The gene encoding ARS-binding factor I is essential for the viability of yeast

Peter R. Rhode, Kevin S. Sweder, Karen F. Oegema, and Judith L. Campbell

Divisions of Chemistry and Biology, California Institute of Technology, Pasadena, California 91125 USA

The gene encoding a yeast ARS-binding protein, ABF I, has been cloned by screening a genomic λgt11 library using monoclonal and polyclonal antibodies against ABF I. ABF I is of interest because it not only binds to ARSs but also to the 5'-flanking region of genes encoding proteins involved in transcription, translation, respiration, and cell-cycle control. The cloned gene has been used to prepare null mutants, which further demonstrate the importance of the ABF I protein by showing that it is essential for vegetative growth. ABF I maps to chromosome V. The DNA sequence of the ABF I gene reveals several motifs characteristic of DNA-binding proteins but shows no overall similarity to any protein of known function.

[Key Words: DNA-binding proteins; DNA replication; ribosomal protein genes; mitochondrial proteins genes; HMR E silencer]

Received August 25, 1989; revised version accepted October 3, 1989.

Eukaryotic DNA replication initiates at multiple sites within the chromosome during the S phase of the cell cycle. In yeast, replication is thought to initiate at specific origins corresponding to autonomously replicating sequences (ARSs), which are operationally defined as DNA elements that allow extrachromosomal maintenance of plasmid DNAs. Physical mapping of replication intermediates formed in vivo and in vitro demonstrate that ARSs serve as origins of replication both on plasmids and in the chromosome, validating the usefulness of ARS elements in experimentally examining the initiation of chromosomal replication (for review, see Newlon 1989).

Mutational analysis of a number of ARSs has indicated that sequences required for ARS function are complex. ARS1 consists of three functional domains: A, B, and C (Celniker et al. 1984). Domain A contains the 11-bp core consensus sequence (A/T TTTATRTTT A/T) (R = A or G) found at all ARSs (Stinchcomb et al. 1981, Broach et al. 1983). Point mutations in the core consensus cause a complete loss of ARS function, defining domain A as essential (see Celniker et al. 1984; Kearsey et al. 1984) for the HO ARS. Domains B and C flank domain A on either side. Domain B consists of ~110 bp of DNA (Srienc et al. 1985). Deletion analysis suggests that domain B consists of multiple functional elements that are important, though not essential, for ARS function (Srienc et al. 1985; Snyder et al. 1987; Diffley and Stillman 1988; Strich et al. 1988). This domain contains many features that may contribute to optimal ARS function: regions of bent DNA, AT-rich DNA, DNA that unwinds relatively easily, a sequence conserved among many ARSs, and multiple copies of close matches to the core consensus (Broach et al. 1983; Palzkill et al. 1986; Snyder et al. 1987; Umek and Kowalski 1987; Palzkill and Newlon 1988; Williams et al. 1988). Domain C, ~200 bp in size, has been shown by deletion analysis to be less important than domain B for ARS function (Celniker et al. 1984; Koshland et al. 1985; Srienc et al. 1985). One functional model for ARS1 describes domain A as an initiation protein recognition site analogous to the 9-bp dnaA protein recognition site at oriC of Escherichia coli. Domain B may contain sequences required for efficient assembly of the initiation complex, for DNA unwinding, or for transcriptional activation of the origin. The function of domain C is presently unknown. On the basis of prokaryotic and viral replication origins, it is reasonable to assume that proteins that bind to these functional domains will be involved in initiating and regulating DNA replication.

Several laboratories have detected an activity that specifically binds to a site in domain B shown by deletion analysis to be important for optimal ARS activity (Shore et al. 1987, Buchman et al. 1988a, Diffley and Stillman 1988, Sweder et al. 1988). This activity, ARS-binding factor I (ABF I) or silencer-binding factor B (SBF-B), also binds to sequences adjacent to at least five other ARSs, including the ARS at the silent mating-type locus, HMR E (Shore et al. 1987, Buchman et al. 1988a, Diffley and Stillman 1988; Sweder et al. 1988). ARSs associated with H2B, H4, HML E did not compete for ABF I binding to HMR E (Buchman et al. 1988a). Given the sensitivity of the assay, this suggests that ABF I has an affinity at least fivefold lower for these ARSs than for HMR E, if it binds at all. Detailed analysis of HMR E function indicated...
that the ABF I-binding site is involved in transcriptional repression of HMR, along with maintenance and centromere-independent segregation of the HMR E-bearing plasmids (Abraham et al. 1984; Feldman et al. 1984; Brand et al. 1985, 1987; Shore et al. 1987; Kimmerly and Rine 1987; Kimmerly et al. 1988). The ABF I-binding site could also activate transcription from the CYC1 promoter, suggesting that ABF I has multiple roles in transcription and replication (Brand et al. 1987). Recently, the interaction of another DNA-binding protein, OBF1, with telomeric ARS elements and a single-copy ARS (ARS121) has been characterized independently (Eisenberg et al. 1988; Francesconi and Eisenberg 1989). Site-directed mutagenesis of ARS121 demonstrated a correlation between OBF1 binding in vitro and the ability of this ARS to function efficiently in vivo (Walker et al. 1989). OBF1 binds the same sites in ARS1 and HMR E as ABF I (Francesconi and Eisenberg 1989), suggesting that these factors are identical.

Although ABF I binds to multiple ARSs, suggesting a role in ARS function, ABF I binding is not limited to ARSs. The ABF I-binding site has been identified as a protein recognition site in the 5'-flanking regions of a number of genes. The common thread linking these genes is that ARS and telomeric ARS are both DNA-binding proteins. As can be seen in Figure 1A, the antibodies recognized two proteins of apparent molecular masses of 130 and 135 kD. These proteins comigrate with the silver-stained doublet observed in purified ABF I preparations and the ARS1 DNA-binding activities detected in Southwestern analysis (Sweder et al. 1988). Subsequent experiments indicated that the rabbit antiserum reacted with several other polypeptides in crude yeast extracts (data not shown); however, the monoclonal antibodies detected only ABF I (e.g., see Fig. 5A, below). To confirm that these antibodies reacted with authentic ABF I, we tested the effects of the antibodies on the electrophoretic mobility of ABF I/ARSI DNA complexes in non-denaturing polyacrylamide gels (Fig. 1B). Addition of rabbit antiserum to the binding reaction caused a significant decrease in the mobility of the ABF I/DNA complex (lane 4), whereas preimmune serum had no effect (lane 3). No complexes were observed if ABF I was omitted from the binding reaction (data not shown). The change in mobility reflects the interaction of the antiserum with ABF I. Similarly, all six of the monoclonal antibodies tested decreased the mobility of the ABF I/DNA complex (lane 7 shows one example). These results indicate that these antibodies react with ABF I in such a way that DNA binding is not disrupted.

Cloning of the ABF1 gene

To isolate the ABF1 gene, we screened a λgt11 yeast genomic library with the polyclonal and monoclonal antibodies described above. Initially, 1.2 x 10⁹ phage were screened with rabbit polyclonal antiserum, resulting in 10 positive clones. These clones were grouped into six different classes by cross hybridization and restriction enzyme mapping. Because the antiserum used in the initial screen reacts with several yeast polypeptides in addition to ABF I, it was expected that several different genes would be isolated. Each class of insert was screened with an ABF I-specific monoclonal antibody (6C11G4), and only one class gave positive signals. One clone in this class, λgt11-15a, contains the 4.4-kb EcoRI yeast DNA insert shown in Figure 2. Preliminary gene mapping experiments suggested that this DNA fragment did not contain the entire ABF1 gene. To obtain the intact ABF1 gene, we used the λgt11-15a insert as a hybridization probe to screen a yeast genomic library prepared in the high copy number plasmid YEp24. Yeast DNA inserts from several positive clones were found by restriction mapping to overlap the λgt11-15a insert. The largest yeast DNA inserts (from YEp24-3a and YEp24-4c) were found to contain the intact ABF1 gene and were used to derive the restriction map shown in Figure 2.

Characterization of the ABF1 gene

Two independent methods were used to map the location of the ABF1 gene within the cloned inserts and to determine the direction of transcription of the putative ABF1 gene. In the first approach, Northern blots of yeast
poly(A)^+ RNA were hybridized to DNA and RNA probes from the cloned DNA of interest. Figure 3 shows that two transcripts, one of 1.1 kb and another of 2.6 kb, were detected in blots probed with the Xgtl1-15a insert. Because of the apparent 135-kD molecular mass of ABF I, we suspected that the 2.6-kb transcript encoded this protein. To localize the genes encoding the two transcripts, a series of smaller DNA fragments within the 15a insert [labeled a–g in Fig. 2] were used as hybridization probes. The two transcripts localized to adjacent regions within the cloned DNA [Fig. 3, lanes a–g]. The EcoRI fragment encoding both transcripts [Fig. 2] was isolated from xgtl1-15a and subcloned in both orientations adjacent to the T7 RNA polymerase promoter in the vector Bluescript. Strand-specific RNA probes of the 15a insert were synthesized in vitro and were used to determine the orientations of the 2.6- and 1.1-kb yeast genes. Probes complementary to the top strand of the 15a insert [in the orientation shown in Fig. 2] detected the 2.6-kb transcript, whereas those complementary to the bottom strand detected the 1.1-kb transcript [Fig. 3, lanes T and B]. Results of these experiments are summarized above the restriction map in Figure 2. The 2.6-kb mRNA is transcribed from left to right, and all but ~200 bp of the 5' end are within the 15a insert. The 1.1-kb mRNA is derived from an adjacent gene that is transcribed in the opposite direction.

Our second approach to identifying the ABF1-coding sequence involved characterization of polypeptides produced in bacteria from the cloned yeast DNA inserts. A xgtl1-15a-derived EcoRI fragment encoding both the 2.6- and 1.1-kb transcripts was subcloned into the Bluescript polylinker such that the 5' end of the 2.6-kb yeast gene was fused downstream of a sequence encoding 37 amino-terminal amino acids of β-galactosidase. The resulting plasmid was called pBS-15a [Fig. 2]. The plasmid was introduced into E. coli, and crude cell lysates prepared from cultures induced with isopropyl-β-D-thiogalactopyranoside (IPTG) and from uninduced cells were analyzed by protein blotting with anti-ABF I polyclonal

Figure 2. Restriction map of yeast ABF I genomic region and structure of cloned inserts. The restriction map (top) was determined from the largest genomic clone. The symbols for the restriction enzymes are B (BamHI), Bg (BglII), H (HindIII), K (KpnI), P (PstI), R (EcoRI), and S (Spel). The relative boundaries and direction of the 2.6-kb ABF I and 1.1-kb genes, as defined by Northern analysis, are indicated by the wavy lines. The lettered brackets under the map indicate the DNA fragments used as hybridization probes. The horizontal bars show the positions and sizes of yeast inserts in xgtl1 and YEp24. Also shown are yeast inserts subcloned into the lacZ gene of the Bluescript expression vector. These constructs were used in the expression of fusion proteins in E. coli.
ABF I epitopes (lane 3). Expression of these polypeptides from cells containing pBS-15a, even in the absence of ABF I cross-reactive material (lanes 1 and 2). In extracts containing the vector without insert did not contain anti-ABF I cross-reactive polypeptide-specific to the anti-ABF I monoclonal antibodies described above were also examined [Fig. 4B]. As observed with the polyclonal antiserum, two of the monoclonal antibodies recognized polypeptides in extracts of cells carrying pBS-15a or pBS-15aΔ2 but not react with extracts from cells containing the vector alone. Interestingly, the pattern of bands recognized by each monoclonal antibody differed, suggesting that the antibodies react with different epitopes. Protein blot analysis of the truncated polypeptides showed that mAb 2E7E9 recognized polypeptides in each of the extracts [Fig. 4B, lanes 3–7], including the extract from cells expressing only 400 bp of yeast DNA at the 5' end of the 15a insert. In contrast, mAb 6C11G4 reacted with polypeptides specific to the pBS-15aΔ2 insert [lane 10] but failed to recognize the ABF I-related polypeptides in extracts from cells expressing the smaller 15a-derived inserts [lanes 11–14]. These data indicate that the individual monoclonal antibodies recognize different determinants on the bacterially produced protein and provide additional evidence that the yeast DNA insert contains authentic ABF I-coding sequences.

Overproduction of ABF I in yeast

To verify that the cloned yeast DNA inserts contained the ABF1 gene, we transformed yeast with the YEp24-3a and YEp24-4c clones and looked for overproduction of ABF I. Because of their specificity for ABF I, monoclonal antibodies were used as probes to determine the relative quantities of ABF I in crude yeast extracts. As shown in Figure 5A, extracts from transformants carrying either YEp24-3a [lane 2] or YEp24-4c [lane 3] contained levels of ABF I three- to fourfold higher than extracts from transformants carrying the vector alone [lane 1]. Both the 130- and 135-kD ABF I polypeptides were overexpressed, consistent with their being products of the same gene. Because of the large amounts of nonspecific DNA-binding proteins present in these crude extracts, it was not possible to correlate increased ARS1-specific binding activity with overproduction at this level of ABF1 expression.

To increase the level of ABF I overproduction, we fused the ABF I open reading frame (ORF) to the yeast GAL1 promoter and introduced the construct into yeast on a centromere-containing plasmid. The GAL1 promoter is strongly activated by growth in media containing galactose. Yeast transformants containing the GAL1/ABF1 fusion or the vector alone were grown on media containing raffinose (uninduced), followed by the addition of galactose. Immunoblot analysis of whole-cell extracts showed little detectable ABF I in strains grown in the absence of galactose induction [Fig. 5A, lane 5] or in the control strain carrying vector alone after galactose induction [data not shown]. However, >20-fold overproduction of ABF I was observed in the strain carrying the GAL1/ABF1 fusion, following galactose induction [lane 6]. We also quantitated the levels of ARS1-specific
Rhode et al.

**Figure 4.** Antibodies recognize bacterially produced β-gal/ABF I fusion proteins. Yeast DNA inserts (shown in Fig. 2) were subcloned into the lacZ gene of the expression vector Bluescript. Bacterial extracts were prepared from uninduced and IPTG-induced cultures, electrophoresed on a 10% SDS–polyacrylamide gel, and transferred to nitrocellulose. (A) Immunoblot probed with anti-ABF I polyclonal antisera. Extracts were prepared from cultures carrying Bluescript (lanes 1 and 2), pBS-15a (lanes 3 and 4), pBS-15Δ2 (lanes 5 and 6), pBS-15ΔK (lanes 7 and 8), pBS-15ΔA (lanes 9 and 10), pBS-15ΔA3 (lanes 11 and 12), and pBS-15ΔS (lanes 13 and 14). Extracts shown in lanes 1, 3, 5, 7, 9, 11, and 13 were from cultures grown in the absence of IPTG, extracts shown in lanes 2, 4, 8, 10, 12, and 14 were from cultures grown in the presence of 10 mM IPTG. (B) Immunoblots probed with monoclonal antibodies. Extracts were prepared from IPTG-induced cultures carrying Bluescript (lanes 1 and 8), pBS-15a (lanes 2 and 9), pBS-15Δ2 (lanes 3 and 10), pBS-15ΔK (lanes 4 and 11), pBS-15ΔA (lanes 5 and 12), pBS-15ΔA3 (lanes 6 and 13), or pBS-15ΔS (lanes 7 and 14). mAb 2E7E9 (lanes 1–7) and mAb 6C11G4 (lanes 8–12) were used as probes. A weak reaction between 2E7E9 and extracts from pBS-15ΔS was visible in the original blot. The positions of protein standards are shown.

Binding proteins in these extracts by gel retardation, as shown in Figure 5B. When the crude extracts were incubated with an ARSl –domain B fragment (lanes 2–5), we observed a single DNA–protein complex that comigrated with the complex formed with purified ABF I (lane 10). This binding activity was 20-fold greater in extracts from galactose-induced cultures of strains carrying the GAL1/ABF1 fusion (lanes 4 and 5) than in control extracts (lanes 2 and 3), consistent with the protein data. Competition with an oligonucleotide specific to the ABF I-binding site verified that this DNA-binding activity was ABF I (lanes 6–9). The results provide proof that the cloned DNA contains the ABF1 gene.

**Analysis of the ABF1 sequence**

We determined the nucleotide sequence of 3 kb, starting from the left-most SpeI site shown in Figure 2. The sequence spans the region coding for the 2.6-kb ABF1 gene defined above and part of the 1.1-kb gene (Fig. 6). Sequence numbering begins at the SpeI site. The sequence contains a single, large ORF (nucleotides 190–2488) with a 5' end that coincides with the 5' limit of the ABF1 gene defined by Northern analysis. Direct expression of the BglII–SpeI fragment in E. coli by fusion to an E. coli promoter results in production of an ABF I-related protein that comigrates with the 130-kD polypeptide of yeast ABF I (data not shown), indicating that this sequence is sufficient to code for ABF I. The first ATG codon at nucleotide 295 is preceded by an A at the –3 position that is conserved and a G at +4 that is partially conserved in translation start sites (Kozak 1983). Therefore, we propose that this ATG serves as the initiator for ABF I. No conserved splice site sequences were found in the DNA sequence. The termination codon TAG (nucleotide 2489) is immediately upstream of a region of A/T-rich DNA, possibly involved in mRNA termination. Consistent with the position of the 1.1-kb gene, another ORF of the opposite orientation was observed 3' of the ABF1 gene. This ORF has a termination codon at nucleotide 2666 and extends beyond the end of the sequenced DNA.
The ABFI gene codes for a 731-amino-acid protein with a predicted molecular weight of 81,662. This is considerably less than the molecular weight of 135,000 estimated by SDS–polyacrylamide gel electrophoresis. We also observed discrepancies between the predicted and observed molecular weights of the E. coli-produced β-gal/ABFI fusion proteins. For example, sequence analysis predicted that pBS-15aAR and pBS-15aAK constructs could encode truncated β-gal/ABFI fusion products with molecular masses of 87 and 69 kD, respectively. The molecular masses observed in Figure 4A were 120 and 87 kD, respectively. Therefore, it seems unlikely that the discrepancy seen with ABFI is attributable to either post-translational modification occurring in yeast or to the conformation of the intact protein. Similar anomalous migration on SDS gels has been reported for a number of other yeast DNA-binding proteins (Hope and Struhl 1985; Shore and Nasmyth 1987; Wiederrecht et al. 1988; Moye-Rowley et al. 1989).

In comparing the sequence with available protein and DNA data bases, we found no strong homologies. Inspection of the ABFI amino acid sequence did reveal a zinc finger motif, H-X₁₁-H-X₁₁-C-X₆₋₄-C [residues 57–71], that matches the H-X₁₁₋₄-H-X₁₁₋₄-C-X₆₋₄-C consensus [Berg 1986; Klug and Rhodes 1987]. This zinc finger motif is similar in size to the CCHC box found in retroviral gag-encoded nucleic acid-binding proteins. The role that this region plays in DNA binding remains to be determined. The protein contains no obvious regions similar to the helix–turn–helix motifs or leucine zippers characteristic of some DNA-binding proteins [Pabo and Sauer 1984; Landschulz et al. 1988].

The codon usage [59 of 61 possible codons used] shows none of the bias observed in highly expressed yeast genes [Bennetzen and Hall 1981], consistent with the finding that the ABFI gene is expressed at low levels. Because of a high proportion of aspartic acid residues [11.4%], ABFI is very acidic with a net charge of −60 at neutral pH and a calculated pI of 4.74. The distribution of charge is not random. The amino-terminal region [residues 1–75] is basic [net charge +5], as is a small region in the middle of the protein [residues 412–435, charge +10]. The acidic amino acids predominate at the carboxyl terminus [residues 552–731, charge −35] and between residues 116 and 268 [charge −28]. ABFI is also rich in asparagine [14.8%] and serine [9.8%]. Four regions of the protein contain small clusters of asparagine residues.

The ABFI gene is essential for viability

To determine whether the ABFI and 1.1-kb genes are essential for yeast growth, we disrupted the chromosomal copies of each of these genes. First, a series of insertion mutations in the 15a fragment were generated in E. coli using the Tn3–LEU2 shuttle mutagenesis system developed by Seifert et al. [1986a,b]. Figure 7A shows the insertion sites of the yeast LEU2 gene for five of the mutants isolated. Two of the mutants contain insertions disrupting the ABFI gene; the remainder contain insertions in the 1.1-kb gene. Second, insertion mutations in the ABFI gene were introduced into the genome of a homoyzgous leu2 diploid strain. Southern analysis of DNA isolated from Leu+ transformants indicated that one copy of the chromosomal ABFI gene contained the
Rhode et al.

LEU2 insertion. Figure 7B shows a Southern blot of BglII-digested DNA hybridized to the 15a insert. Two BglII fragments (3.3 and 4.1 kb) were detected in the undisrupted diploid strain, consistent with the restriction analysis of the cloned inserts. Strains transformed with either of the two ABF1::LEU2 disruptions contained one intact chromosomal copy of the ABF1 gene and one copy containing a 7.6-kb BglII fragment indicative of the 3.5-kb BglII-LEU2 insertion [lanes 2 and 3]. These strains were sporulated and subjected to tetrad analysis. Almost all of the tetrads (23 of 25 for the abfl::LEU2-1 disruption and 21 of 22 for the abfl::LEU2-3 disruption) gave only two viable spores; the remainder gave only one viable spore. The nonviable spores failed to grow beyond the two- to four-cell stage. The two viable spores were Leu−. These results indicate that ABF1 is an essential, single-copy gene.

To test further the role of ABF1 in yeast growth, we constructed strains from which we could remove the functional copy of the ABF1 gene during vegetative growth. A ura3/ura3 diploid strain heterozygous for the abfl::LEU2 disruption was transformed to Ura+ with a plasmid carrying the URA3 and ABF1 genes [YEp24-4c]. Following sporulation, four viable spores (two Leu+ and two Leu−) were observed in each tetrad dissected, indicating that the plasmid-borne ABF1 gene could complement the abfl::LEU2 disruption. When these strains were replica-plated on medium containing 5-fluoro-orotic acid (5-FOA) to select against the URA3-bearing plasmid, growth was observed only in Leu+ strains, which presumably carry an intact ABF1 gene. No viable Leu− cells were observed on 5-FOA plates, indicating that the strains carrying the abfl::LEU2 disruption could not form colonies in the absence of the plasmid carrying ABF1. These results confirm that the ABF1 gene is essential for vegetative growth, and they suggest...
DNA-binding protein that binds to functionally important regions at several yeast ARSs (Sweder et al. 1988). In the current work, we used antisera directed against ABF I to screen a yeast genomic αgt11 library to isolate the gene encoding this protein. The identity of the cloned gene was confirmed by two methods. First, two monoclonal antibodies specific to ABF I were found to react to clone-derived polypeptides produced in E. coli. Further experiments showed that the antigenic determinants for these antibodies map to opposite ends of the protein.

Second, controlled expression of the cloned gene in yeast resulted in overproduction of the ABF I protein and DNA-binding activity.

**ABF I contains a zinc finger motif**

The ABF I gene codes for a 731-amino-acid protein that shows no strong homologies to sequences present in available protein or DNA data bases. ABF I does contain a region that matches the zinc finger DNA-binding motif, however. These metal-binding, nucleic acid-binding domains were first described for the Xenopus transcription factor TFIIIA (Brown et al. 1985; Miller et al. 1985) and were subsequently found in a number of transcriptional activators and DNA-binding proteins (for review, see Berg 1986; Klug and Rhodes 1987; Evans and Hollenberg 1988). Zinc fingers have been analyzed by a wide variety of methods leading to the proposal that two pairs of Cys and/or His residues in each finger coordinate a zinc(II) ion, and the resulting metalloprotein structure interacts with the DNA. Two major classes of zinc fingers have been well characterized. The Cys₂/His₂ finger [consensus, C-X₄,C-X₃,F-X₃,L-X₂-H-X₂-H] is found in TFIIIA, the yeast transcription factors ADR1 and SWI5, the human transcription factor SP1, and proteins encoded by various Drosophila developmental genes. The Cys₂/Cys₂ finger [C-X₂,C-X₃,F-X₃,L-X₂-H-X₂-H] is found in the steroid receptor superfamily and the yeast transcription factors GAL4, PPR1, ARGR11, and LAC9. As compared to other yeast DNA-binding proteins, ABF I is novel in that its zinc finger motif belongs to a third class found in the single-stranded nucleic acid-binding proteins, encoded by retroviral gag genes and the T4 phage gene 32 [Berg 1986; Giedroc et al. 1986]. This class of ‘stubbier’ fingers contains only four to five residues in the loop between the Cys and/or His pairs. Studies have shown that this motif is capable of binding zinc (Green and Berg 1989); however, site-specific DNA binding remains to be demonstrated. Further studies to localize the ABF I DNA-binding domain should determine whether this motif plays a role in DNA binding.

In examining the ABF I amino acid sequence, we found several features that are shared with other yeast DNA-binding proteins. First, ABF I contains several regions that are high in acidic residues. These regions may be functionally analogous to the acidic activation sequences found in transcription factors such as GAL4 and GCN4 (Hope and Struhl 1986; Ma and Ptashne 1987). Current models of transcriptional activation propose an interaction between these regions and the transcription...
In the proper context, the B element can also function as an upstream activation sequence [UAS] element [Brand et al. 1987]. Thus, these studies demonstrated that ABF I can act in transcriptional activation and repression, as well as stimulating replication.

Since the initial characterization of ABF I, a number of independently identified proteins (OBFl, GF1, SUF, TAF, and Y protein) have been found to bind to DNA sites conforming to the ABF I consensus [RTCPRN,ACG]. OBFl was shown to bind telomeric ARSs [ARS120, ARS131C, and ARS131S] and the single-copy ARS121 [Eisenberg et al. 1988; Francesconi and Eisenberg 1989]. Linker substitution mutagenesis at ARS121 demonstrated that the OBFl-binding site was required for optimal ARS activity [Walker et al. 1989]. Other factors were identified on the basis of their binding to various promoter elements. GF1 binds to the 5'-flanking sequences in a number of nuclear genes encoding imported mitochondrial proteins [Dorsman et al. 1988]; SUF and TAF bind to promoters of genes encoding ribosomal proteins S33 and L3 [TMC1 gene], respectively [Vignais et al. 1987; Hamil et al. 1988]; and Y protein binds to the promoter of the phosphoglycerate kinase gene [Stanway et al. 1987]. In several cases, these binding sites were located in regions that regulated transcription. For example, Hamil et al. (1988) showed that the TAF-binding site could act as a UAS element in the TCM1 promoter or in a heterologous promoter that lacked a functional UAS. Point mutations at this site that reduced TAF binding also decreased UAS activity in vivo.

Several lines of evidence suggest that these factors are the same protein. The mobilities in SDS gels suggest that ABF I, OBFl, and TAF are in the same 125- to 150-kD range [Diffley and Stillman 1988; Hamil et al. 1988; Sweeder et al. 1988; Francesconi and Eisenberg 1989]. Competitive binding and DNase I footprinting analyses established that ABF I [SBF-B], OBFl, and GF1 bind to identical sites at ARS1 and HMR E [Diffley and Stillman 1988; Dorsman et al. 1988; Sweeder et al. 1988; Francesconi and Eisenberg 1989]. Recently, partially purified GF1 was also shown to specifically bind SUF- and TAF-binding sites [Dorsman et al. 1989]. Furthermore, linker substitution or point mutations in the binding sites cause a concomitant loss of ABF I, OBFl, GF1, or TAF binding and biological activity only when the mutations fall within the ABF I consensus [Hamil et al. 1988; Kimmerly et al. 1988; Dorsman et al. 1989; Sweeder et al. 1988; Francesconi and Eisenberg 1989]. These data are consistent with a role of ABF I in regulating transcription and/or replication at these sites. However, it cannot be ruled out that other proteins with ABF I-like binding properties mediate some of these functions. Direct proof of ABF I function will require characterization of conditional abf1 mutants. In vivo.

Several lines of evidence suggest that ABF I is rich in asparagine residues. Similar amino acid compositions have been reported for RAP I and HSTF [Shore and Nasmyth 1987; Wiederrecht et al. 1988]. The asparagine residues are unevenly distributed such that four small asparagine clusters are apparent in ABF I. Secondary structure analysis based on the Chou-Fasman algorithm [Chou and Fasman 1977] predicts strong turn potential at these clusters, suggesting that these regions have unusual conformational characteristics. No functional role for these regions has been defined.

Roles of ABF I in transcription and replication

Recent indications suggest that ABF I binds to a variety of genetic elements that serve diverse functions in regulating DNA replication and gene expression. The fact that ABF I was determined to be essential for growth supports this possibility. Deletion analyses have shown that the regions containing the ABF I-binding site stimulate [but are not essential for] the activity of ARS1 and the ARS at HMR E [Abraham et al. 1984; Celniker et al. 1984; Brand et al. 1985, 1987; Srienc et al. 1985; Snyder et al. 1987; Diffley and Stillman 1988; Strich et al. 1986]. In addition, the ABF I-binding site at HMR E [B element] has been shown to be important in silencer activity and to possess a segregation function [Brand et al. 1985, 1987; Kimmerly and Rine 1987; Kimmerly et al. 1988].
regions of genes transcribed by RNA polymerase II. These included genes encoding proteins involved in transcription [subunits of RNA polymerase I and III], translation [ribosomal proteins, aa-tRNA synthetases], glycolysis [PGK, ENO2, TDH3], respiration [mitochondrial proteins], cell structure (β-tubulin), and cell-cycle progression (CDC25, CDC16, calmodulin). In many respects, ABF I-binding sites are similar to those found for the silencer DNA-binding protein, RAP I [repressor–activator protein] [also called GRFl and TUF] [Huet and Sentenac 1987; Shore and Nasmyth 1987; Shore et al. 1987; Buchman et al. 1988a,b; Kimmerly et al. 1988]. This protein acts as a transcriptional activator and repressor, coordinative expression of as many as 45 genes that code for proteins involved in cell growth [Buchman et al. 1988b; Capieaux et al. 1989]. Both ABF I- and RAP I-binding sites are found in the same promoters of several genes. On the basis of these observations, it is tempting to speculate that ABF I and RAP I coordinately regulate transcription of growth-related genes and initiation of replication in response to growth conditions. The dual role of a protein in transcriptional control and replication is not unique. In mammalian cells, several proteins have been shown to regulate transcription, as well as stimulate viral replication [Nagata et al. 1982; Rawlins et al. 1984; Jones et al. 1987; Santoro et al. 1988].

Possible mechanisms of ABF I action

Several models could explain the diverse roles of ABF I in regulating transcription and replication. First, ABF I may exist in several functionally distinct states. In crude yeast extracts, ABF I is present in two forms, both capable of specifically binding DNA. We have shown that both are products of the same gene. Bacterially produced ABF I comigrates with the 130-kD form [data not shown], suggesting that protein modification in yeast results in the 135-kD species. The nature of this change is not known, though modifications, such as phosphorylation, have been observed to affect the mobility of other DNA-binding proteins [Hoeffler et al. 1988; Sorger and Pelham 1988]. The different forms of ABF I may mediate different functions or represent different functional states of the protein. Recent results suggest that growth in different carbon sources affects the relative abundance of the two forms of ABF I [K. Oegema, unpublished]. This change may be analogous to the phosphorylation and activation of the heat shock transcription factor observed after heat shock [Sorger et al. 1987; Sorger and Pelham 1988]. Second, specific protein–protein interactions with other DNA-binding factors [and possibly other proteins] may dictate ABF I function. The context of the ABF I-binding site is clearly important. For example, in combination with a RAP I-binding site at HMR E, the ABF I-binding site is involved in repressing transcription. However, either binding site alone acts as a UAS in a heterologous promoter. Similar observations have been made for the DNA-binding protein, MCM1 [also called PRTF/GRM] [Jarvis et al. 1989; Passmore et al. 1989]. The MCM1-binding site can function as a generic UAS, as a cell-type-specific UAS in conjunction with an α1-binding site, as a cell-type-specific repressor element with an α2-binding site (Bender and Sprague 1987; Jarvis et al. 1988; Keleher et al. 1988). Interaction between DNA-binding proteins could prevent or enhance direct interactions with transcription [or replication] machinery or alter the local chromatin structure, making transcription more or less likely.

ABF I may play a role in the higher order structure of the genome. Because ABF I-binding sites have been found in regions with no apparent ARS or transcriptional regulatory elements [Buchman et al. 1988a], it has been proposed that these sites serve as boundaries between adjacent regions of the genome. The fact that the ABF I-binding site plays a role in the segregation of HMR E–ARS plasmids also suggests that ABF I is associated with a structural component of the nucleus [i.e., the nuclear scaffold]. However, any such association must be weak, as Grasser and co-workers recently found that DNA fragments containing ABF I-binding sites did not bind to isolated nuclear scaffold nor was ABF I-binding activity detected in scaffold protein preparations [Amati and Gasser 1988; Hofmann et al. 1989].

In regulating ARS activity, ABF I-binding sites may be functionally analogous to transcriptional enhancers found adjacent to eukaryotic viral origins. It has been proposed that these regions may stimulate transcription, activating the origin [DePamphilis 1988]. Alternatively, factors binding to these enhancers may [directly or indirectly] facilitate interactions between the origin and proteins involved in initiation of replication. Because yeast initiator proteins have not been characterized, it will be interesting to examine proteins that interact with ABF I.

Materials and methods

Strains and media

Bacterial strains TG-1 [Amersham] and DH5αF’ [BRL] were used in cloning, overproduction of ABF I fusion protein, and isolation of plasmids used in sequencing. The λgt11 genomic library was grown in Y1090 [Young and Davis 1983]. E. coli strains RDP146 and NS21145m were used in the Tn3 insertion mutagenesis [Seifert et al. 1986a].

ABF I was purified from a protease-deficient strain PEP4D [MATa / α his1 / + trp1 / + pro1-126 pro1-126 pep4-3 / pep4-3 pbr 1-1122 / pbr1-1122 can1 / can1 [Jones 1977]]. All other experiments were carried out in the yeast strain SEY6210.5 [MATα / a leu2-3, 112 / leu2-3, 112 ura3-52 / ura3-52 his3-Δ200 / his3-Δ200 tsp1-901 / tsp1-901 lys2-801 / + ade2-101 / + suc2-91 / + mel1 / mel1 [S. Emr, unpublished]] or haploid derivatives of it, except the galactose induction of the GAL1/ABFI fusion, which was carried out in YM603 [a ura3-52 lys2-8D1 met his3 ade2-101 reg1-501 (Celniker et al. 1984)].

Bacterial media were made as described by Miller [1972]. Yeast cells were grown on media [YPD, yeast extract, peptone, dextrose, SD, synthetic minimal] described by Sherman et al. [1979]. Diploid strains were sporulated on a solid sporulation media. Counter-selection against plasmids containing the URA3 gene was performed in media containing 1 mg/ml 5-FOA [Boeke et al. 1984]. For galactose induction of GAL1/ABFI gene fusions, YM603/p68g-3a [see below] strains were grown in YP media containing 2% raffinose to AS95 = 0.8. Glucose-free ga-
lactose was added to a final concentration of 2%, and cells were harvested 4 hr later.

DNA manipulations

λDNA was purified using Lambda Sorb [Promega Biotec]. DNA fragments were cloned into Bluescript SK (−) [Stratagene] by standard techniques [Maniatis et al. 1982]. Insertion of the EcoRI yeast DNA fragment from Agt11-15a into the EcoRI site of the Bluescript polylinker resulted in fusion of the ABF 1 ORF with the first 37 codons of lacZ. This plasmid is referred to as pBS-15a (the insert cloned in the opposite orientation is referred to as pBS-15a‘). Nestled deletions used in DNA sequencing were constructed from pBS-15a and pBS-15a‘ by the method of Henikoff et al. [1984]. The GAL1/ABF1 fusion was made by inserting the 3.2-kb BglII fragment of Yep24-3a into the BamHI site of PSEYC68-GAL [Emr et al. 1986] to form p68g-3a. To generate a target vector for Tn3 mutagenesis, we subcloned the 4.4-kb PstI–HindIII fragment of pBS-15a‘ into PstI–HindIII-linearized pHS55 (Seifert et al. 1986a), forming pHS55-15a.

The ABF1 3′ deletion series shown in Figure 2 was generated by subcloning fragments of pBS-15a and pHS55-15a:: Tn3–LEU2 into the lacZ gene of Bluescript as follows: The 425-bp EcoRI–StuI fragment from pBS-15a was inserted into EcoRI/HindII-digested Bluescript to give pBS-15aAΔS, the 1275-bp EcoRI–KpnI fragment from pBS-15a was inserted into EcoRI/KpnI-digested Bluescript to give pBS-15aAΔK, and the 1729-bp EcoRI–EcoRV fragment from pBS-15a was inserted into EcoRI–EcoRV-digested Bluescript to give pBS-15aAΔR. We used the SalI site, 58 bp from the end of the Tn3–LEU2 mini transposon [Seifert et al. 1986a], to subclone fragments of the ABF1 gene from the pHS55-15a:: Tn3–LEU2 insertion mutants. The 735-bp EcoRI–SalI fragment from pHS55-15a:: Tn3–LEU2-3 was inserted into EcoRI/SalI-digested Bluescript to give pBS-15aAΔ3, and the 2540-bp EcoRI–SalI fragment from pHS55-15a:: Tn3–LEU2-2 was inserted into EcoRI/SalI-digested Bluescript to give pBS-15aAΔ2.

Preparation and use of anti-ABF1 antibodies

ABF 1 was purified as described previously [Sweder et al. 1988]. A 4-lb female New Zealand rabbit was immunized with 50 μg of ABF 1. Two 25-μg boosts were given 8 and 10 weeks later. Serum was obtained 9–15 days after the final boost.

Mouse monoclonal antibodies against ABF 1 were raised in RBF/Dn mice. A mouse was given three 10-μg injections of ABF 1 at 2-week intervals. A final 5-μg boost was given 2 weeks later. Three days after the final boost, the mouse was sacrificed and the spleen cells were fused with HL-1 myeloma cells. Antibody-producing hybridomas were selected on AAT medium [adenine, aminopterin, thymidine (Taggart and Samloff 1983)] containing the yeast LEU2 gene. The resulting transconjugate contains a gene encoding Tn3 transposase. The resulting derivative was tested for resistance to 10 mM IPTG to A595 = 1.0 in 5 ml of Luria broth containing ampicillin [100 μg/ml]. The cultures were harvested (6000 g, 5 min), and the cell pellets were lysed in 300 μl of SDS–gel loading buffer. Following incubation for 10 min at 90°C, the lysates were passed twice through a 25-gauge needle to reduce viscosity. Fifty microliters of lysate was loaded onto a 10% SDS–polyacrylamide gel. The proteins were separated by electrophoresis and transferred to a nitrocellulose membrane using a trans-blott apparatus [Bio-Rad]. Filters were blocked by incubating for 1 hr in 5% non-fat dry milk in TBS. The filters were then incubated for 1 hr with anti-ABF 1 polyclonal antiserum or monoclonal antibodies in 5% non-fat dry milk/TBS. Following three washes in TBS, the filters were incubated for 1 hr with an alkaline phosphatase-conjugated secondary antibody, washed three times with TBS, and incubated for 10–30 min with the NBT/BCIP developing reagent. The developing reagent consists of 15 mg NBT (dissolved in 1 ml 70% dimethylformamide) and 7.5 mg BCIP (dissolved in 1 ml dimethylformamide) per 50 ml of 0.1 M NaHCO3 [pH 9.8], 1 mM MgCl2.

Yeast extracts were prepared from 100-ml cultures. After centrifuging [3000 g, 10 min], cells were resuspended in 0.2 ml of buffer A [20 mM Hepes (pH 7.4), 10% glycerol, 1 mM 2-mercaptoethanol, 1 mM EDTA, 1 mM phenylmethylsulfonyl fluoride (PMSF)] containing 1 mM KCl. Lysis was achieved by vortexing the cells three times for 30 sec in the presence of 0.3 grams of glass beads. The lysate was microfuged for 5 min and the supernatant saved. An additional 0.2 ml of buffer A containing 1 mM KCl was added to the pellet and the lysate was vortexed three times for 30 sec. After centrifugation, the supernatants were combined. In some cases, extracts were desalted against buffer A containing 50 mM KCl, using Centricron-10 concentrators. Fifty micromgrams of extract was electrophoresed on 7.5% SDS–polyacrylamide gels, and immunoblotting was performed with monoclonal antibodies, as described above.

Screening of a kg11 library

Antibodies that recognized E. coli proteins were removed from the polyclonal antiserum, as described in Johnson et al. (1985). A kg11 library of genomic yeast DNA fragments was screened on nitrocellulose filters, as described in Johnson et al. (1985), except that all washes were done in TTBS [0.1% Tween 20 in Tris-buffered saline: 50 mM Tris-HCl (pH 7.6), 150 mM NaCl, 0.1% Tween 20, 0.02% sodium azide]. The overnight culture was diluted into medium without antibiotics and mated with the strain NS2114Sm. This
Hybridization procedures

A yeast genomic library in the plasmid YEp24 [Carlson and Botstein 1982] was screened by hybridization to the \( \lambda gt11-15a \) insert, using colony hybridization procedures previously [Hanahan and Meselson 1980; Johnson et al. 1985]. Approximately 10\(^8\) E. coli transformants were spread on 1-agar plates (150 mm) containing 100 \( \mu \text{g/ml} \) ampicillin and were incubated at 37°C for overnight amplification. Cell lysis, denaturation at 85°C for 5 min in 0.2 N NaOH and 0.2 mM EDTA, allowed for easier isolation of plasmids containing inserts. Restriction analysis of plasmid DNA from 24 isolates revealed that the sites of Tn3 insertion were distributed throughout the 15a insert. DNA from five different insertion mutants was digested with NotI, and the 15a::Tn3::LEU2 fragments were isolated by electroelution. These were used in the fragment-mediated transformation procedure described by Rothstein (1983) to disrupt the \( ABF1 \) gene in the yeast strain SEY6210.5.

Other methods

Double-stranded plasmid DNA sequencing was performed by the dideoxy chain termination method (Sanger et al. 1977), using \( \alpha \)-\( ^35 \)S-labeled dATP and modified T7 DNA polymerase [Feinberg and Vogelstein 1983]. Riboprobes were synthesized from PstI-linearized pBS-15a and pBS-15a' using T7 RNA polymerase and \( [\alpha-^35 \text{P}] \)UTP, according to methods provided by the supplier (Stratagene).

Acknowledgments

We thank Carolyn Cabanski for technical assistance. We also thank Caltech’s Monoclonal Antibody Facility for generating the monoclonal antibodies used in this study and Dr. Bruce Birren for providing yeast chromosomal DNA blots. We are grateful to Drs. Colin Gordon, Karen Sitney, Scott Moyer-Rowley, Greg Wiederecht, and Keith Harshman for providing libraries, plasmids, and strains and for helpful advice. We thank Drs. Rati Verma, Scott Moyer-Rowley, and Greg Wiederecht for critically reading the manuscript. This work was supported by a grant from the National Institutes of Health (GM-25508), a Procter and Gamble postdoctoral fellowship to P.R.R., and the National Institutes of Health [BRSG RR07003].

References

Abraham, J., K.A. Nasmyth, J.N. Strathern, A.J.S. Klar, and J.B. Hicks. 1984. Regulation of mating type information in yeast. J. Mol. Biol. 176: 307–331.
Amati, B.D. and S.M. Gasser. 1988. Chromosomal ARS and CEN elements bind specifically to the yeast nuclear scaffold. Cell 54: 967–978.
Bender, A. and G.F. Sprague, Jr. 1987. MATa 1 protein, a yeast transcription activator, binds synergistically with a second protein to a set of cell-type-specific genes. Cell 50: 681–691.
Bennetzen, J. and B. Hall. 1981. Codon selection in yeast. J. Biol. Chem. 257: 3026–3031.
Berg, J.M. 1986. Potential metal-binding domains in nucleic acid binding proteins. Science 232: 485–487.
Bocke, J.D., F. LaCroute, and G.R. Fink. 1984. A positive selection for mutants lacking orotidine-5’-phosphate decarboxylase activity in yeast: 5-Fluoro- orotic acid resistance. Mol. Gen. Genet. 197: 345–346.
Brand, A.H., L. Breeden, J. Abraham, R. Sten glanz, and K. Nasmyth. 1985. Characterization of a ‘silencer’ in yeast: A DNA sequence with properties opposite to those of a transcriptional enhancer. Cell 41: 41–48.
Brand, A.H., G. Micklem, and K. Nasmyth. 1987. A yeast silencer contains sequences that can promote autonomous plasmid replication and transcriptional activation. Cell 51: 709–719.
Broach, J.R., Y.-Y. Li, J. Feldman, M. Jayaram, J. Abraham, K.A. Nasmyth, and J.B. Hicks. 1983. Localization and sequence analysis of yeast origins of DNA replication. Cold Spring Harbor Symp. Quant. Biol. 47: 1165–1173.
Brown, R.S., C. Somder, and P. Argos. 1985. The primary structure of transcription factor TFIIIA has 12 consecutive repeats. FEBS Lett. 186: 271–274.
Buchach, A.R., N.F. Lue, and R.D. Kornberg. 1988b. Connections between transcriptional activators, silencers and telomeres as revealed by functional analysis of a yeast DNA binding protein. Mol. Cell. Biol. 8: 5086–5099.
Capiaux, E., M.-L. Vignais, A. Sentenac, and A. Goffeau. 1989. The yeast H+–ATPase gene is controlled by the promoter binding factor TUF. J. Biol. Chem. 264: 7437–7446.
Carlson, M. and D. Botstein. 1982. Two differentially regulated mRNAs with different 5’ ends encode secreted and intracellular forms of yeast invertase. Cell 28: 145–154.
Celniker, S.E., K. Sweder, F. Sriend, J.E. Bailey, and J.L. Campbell. 1984. Deletion mutations affecting autonomously rep-
Rhode et al.

licating sequence ARS1 of Saccharomyces cerevisiae. Mol. Cell. Biol. 4: 2455–2466.
Chou, P.Y. and G.P. Fasman. 1977. β-turns in proteins. J. Mol. Biol. 115: 135–175.
DePamphilis, M.L. 1988. Transcriptional elements as components of eukaryotic origins of DNA replication. Cell 52: 635–638.
Diffley, J.F.X. and B. Stillman. 1988. Purification of a yeast protein that binds to origins of DNA replication and a transcriptional silencer. Proc. Natl. Acad. Sci. 85: 2120–2124.
Dorsman, J.C., W.C. vanHeeswijk, and L.A. Grivell. 1988. Identification of two factors which bind to the upstream sequences of a number of nuclear genes coding for mitochondrial proteins and to genetic elements important for cell division in yeast. Nucleic Acids Res. 16: 7287–7301.
Dorsman, J.C., M.M. Doorenbosch, C.T.C. Maurer, J.H. deWinde, W.H. Mager, R.J. Planta, and L.A. Grivell. 1989. An ARS/silencer binding factor also activates two ribosomal protein genes in yeast. Nucleic Acids Res. 17: 4917–4923.
Eisenberg, S., C. Civalier, and B.K. Tye. 1988. Specific interaction between a Saccharomyces cerevisiae protein and a DNA element associated with certain autonomously replicating sequences. Proc. Natl. Acad. Sci. 85: 743–746.
Emr, S., A. Vassarotti, J. Garrett, B. Geller, M. Takeda, and M. Douglas. 1986. The amino terminus of the yeast Fl-ATPase β-subunit functions as a mitochondrial import signal. J. Biol. Chem. 261: 523–533.
Evans, R.M. and S.M. Hollenberg. 1988. Zinc fingers: Gilt by association. Cell 52: 1–3.
Feldman, J.B., J.B. Hicks, and J.R. Broach. 1984. Identification of a cell-type specific repressor a2 acts cooperatively with a non-cell-type-specific protein. Cell 53: 927–936.
Kimmerly, W.J. and J. Rine. 1987. Replication and segregation of plasmids containing cis-acting regulatory sites of silent mating-type genes in Saccharomyces cerevisiae are controlled by the SIR genes. Mol. Cell. Biol. 7: 4225–4237.
Kimmerly, W., A. Buchman, R. Kornberg, and J. Rine. 1988. Roles of two DNA-binding factors in replication, segregation and transcriptional repression mediated by a yeast silencer. EMBO J. 7: 2241–2253.
Klug, A. and D. Rhodes. 1987. Zinc fingers: A novel protein motif for nucleic acid recognition. Trends Biochem. Sci. 12: 464–469.
Koshland, D., J.C. Kent, and L.H. Hartwell. 1985. Genetic analysis of the mitotic transmission of minichromosomes. Cell 40: 393–403.
Kozak, M. 1983. Comparison of initiation of protein synthesis in prokaryotes, eukaryotes and organelles. Microbiol. Rev. 47: 1–45.
Kraft, R., J. Tardiff, K.S. Krauter, and L.A. Leinwand. 1988. Using mini-prep plasmid DNA for sequencing double stranded templates with Sequenase. BioTechniques 6: 544–547.
Lai, E., B.W. Birren, S.M. Clark, M.I. Simon, and L. Hood. 1989. Pulsed field gel electrophoresis. BioTechniques 7: 34–42.
Landshultz, W.H., P.F. Johnson, and S.L. McKnight. 1988. The leucine zipper: A hypothetical structure common to a new class of DNA binding proteins. Science 240: 1759–1764.
Ma, J. and M. Ptashne. 1987. A new class of yeast transcriptional activators. Cell 51: 113–119.
Mamiani, T., E. Fritsch, and J. Sambrook. 1982. Molecular cloning: A laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.
Miller, J.H. 1972. Experiments in molecular cloning. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.
Miller, J., A.D. McLachlan, and A. Klug. 1985. Repetitive zinc-binding domains in the protein transcription factor IIIA from Xenopus oocytes. EMBO J. 4: 1609–1614.
Mitchell, P.J. and R. Tjian. 1989. Transcriptional regulation in mammalian cells by sequence-specific DNA binding proteins. Science 245: 371–378.

1938 GENES & DEVELOPMENT
Yeast gene for ARS-binding factor I

Moye-Rowley, W.S., K.D. Harshman, and C.S. Parker. 1989. Yeast YAPI encodes a novel form of the jau family of transcriptional activator proteins. *Genes Dev.* 3: 283–292.

Nagata, K., R.A. Guggenheimer, T. Enomoto, J.H. Lichy, and J. Hurwitz. 1982. Adenovirus DNA replication in vitro: Identification of a host factor that stimulates synthesis of the preterminal protein-dCMP complex. *Proc. Natl. Acad. Sci.* 79: 6438–6442.

Newlont, C.S. 1989. Yeast chromosome replication and segregation. *Microbiol. Rev.* 52: 568–510.

Palzkill, T.G., S.G. Oliver, and C.S. Newlont. 1986. DNA sequence analysis of ARS elements from chromosome III of *Saccharomyces cerevisiae*: Identification of a new conserved sequence. *Nucleic Acids Res.* 14: 6247–6264.

Palzkill, T.G. and C.S. Newlont. 1988. A yeast replication origin consists of multiple copies of a small conserved sequence. *Cell* 43: 441–450.

Passmore, S., R. Elble, and B.K. Tye. 1989. A protein involved in minichromosome maintenance in yeast binds a transcriptional enhancer conserved in eukaryotes. *Genes Dev.* 3: 921–935.

Rawlins, D.R., P.J. Rosenfeld, R.J. Wides, M.D. Challberg, and T.J. Kelly, Jr. 1984. Structure and function of the adenovirus origin or replication. *Cell* 37: 309–319.

Rothstein, R. 1983. One-step disruption in yeast. *Methods Enzymol.* 101: 202–211.

Sanger, R., S. Nicklen, and A. Coulson. 1977. DNA sequencing with chain-terminating inhibitors. *Proc. Natl. Acad. Sci.* 74: 5463–5467.

Santoro, C., N. Mermod, P.C. Andrew, and R. Tjian. 1988. A family of human CCAAT-block-binding proteins active in transcription and DNA replication: Cloning and expression of multiple cDNAs. *Nature* 334: 218–224.

Seifert, H.S., M. So, and F. Heffron. 1986a. Shuttle mutagenesis: A method of introducing transposons into transformable organisms. In *Genetic engineering: Principle and methods* (ed. J.K. Setlow and A. Hollaender). Plenum Press, New York.

Seifert, H.S., E. Chen, M. So, and F. Heffron. 1986b. Shuttle mutagenesis: A method of transposon mutagenesis for *Saccharomyces cerevisiae*. *Proc. Natl. Acad. Sci.* 83: 735–739.

Sherman, F., G.R. Fink, and J. Hinks. 1979. *Methods in yeast genetics*. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

Shore, D. and K. Nasmyth. 1987. Purification and cloning of a DNA binding protein from yeast that binds to both silencer and activator elements. *Cell* 51: 721–732.

Shore, D., D.J. Stillman, A.H. Brand, and K.A. Nasmyth. 1987. Identification of silencer binding proteins from yeast: Possible roles in SIR control and DNA replication. *EMBO J.* 6: 461–467.

Snyder, M., A.R. Buchman, and R.W. Davis. 1987. Bent DNA at a yeast autonomously replicating sequence. *Nature* 324: 87–89.

Sorger, P.K., M.J. Lewis, and H.R.B. Pelham. 1987. Heat shock factor is regulated differently in yeast and HeLa cells. *Nature* 329: 81–84.

Sorger, P.K. and H.R.B. Pelham. 1988. Yeast heat shock factor is an essential DNA-binding protein that exhibits temperature-dependent phosphorylation. *Cell* 54: 855–864.

Srienc, F., J.E. Bailey, and J.L. Campbell. 1985. Effect of ARS1 mutations on chromosome stability in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* 5: 1676–1684.

Stanway, C., J. Mellor, J.E. Ogden, A.J. Kingsman, and S.M. Kingsman. 1987. The UAS of the yeast PGK gene contains functionally distinct domains. *Nucleic Acids Res.* 15: 6855–6873.

Stinchcomb, D.T., C. Mann, E. Selker, and R.W. Davis. 1981. DNA sequences that allow the replication and segregation of yeast chromosomes. *ICN-UCLA Symp.* 22: 473–488.

Strich, R.M., M. Wootner, and J.F. Scott. 1986. Mutations in ARS1 increase the rate of simple loss of plasmids in *Saccharomyces cerevisiae*. *Yeast* 2: 169–178.

Sweder, K.S., P.R. Rhode, and J.L. Campbell. 1988. Purification and characterization of proteins that bind to yeast ARSs. *J. Biol. Chem.* 263: 17270–17277.

Taggart, R. and J. Samloff. 1983. Stable antibody-producing murine hybridomas. *Science* 219: 1228–1230.

Umek, R.M. and D. Kowalski. 1987. Yeast regulatory sequences preferentially adopt a non-B conformation in supercoiled DNA. *Nucleic Acids Res.* 15: 4467–4480.

Vignais, M.L., L.P. Woudt, G.M. Wassenaar, W.H. Mager, A. Sentenac, and R.J. Planta. 1987. Specific binding of TUF factor to upstream activation sites of yeast ribosomal protein genes. *EMBO J.* 6: 1451–1457.

Walker, S.S., S.C. Francesconi, B.K. Tye, and S. Eisenberg. 1989. The OBFI protein and its DNA-binding site are important for the function of an autonomously replicating sequence in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* 9: 2914–2921.

Wiederrecht, G., D. Seta, and C.S. Parker. 1988. The isolation of the gene encoding the S. cerevisiae heat shock transcription factor. *Cell* 54: 841–853.

Williams, J.S., T.T. Eckdahl, and J.N. Anderson. 1988. Bent DNA functions as a replication enhancer in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* 8: 2763–2769.

Young, R. and R. Davis. 1983. Efficient isolation of genes by using antibody probes. *Proc. Natl. Acad. Sci.* 80: 1194–1198.

GENES & DEVELOPMENT 1939
The gene encoding ARS-binding factor I is essential for the viability of yeast.

P R Rhode, K S Sweder, K F Oegema, et al.

Genes Dev. 1989, 3: Access the most recent version at doi:10.1101/gad.3.12a.1926