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Transcriptional Control of the *Saccharomyces cerevisiae ADH1* Gene by Autonomously Replicating Sequence Binding Factor 1

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Abstract. Autonomously replicating sequence (ARS)-binding factor 1 (ABF1) is a multifunctional protein involved in transcriptional activation and repression, as well as DNA replication, in yeast. The *ADH1* gene, encoding alcohol dehydrogenase 1, contains two ABF1 consensus binding sites in the promoter and the coding regions. To examine the effect of ABF1 on expression of the *ADH1* gene, we constructed an *ADH1-lacZ* fusion plasmid. Both ABF1 binding sites appeared to be transcriptional activators because deletions and mutations of these sites decreased transcriptional activity. The ABF1 binding sites also acted in an orientation-independent manner when a synthetic ABF1 binding site was inserted into the yeast *CYC1* gene lacking its transcriptional activation region. A gel mobility shift assay showed that ABF1 bound *in vitro* to both ABF1 binding sites in the promoter and coding regions. In a glycerol medium the degree of activation by ABF1 was higher than in a glucose medium. The expression of *ADH1* was activated synergistically by both ABF1 binding sites. These observations suggest that ABF1 transactivates the *ADH1* gene through its binding sequences in both the promoter and coding regions.

In *Saccharomyces cerevisiae* the coordinate expression of genes with related functions is accomplished via the interaction of specific DNA binding proteins with conserved sequences located within the promoter regions. Recently, a class of abundant sequence-specific DNA binding proteins was identified. One such protein, autonomously replicating sequence (ARS) binding factor 1 (ABF1), was initially identified by interactions with *ARS1* and *HMRE* silencer/*ARS* which are required for optimum ARS activity [5, 9, 25, 28]. Subsequent studies have shown that ABF1 binding is not limited to ARS elements. The ABF1 binding site has been identified in the 5′-flanking regions of a number of genes [6, 7, 14, 15, 26]. These genes include those encoding RNA polymerase, ribosomal proteins, and glycolytic enzymes, all of which are proteins involved in cell growth. Comparison of a number of ABF1-binding sites has revealed that 5′-RTCRYNNNNNACG-3′ (R = A or G, Y = C or T, N is any nucleotide) is the consensus sequence high-affinity recognition by ABF1 [10] and that the role of the ABF1 binding site is as a constitutive upstream activation sequence for several genes [6, 14, 26]. Recently, ABF1 binding sites were observed in the coding region of the *LPD1* gene [30] for which the expression level was slightly repressed by ABF1 binding within the coding region [27].

The gene encoding the glycolytic enzyme *ADH1* is one of the most highly expressed genes in yeast cells. Although *ADH1* gene expression was originally considered to be constitutive [11], more recent experiments have demonstrated that it is regulated by the nature of the carbon source in the growth medium [8]. However, the carbon source-related element controlling the *ADH1* gene has not yet been characterized. Sequence analysis of the *ADH1* gene has revealed the existence of ABF1 binding sites in the 5′-flanking region and the coding region. In this study it is demonstrated that ABF1 binding sequences in the promoter and the coding regions are responsible for transactivation of the *ADH1* gene and that they mediate carbon source-dependent activation of this gene.

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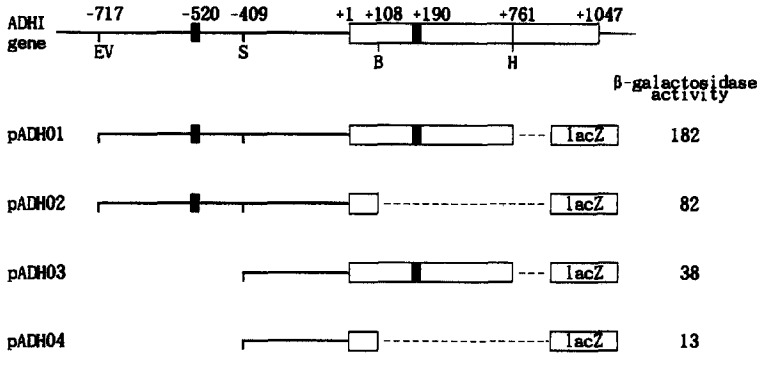


Fig. 1. Construction of *ADH-lacZ* fusions and expression of β-galactosidase activity in SHY4 cells. The *ADH1* gene contains two ABF1 binding sites in the promoter (between −520 and −508 with respect to start codon) and the coding region (between +190 and +202). Each *ADH1-lacZ* fusion was transformed into strain SHY4, and the cells were grown in YEPD (2% glucose). β-galactosidase-specific activities are expressed in units per mg of protein and are averages of three determinations with a range of less than 10%. Solid box represents the ABF1 binding site. Restriction sites illustrated include: *Bcl*I(B); *Eco*RV(EV); *Hin*dIII(H); *Sph*I(S).

Materials and Methods

Strains and growth conditions. The *S. cerevisiae* strains used in this study were SHY4 (*MATa, ste-VC9, ura3-52, trp1-289, leu2-3, leu2-112, his3-Δ1*) [3] and JCA35(*MATa, trp1Δ, his3Δ200, ura3-52, lys2-801, ade2-1, gal, abf1-5, his3*) [23]. JCA35 (*abf1-5*) is an *abf1* temperature-sensitive mutant strain. *Escherichia coli* HB101 (*F−, r−, m−, recA13*) and JM83 (*ara, Δ(lac-proAB), rspL, Φ80lacZ ΔM15*) were used for maintenance and amplification of plasmids. Plasmids were introduced into *E. coli* strains by the $CaCl_2$ transformation method [24]. *E. coli* cells were grown at 37°C in LB medium. Yeast DNA transformation was carried out by the method of Hill et al. [16]. Yeast strain SHY4 was grown at 30°C with shaking in a liquid medium containing 2% bactopeptone, 1% yeast extract, supplemented with 2% glucose (YEPD) or 3% glycerol (YEPG), respectively. Strain JCA35 (*abf1-5*) was cultured at a semi-permissive temperature (30°C). Yeast cells were washed with a medium containing 1% yeast extract and 2% bactopeptone before transfer to different carbon sources.

Cloning and construction of fusion plasmids. A yeast genomic library was screened and a full-length *ADH1* gene was cloned by colony hybridization with a DNA probe containing the promoter region of the *ADH1* gene [2]. The *Eco*RV-*Hin*dIII fragment containing the sequence −717 to +761 of *ADH1* was obtained and inserted into pYJ5β. The resulting plasmid pADH01 was an in-frame *ADH1-lacZ* fusion vector. The *Bcl*I-*Hin*dIII and *Eco*RV-*Sph*I fragments of pADH01 were deleted, and the resulting plasmids were designated pADH02 and pADH03, respectively. The *Sph*I/*Bcl*I fragment was inserted into pYJ5β to give plasmid pADH04 (Fig. 1). The fragments that were used to construct the *ADH1-lacZ* fusion plasmids were modified to adjust the reading frame. Plasmid pCYCABF1 was made by inserting the synthesized oligonucleotides (5′-TCGAGTCGCTGGTCACGAG-3′) corresponding to the ABF1 binding site within the coding region of the *ADH1* gene into the *Xho*I site of pRSCYC. Plasmid pRSCYC, lacking all *CYC1* activation sequences, was produced from pLG669-Z [13] by digestion with *Xho*I, and the selectable marker gene was changed from *URA3* to *TRP1*.

Enzyme assays. β-galactosidase assays were performed with ONPG (Sigma Chemical Co.) according to Jung et al. [17]. β-galactosidase activities were represented as nmol of ONP produced min^{-1} (mg protein)$^{-1}$. All samples were assayed in duplicate or triplicate, and the experiments were repeated at least three times. Standard spectrophotometric assays for ADH1 were performed as described by Tornow and Santangelo [29].

Gel mobility shift assays. Preparation of protein extracts was performed according to the method of Luche et al. [19]. The protein concentration of yeast extracts was determined by the method of Bradford [4]. Gel mobility shift assays were carried out as described by Graves et al. [12].

Site-directed mutagenesis of ABF1 binding sites. Oligonucleotide-directed mutagenesis was performed according to the method of Kunkel et al. [18]. The C(−518) and A(−510) bases of the ABF1 binding site in the promoter region were changed to A and C, respectively. The sequence of the ABF1 binding site within the coding region was changed from C(+192) to A and from A(+200) to C. The base changes were confirmed by DNA sequence analysis.

Results

ABF1 as a transcriptional activator of the *ADH1* gene. The *ADH1* gene contains two ABF1 binding sites within the promoter and the coding regions. The *ADH1-lacZ* fusion plasmids were constructed to see the effect of ABF1 on the expression of the *ADH1* gene. Fig. 1 is a summary of the results of β-galactosidase assays for strain SHY4 carrying *ADH1-lacZ* fusion plasmids. Deletion of the sequence between −717 and −410 (with respect to the start codon) resulted in a 4.7-fold reduction of β-galactosidase-specific activity. A similar level of reduction was also observed in ADH1 enzyme (data not shown). Deletion of the sequence located between +109 and +761 resulted in an approximate 2.2-fold decrease in β-galactosidase activity. These results indicate that the activating sequences are located in the upstream region and the coding sequence. Since the ABF1 protein is abundant in strain SHY4, the *abf1* temperature-sensitive mutant JCA35(*abf1-5*) [23] was used to elucidate the exact function of ABF1 on *ADH1* gene expression. Each of four different in-frame *ADH1-lacZ* fusion plasmids was cotransformed with and without YEP24-3a into strain JCA35(*abf1-5*). These transformants carrying YEP24-3a, the ABF1 expression vector, contained levels of ABF1 approximately 4-fold higher than transformants carrying the vector alone (YEP24) [22]. Transformants carrying pADH01 and YEP24-3a exhibited a 4.8-fold greater increase in β-galactosidase activities than transformants carrying pADH01 and YEP24 (Table 1 and Fig. 2). In the case

Table 1. The effects of ABF1 on the expression of *ADH-lacZ* fusions in strain JCA35 (*abf1-5*)[a]

Plasmids	YEPD[b] β-galactosidase activity[d] +YEP24 (Control)	+YEP-3a[e] (ABF1)	Ratio[f]	YEPG[c] β-galactosidase activity +YEP24 (Control)	+YEP24-3a (ABF1)	Ratio[f]
pADH01	33.8	162.2	4.8	15.4	134	8.7
pADH02	26.6	69.2	2.6	12.3	66.4	5.4
pADH03	11.5	43.7	3.8	7.8	71.8	9.2
pADH04	8.6	12.9	1.5	7.2	13.0	1.8

[a] Strain JCA35 is an *abf1* temperature-sensitive mutant.
[b] YEPD is rich medium containing 2% (wt/vol) glucose.
[c] YEPG is rich medium containing 3% (vol/vol) glycerol.
[d] The specific activities of β-galactosidase are expressed in units per mg of protein and are the results of at least three independent experiments; the range of these values was less than 15%.
[e] YEP24-3a contains the genomic *ABF1* gene and expresses ABF1 protein.
[f] Ratio of β-galactosidase activity in strain JCA35(*abf1-5*) with *ADH-lacZ* fusion carrying the YEP24-3a versus YEP24 plasmid.

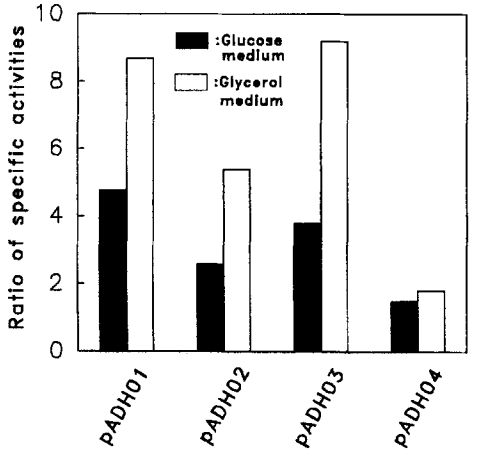


Fig. 2. Effect of ABF1 on the expression of *ADH-lacZ* fusions in different carbon sources. Each plasmid was cotransformed into mutant strain JCA35(*abf1-5*) with YEP24 and YEP24-3a, respectively, and assayed for β-galactosidase activity. Solid bar represents the ratio of β-galactosidase activity of transformants carrying YEP24-3a vs. YEP24, grown on YEPD medium (2% glucose). Open bar represents the ratio of β-galactosidase activity for cells carrying YEP24-3a vs. YEP24, grown on YEPG medium (3% glycerol).

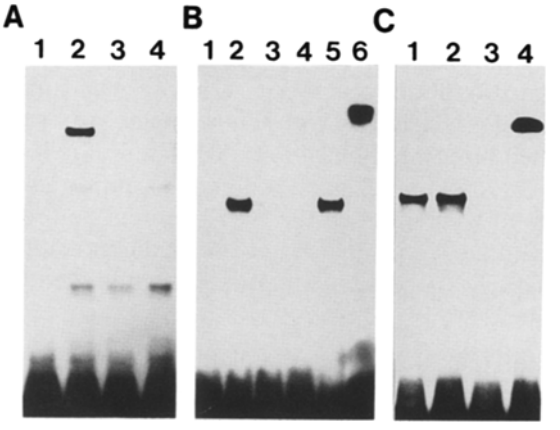
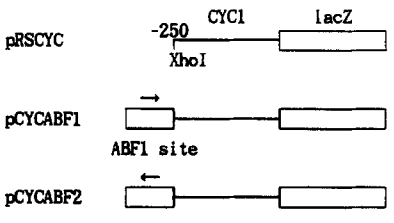


Fig. 3. Gel mobility shift assay of ABF1 binding site of *ADH1* gene. (**A**) A ^{32}P-labeled fragment (between −490 and −546 with respect to the start codon) containing the ABF1 binding site was incubated with 40 μg of cell extract from SHY4 cells in the presence or absence of competitor DNA. Lane 1, no extract; 2, no competitor DNA; 3, 50-fold excess of consensus oligonucleotide; 4, 50-fold excess of ARS1-ABF1 DNA. (**B**) 40 μg of cell extract from SHY4 cells was incubated with a ^{32}P-labeled, double-stranded synthetic oligonucleotide corresponding to ABF1 binding site within the coding region and either antiserum to ABF1 or preimmune serum. Lane 1, no extract; 2, no competitor DNA; 3 and 4, 50-fold and 100-fold excess of consensus oligonucleotide respectively; 5, 1:5 dilution of preimmune serum; 6, 1:5 dilution of anti-ABF1 serum. (**C**) ^{32}P-labeled oligonucleotide was used as probe, and anti-ABF1 serum was added before or after binding reaction. Lane 1, extract; 2, preimmune serum; 3, anti-ABF1 serum was added before binding reaction; 4, anti-ABF1 serum was added after binding reaction.

of pADH02 and pADH03, 2.6- and 3.8-fold increases were observed, respectively, confirming that the ABF1 binding site has a weak UAS activity [23]. Both ABF1 sites of the promoter and coding region function additively to boost the overall expression of the gene. The β-galactosidase activity was not significantly increased in the strain transformed with pADH04 (Table 1). The enzyme levels of pADH01 and pADH03 were higher than the levels of pADH02 and pADH04, respectively (Table 1 and Fig. 1), indicating that ABF1 exerted its influence on the sequence between +108 and +761.

ABF1 binds to the consensus sequences in the promoter and the coding regions. The binding of ABF1 to the consensus sites of both the promoter and the coding regions was determined by gel retardation assays (Fig. 3). A fragment containing the ABF1



	YEPD			YEPG		
	+YEP24	+YEP24-3a	Ratio	+YEP24	+YEP24-3a	Ratio
pRSCYC	3.5	4.2	1.2	3.2	4.6	1.4
pCYCABF1	4.5	17.1	3.8	3.5	27.3	7.8
pCYCABF2	4.2	16.4	3.9	3.8	34.6	9.1

Fig. 4. Effect of ABF1 on the expression of *CYC1-lacZ* gene on different carbon sources. pRSCYC containing a UAS-less *CYC1-lacZ* gene fusion was constructed from pLG669-Z. An oligonucleotide corresponding to the ABF1 binding site in the coding region of *ADH1* gene was inserted at the *Xho*I site of pRSCYC, and its orientation is designated by the direction of arrow. Each plasmid was transformed into ABF1 temperature-sensitive mutant strain JCA35(*abf1-5*) with or without ABF1 expression vector (YEP24-3a), and β-galactosidase assay was performed. The range of these values was less than 10%. Cells were grown either on YEPD (2% glucose) or on YEPG (3% glycerol).

binding site between −490 and −546 formed one specific DNA-protein complex (Fig. 3A). The synthetic ABF1 binding site as a competitor abolished the protein-DNA complex (Fig. 3A, lane 4). A double-stranded synthetic oligonucleotide corresponding to the ABF1 binding sequence between +190 and +202 also formed one specific DNA-protein complex (Fig. 3B). Each DNA-protein complex was supershifted after addition of anti-ABF1 serum (Fig. 3B, lane 6, and Fig. 3C, lane 4). When this antiserum was allowed to pre-adsorb the ABF1 protein before the labeled DNA fragments were added, the formation of specific DNA-protein complexes was inhibited (Fig. 3C, lane 3).

Modulation of transcriptional activation by the carbon source. When the carbon source was changed from glucose to glycerol, the levels of β-galactosidase expression were moderately decreased (Table 1). In the case of pADH03 the specific β-galactosidase activity of cells cultured in a glycerol medium was higher than the activity of cells cultured in a glucose medium. This result indicates that a site, possibly the ABF1 binding site in the coding region, is responsible for efficient transactivation of this gene. Activation in a glycerol medium was approximately two-fold higher than in a glucose medium (Fig. 2).

Carbon source-dependent activation of ABF1 after fusion of the ABF1 binding site to a UAS-less *CYC1-lacZ* gene fusion was also investigated. An oligonucleotide corresponding to the ABF1 binding site in the coding region of the *ADH1* gene was inserted into the UAS-less *CYC1* promoter-*lacZ* fusion gene of the pRSCYC plasmid (pCYCABF1) (Fig. 4). When plasmid pCYCABF1 was cotransformed with YEP24-3a, the β-galactosidase level was 4-fold higher than the level obtained with pRSCYC. A base level of β-galactosidase activity was observed in strain JCA35(*abf1-5*) carrying pRSCYC with YEP24 or YEP24-3a (Fig. 4). This result indicates that ABF1 can function as a *trans*-activator. When a consensus oligonucleotide fusion plasmid (pCYCABF1) was cotransformed with YEP24-3a, the β-galactosidase activity was increased 3.8-fold compared with transformation with YEP24; with a change in the carbon source from glucose to glycerol, a 7.8-fold increase in β-galactosidase activity was observed. The activation ratio in a glycerol medium was approximately two times higher than in a glucose medium (Fig. 4). This result is consistent with the result using the *ADH1-lacZ* fusion plasmid. The β-galactosidase activity of pCYCABF2 containing a reverse orientation of the ABF1 binding site was similar to the activity of pCYCABF1 (Fig. 4). This result shows that the ABF1 binding site functions in an orientation-independent manner.

Effect of mutation of the ABF1 binding sequences. Mutations of ABF1 binding sites were introduced to examine the role of the ABF1 binding sites in regulating the *ADH1* gene (Fig. 5). The C(−518) and A(−510) bases of the ABF1 binding site in the promoter were changed to A and C, respectively (Fig. 5A). The sequence of the ABF1 binding site within the coding region was also changed from C(+192) to A and from A(+200) to C (Fig. 5A). The effects of these mutations on β-galactosidase activities are shown in Fig. 5B. In a parallel study of these plasmids with mutations, a two-fold reduction of β-galactosidase activity in comparison with the wild-type plasmid was observed. The effect of the ABF1 binding site in the promoter region was similar to that of the ABF1 binding site in the coding region (Table 1 and Fig. 5B). Mutations in each plasmid completely abolished the effect of ABF1. These results indicate that ABF1 exerts its function on both ABF1 binding sites in the promoter and the coding regions of the *ADH1* gene.

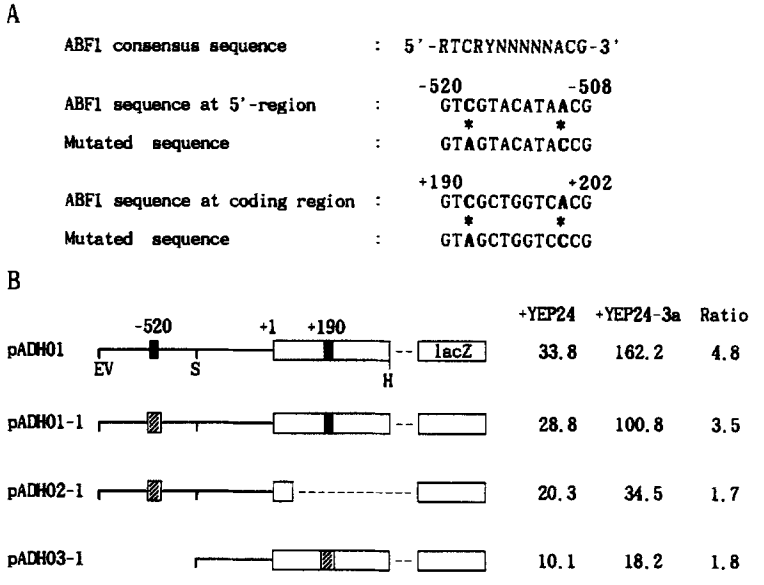


Fig. 5. Mutation effect of ABF1 binding site on the expression of *ADH-lacZ* fusion. (**A**) Position and changed bases are shown; the third base (C) of consensus sequence was changed to A and the eleventh base(A) was changed to C. The asterisks (*) represent the altered base. (**B**) All cultures were grown on YEPD (2% glucose). Each plasmid was cotransformed into strain JCA35 (*abf1-5*) with YEP24 or YEP24-3a, respectively. The range of β-galactosidase activities was less than 10%. The mutation was indicated by hatched box. Solid box represents the ABF1 binding site.

Discussion

In this study the effect of ABF1 on regulation of the *ADH1* gene was analyzed. The β-galactosidase activities for deletion constructs in strain JCA35(*abf1-5*) with and without YEP24-3a indicated that ABF1 acts as a transcriptional activator through ABF1 binding sites in the promoter and the coding regions of the *ADH1* gene (Table 1). Deletion of the ABF1 binding site in the promoter region resulted in a greater reduction of β-galactosidase activity than in the case of deletion of the coding region. This might be due to involvement of other factors in *ADH1* gene regulation [29].

ADH1 has often been considered to be a constitutive gene. However, the expression level decreased when cells were cultured on a nonfermentable carbon source. The extent of this decrease was relatively moderate, as shown by Beier and Young [1]. In glycerol medium, the expression levels of *ADH1* were decreased compared with the levels of cells grown in a glucose medium (Table 1). Activation levels of pADH01, pADH02, and pADH03 were increased by ABF1 more than 2-fold in a glycerol medium compared with a glucose medium (Table 1 and Fig. 2). The phosphorylation form of ABF1 has been shown to be different depending on the carbon source [17, 26]. This difference may be due to the different level of phosphorylation of ABF1.

Yeast *PGK*, *PYK1*, and *LPD1* genes appear to have transcriptional enhancers within their coding regions [20, 21, 30]. Recently, ABF1 binding sites in the coding region of the *LPD1* gene were shown to repress slightly expression of the gene [27]. However, for the *ADH1* gene, the ABF1 binding site activated the expression of the *ADH1* gene approximately 4-fold. Alteration of the base sequence of the ABF1 binding sequence in the coding region by site-directed mutagenesis without affecting its coding potential abolished binding of ABF1 to these sites (data not shown) and reduced enzyme activity in the *ADH-lacZ* fusion plasmids (Table 1 and Fig. 5B). ABF1 action as a *trans*-activator through the 5'- and coding regions of the *ADH1* gene and ABF1 function as a binding site in an orientation-independent manner are indicated by the results of this study.

ACKNOWLEDGMENTS

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Literature Cited

1. Beier DR, Young ET (1982) Characterization of a regulatory region upstream of the *ADR2* locus of *S. cerevisiae*. Nature 300:724–728
2. Bennetzen JL, Hall BD (1982) The primary structure of the *Saccharomyces cerevisiae* gene for alcohol dehydrogenase I. J Biol Chem 257:3018–3025
3. Botstein D, Falco SC, Stewart SE, Brennan M, Scherer S, Stinchcomb OT, Struhl K, Davis RW (1979) Sterile host yeast. Gene 8:17–24
4. Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of dye-ligand binding. Anal Biochem 72:248–254
5. Buchman AR, Kimmerly WJ, Rine J, Kornberg RD (1988) Two DNA-binding factors recognize specific sequences at silencers, upstream activating sequences, autonomously repli-

cating sequences and telomeres in *Saccharomyces cerevisiae*. Mol Cell Biol 8:210–225
6. Della Seta F, Ciafre S-A, Marck C, Santoro B, Presutti C, Sentenac A, Bozzoni I (1990a) The ABF1 factor is the transcriptional activator of the *L2* ribosomal protein genes in *Saccharomyces cerevisiae*. Mol Cell Biol 10:2437–2441
7. Della Seta F, Treich I, Buhler J-M, Sentenac A (1990b) ABF1 binding sites in yeast RNA polymerase genes. J Biol Chem 265:15168–15175
8. Denis CL, Ferguson J, Young ET (1983) mRNA levels for the fermentative alcohol dehydrogenase of *Saccharomyces cerevisiae* decrease upon growth on a nonfermentable carbon source. J Biol Chem 258:1165–1171
9. Diffley JFX, Stillman B (1988) Purification of a yeast protein that binds to origins of DNA replication and a transcriptional silencer. Proc Natl Acad Sci USA 85:2120–2124
10. Dorsman JC, Doorenbosch MM, Maurer CTC, de Winde JH, Mager WH, Planta RJ, Grivell LA (1989) An ARS/silencer binding factor also activates two ribosomal protein genes in yeast. Nucleic Acids Res 17:4917–4923
11. Fowler PW, Ball AJS, Griffiths DE (1972) The control of alcohol dehydrogenase isozyme synthesis in *Saccharomyces cerevisiae*. Can J Biochem 50:35–43
12. Graves BJ, Johnson PF, McKnight SL (1986) Homologous recognition of a promoter domain common to the MSV LTR and the HSV tk gene. Cell 45:565–576
13. Guarente L, Ptashne M (1981) Fusion of *Escherichia coli lacZ* to the cytochrome *c* gene of *Saccharomyces cerevisiae*. Proc Natl Acad Sci USA 78:2199–2203
14. Halfter H, Muller U, Winnacker E-L, Gallwitz D (1989) Isolation and DNA-binding characteristics of a protein involved in transcription activation of two divergently transcribed, essential yeast genes. EMBO J 8:3029–3037
15. Hamil KG, Nam HG, Fried HM (1988) Constitutive transcription of yeast ribosomal protein gene *TCM1* is promoted by uncommon *cis*- and *trans*-acting elements. Mol Cell Biol 8:4328–4341
16. Hill J, Ian KA, Donald G, Griffiths DE (1991) DMSO-enhanced whole cell yeast transformation. Nucleic Acids Res 20:5791
17. Jung SY, Yoo HY, Kim YH, Kim J, Rho HM (1994) The glucose-dependent transactivation activity of ABF1 on the expression of the *TDH3* gene in yeast. Curr Genet, in press
18. Kunkel TA, Roberts JD, Zakour RA (1987) Rapid and efficient site-specific mutagenesis without phenotypic selection. Methods Enzymol 154:367–382
19. Luche RM, Sumrada R, Cooper TG (1990) A *cis*-acting element present in multiple genes serves as a repressor protein binding site for the yeast *CAR1* gene. Mol Cell Biol 10:3884–3895
20. Mellor J, Dobson MJ, Kingsman AJ, Kingsman SM (1987) A transcriptional activator is located in the coding region of the yeast *PGK* gene. Nucleic Acids Res 15:6243–6259
21. Purvis IJ, Loughlin L, Bettany AJE, Brown AJP (1987) Translation and stability of an *Escherichia coli* β-galactosidase mRNA expressed under the control of pyruvate kinase sequences in *Saccharomyces cerevisiae*. Nucleic Acids Res 15:7963–7973
22. Rhode PR, Sweder KS, Oegema KF, Campbell JL (1989) The gene encoding ARS-binding factor 1 is essential for the viability of yeast. Genes Dev 3:1926–1939
23. Rhode PR, Elsasser S, Campbell JL (1992) Role of multifunctional autonomously replicating sequence binding factor 1 in the initiation of DNA replication and transcriptional control in *Saccharomyces cerevisiae*. Mol Cell Biol 12:1064–1077
24. Sambrook J, Fritsch EF, Maniatis T (1989) Molecular cloning. A Laboratory Manual. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory
25. Shore D, Stillman DJ, Brand AH, Nasmyth KA (1987) Identification of silencer binding proteins from yeast: possible roles in SIR control and DNA replication. EMBO J 6:461–467
26. Silve S, Rhode PR, Coll B, Campbell J, Poyton RO (1992) ABF1 is a phosphoprotein and plays a role in carbon source control of *COX6* transcription in *Saccharomyces cerevisiae*. Mol Cell Biol 12:4197–4208
27. Sinclair DA, Kornfeld GD, Dawes IW (1994) Yeast intragenic transcriptional control: activation and repression sites within the coding region of the *Saccharomyces cerevisiae LPD1* gene. Mol Cell Biol 14:214–225
28. Sweder KS, Rhode PR, Campbell JL (1988) Purification and characterization of proteins that bind to yeast ARSs. J Biol Chem 263:17270–17277
29. Tornow J, Santangelo GM (1990) Efficient expression of the *Saccharomyces cerevisiae* glycolytic gene *ADH1* is dependent upon a *cis*-acting regulatory element (UAS_{RPG}) found initially in genes encoding ribosomal proteins. Gene 90:79–85
30. Zaman Z, Brown AJP, Dawes IW (1992) A 3′ transcriptional enhancer within the coding sequence of a yeast gene encoding the common subunit of two multi-enzyme complexes. Mol Microbiol 6:239–246