicons in *S. cerevisiae*. We have demonstrated that a synthetic binding site containing the nucleotide sequence of the wild-type site I (Fig. 3) can substitute for the entire 5′ domain in enhancing origin activity. This stimulation was not dependent on the relative orientation of the synthetic DNA-binding site and could occur at long distances, as far as 1.2 kb upstream to the ARS essential sequence. A possible explanation for the enhancement of replication is that the OBF1 DNA-binding site inhibits the progress of a putative transcript into the ARS

that may interfere with ARS function as an origin of replication. The impediment of ARS function by transcription through the ARS and initiation of transcription in yeast at a site located near the origin of replication of pBR322-derived plasmids have been previously demonstrated (50, 51). To address this question we have placed the OBF1 DNA-binding site at the *Hin*dIII site, 3′ to the essential element and the 3′ auxiliary domain (data not shown). The stimulation of ARS function in this construct was equal to that of the Ch(−) plasmid (Fig. 4). This rules out the possibility that the enhancement of replication is an indirect result of a potential transcriptional interference by the OBF1 DNA-binding site.

The ARS enhancer might be involved in the regulation of the temporal order of origin activation, believed to occur in yeast (52). The differential binding affinities of OBF1 as well as the number of ARS enhancer sequences present in different origins (16, 17) may have an impact on this regulation. Some known origins (ARS *H4*) do not appear to contain the ARS enhancer sequence. However, since this enhancer can exert its function at rather long distances, the enhancer sequence may have been missed during the ARS isolation, and this question, probably, should be reevaluated.

How enhancers work at long distances is not clear. Recently Cheng and Kelly (53) suggested that transcriptional enhancers present at viral origins of replication stimulate replication by perturbing the distribution of nucleosomes in the adjacent sequences, thus leaving the origin exposed for interaction with the replication machinery. It is expected, however, that such a stimulatory mechanism will be sensitive to the distance of the enhancer from the origin core sequence. Therefore, this mechanism seems less likely for the ARS enhancer because the enhancer works when present at long distances from the essential element of the ARS. It seems more probable that in order to stimulate the origin of replication, the ARS enhancer and its cognate protein have to interact with either the 3′ auxiliary domain or the origin core itself. This could be accomplished by DNA looping that would place the protein bound to the enhancer in proximity to a target site, enabling a direct interaction of the regulatory protein with a component of the replication apparatus. Such a mechanism has been proposed for the action of some enhancer sequences and their cognate proteins in the regulation of transcriptional activation and repression (54). The final elucidation of the mechanism of the ARS enhancer function in replication, however, will require the identification and characterization of the remaining components of the replication initiation apparatus.

We thank William A. Whalen and Piet A. J. deBoer for the careful reading of the manuscript and their useful comments. S.C.F. was supported by Public Health Service National Research Service Award GMO7407. This work was supported in part by American Cancer Society Grant MV-368 and in part by National Science Foundation Grant DMB-8916358.

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