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Regulation of the yeast *HIS7* gene by the global transcription factor Abf1p

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Abstract The *HIS7* gene of *Saccharomyces cerevisiae* encodes a bifunctional glutamine amidotransferase: cyclase that catalyzes the formation of biosynthetic precursors for histidine and adenine. *HIS7* is activated by Gcn4p upon amino acid starvation and by the Bas1/2p complex in response to adenine limitation. Mutation analysis of the *HIS7* promoter in a $\Delta gcn4$ background revealed a polyd(A/T) stretch and a d(CT) repeat as essential elements for Gcn4p-independent basal *HIS7* transcription. The protein binding this element was enriched and identified as the multifunctional DNA-binding protein Abf1p. Abf1p binds specifically to the d(CT) repeat sequence, which represents a novel Abf1p-binding motif, and protects 17 nucleotides from digestion by DNase I. In addition, Abf1p binding causes bending of the *HIS7* promoter structure.

Key words *Saccharomyces cerevisiae* · Regulation of transcription · Histidine biosynthesis · *HIS7* · Abf1p

Introduction

Promoters of yeast genes are composed of an array of binding sites for activator and repressor proteins that act coordinately in a regulated pattern of transcription. TATA and initiator elements, located near the transcriptional start site, comprise the binding site for general transcription factors and ultimately the RNA polymerase holoenzyme complex (for review see Künzler et al. 1996). Elements located upstream of the TATA

element are recognized by gene-specific transcription factors. Activator proteins can function in vitro and in vivo in simple promoter backgrounds that include only TATA and initiator elements (Carey et al. 1989; Chasman et al. 1989). However, native promoters that are modulated in response to various environmental conditions are far more complex.

Several examples of complex promoter structures are provided by the amino acid biosynthetic genes in *Saccharomyces cerevisiae*. The *ARO3* promoter contains binding sites for the yeast regulatory proteins Gcn4p, Abf1p and the URS1-binding protein (Paravicini et al. 1989; Künzler et al. 1995). Gcn4p and Abf1p maintain basal level expression while the URS1-binding protein represses *ARO3* expression. The *HIS4* gene is activated by Gcn4p in response to amino acid starvation. Bas1p and Bas2p maintain basal level expression and further stimulate initiation under conditions of phosphate or adenine limitation (Arndt et al. 1987; Tice-Baldwin et al. 1989; Daignan-Fornier and Fink 1992). An Rap1p-binding site is required for both Gcn4p-dependent and Bas1p/Bas2p-dependent mechanisms of activation of the *HIS4* gene (Devlin et al. 1991).

Yeast Abf1p (= Baf1p = Gf1p = Obf1p = Sufp = Tafp = Sbf-Bp) (Buchman et al. 1988; Diffley and Stillman 1988; Dorsman et al. 1988; Sweder et al. 1988; Halfter et al. 1989) represents a class of abundant regulatory factors named general regulatory factors. Abf1p-binding sites are essential elements for transcription of a wide range of promoters of genes involved in many different biochemical pathways, which include *ARO3* (Künzler et al. 1995), *TRP3* (Martens and Brandl 1994), *ADH1* (Yoo et al. 1995), *CAR1* (Kovari and Cooper 1991), *L2A* and *L2B* (Della Seta et al. 1990), *COX6* (Trawick et al. 1992), *QCR8* (De Winde et al. 1993), and *TDH3* (Jung et al. 1995). This general regulatory factor plays additional roles in various nuclear processes. Abf1p-binding sites have also been found in other genetic elements including silencers, replication origins, telomeres and centromeres (for review see Doorenbosch et al.

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1992). The pleiotropic function of Abf1p has been attributed to its possible role in chromosomal organization or nuclear architecture (Workman and Buchman 1993).

The *HIS7* gene of *S. cerevisiae* encodes a bifunctional glutamine amidotransferase: cyclase that catalyzes the fifth and sixth step in the de novo histidine biosynthesis pathway (Künzler et al. 1993). This enzyme produces an intermediate of histidine biosynthesis and in addition, as a by-product, 5-aminoimidazole-4-carboxamide ribotide, which is an intermediate of purine biosynthesis. The metabolic role of the *HIS7* gene product at the crossing point of these two biosynthetic pathways is reflected in the transcriptional regulation of the *HIS7* gene. Under conditions of amino acid starvation and adenine limitation, *HIS7* transcription is activated by Gcn4p and Bas1/2p, respectively (Springer et al. 1996). Gcn4p binds to two Gcn4p recognition elements (GCREs) at positions -231/-225 and -144/-139 relative to the translational start codon of the *HIS7* gene. The Bas1/2p complex requires the second GCRE at position -144/-139.

In this report, we present evidence that Gcn4p-independent basal transcription of the *HIS7* gene depends on the general regulatory factor Abf1p. We have purified a protein on the basis of its ability to bind specifically to a d(CT) repeat sequence within the *HIS7* promoter. This protein caused bending of the target DNA and was identified as Abf1p.

Materials and methods

Strains and culture conditions

Yeast was cultivated at 30°C in either YEPD complete medium (Rose et al. 1990) or MV minimal medium (Miozzari et al. 1978). Appropriate supplements were added to the growth medium in recommended amounts (Rose et al. 1990). *Escherichia coli* was cultivated at 37°C in LB medium (Sambrook et al. 1989). LB medium containing 50 mg/l ampicillin was used to select for *E. coli* transformants.

DNA techniques and sequencing

Enzymatic manipulations and cloning of DNA were performed as described in Sambrook et al. (1989). *E. coli* strain DH5 α (Hanahan 1983) was used for the propagation of plasmid DNA. DNA sequences were determined using the method of Tabor and Richardson (1987). Oligonucleotides were synthesized by MWG-Biotech (Ebersberg, Germany).

Polymerase chain reaction (PCR)

The PCR technique for amplification of cloned DNA fragments using sequence-specific oligonucleotides was described previously (Saiki et al. 1988). In this work the technique was exploited for the introduction of site-directed mutations in the *HIS7* promoter and for the synthesis of DNA probes for gel-retardation assays.

Construction of internal deletions of the *HIS7* promoter

The various internal deletion mutations of the *HIS7* promoter were constructed by *Bal31* exonuclease treatment of the linearized

plasmid pME947. Plasmid pME947 was constructed based on the pGEM-7Zf(+) plasmid (Promega, Madison, USA) by insertion of the 1.9 kb *SphI*-*Bam*HI fragment of the *ARO4/HIS7* locus with a *Cl*aI site created at position -406 relative to the translational start codon of the *HIS7* gene. The plasmid was linearized either with *Cl*aI or *Eco*RV and subsequently treated with *Bal31* exonuclease to obtain 5' and 3' deletions of the region, respectively. After cloning of a *Cl*aI/*Hind*III/*Eco*RV adapter, appropriate 5'- and 3'-deletion fragments were combined to obtain the internal deletions of the *HIS7* promoter. This resulted in the plasmids pME975 with a 23 bp deletion at positions -211/-189 relative to the *HIS7* translational start codon and pME976 with a 19 bp deletion at positions -190/-172.

Site-directed mutagenesis of the *HIS7* promoter

Site-directed mutations in the *HIS7* promoter were introduced using the PCR technique (Giebel and Spritz 1990). Oligonucleotides carrying specific mutations (mutated nucleotides in lowercase) in the *HIS7* promoter sequence were: PAT2 [5'-d(GGAGAGA-GAGAAATcccAAaCC)-3'] for mutating the polyd(A/T) stretch pAT to pat; GAGA2 [5'-d(CTAATTAGCCGTGGAGGcGacAGAAA-AATAATCCC)-3'] for mutating the d(CT) repeat CT to ct; and OLCS33 [5'-d(CTCCACGGCTAgTcAtGTGATCATG)-3'] for mutating the putative Mcm1p control element MCE to mce. These oligonucleotides were used as primers in a first PCR reaction together with MUTH7 [5'-d(CGCCATTACCGGTCATG)-3'] or CHE5 [5'-d(TTGAAAGTGGTAACCTACAGTCACTAACCA-ATGCAATTG)-3'], respectively, as second primers and pME947 DNA as template. The resulting first PCR product was subsequently used as primer in a second PCR reaction together with MUTH7 or CHE5, respectively, as second primer and the same template DNA. The final PCR product was cut with *Cl*aI and *Eco*RV and exchanged with the corresponding wild-type *HIS7* fragment on pME947, resulting in plasmids pME1023 (pat), pME1021 (ct) and pME1410 (mce). For the construction of pME1411, which is mutated in both the d(CT) repeat and the putative MCE, an analogous procedure with OLCS33 (mce) as primer and DNA of pME1021 (ct) as template was used. All mutations were verified by sequence analysis.

Construction of translational *HIS7-lacZ* fusions

The respective integrative *HIS7-lacZ* fusion constructs pME1018 (Δ pAT/CT), pME1019 (Δ MCE), pME1028 (pat), pME1026 (ct), pME1414 (mce) and pME1415 (ct/mce) were constructed based on the pME947 derivatives carrying the respective *HIS7* promoter mutations, as described previously for pME696 (Künzler et al. 1993).

Integration of the *HIS7-lacZ* fusion constructs

All *HIS7-lacZ* fusion constructs were integrated in a single copy into the yeast genome at the *ARO4/HIS7* locus of yeast strain RH1548 (*MAT α* *aro3-2 gcn4-101 ura3-52 aro4 his7::URA3*), yielding strains RH1824 (Δ pAT/CT), RH1825 (Δ MCE), RH1832 (pat), RH1830 (ct), RH2158 (mce) and RH2159 (ct/mce). The procedure was described previously for the wild-type *HIS7-lacZ* construct pME696, resulting in strain RH1616 (Künzler et al. 1993).

Construction of Δ *fas1* strains

The plasmid pME1430 was constructed by replacing the 2.7 kb *Bg*III-*FAS1* fragment on plasmid pBF1 by the 1.1 kb *Hind*III *URA3* fragment. The *FAS1* gene of the respective *HIS7-lacZ* fusion strains was replaced by the 2.6 kb *Eco*RI fragment of plasmid pME1430 by homologous recombination at the *FAS1* locus, resulting in strains

RH2166 ($\Delta fas1$, $HIS7::his7-lacZ$), RH2167 [$\Delta fas1$, $HIS7::his7-lacZ$ ($\Delta pAT/CT$)] and RH2168 [$\Delta fas1$, $HIS7::his7-lacZ$ (ct)].

β -Galactosidase assay

β -Galactosidase activities were determined by using permeabilized yeast cells and the fluorogenic substrate 4-methylumbelliferyl- β -D-galactoside as described earlier (Künzler et al. 1993). Routinely, yeast cells were cultivated overnight in minimal medium supplemented with the recommended amount of appropriate vitamins (Rose et al. 1990), diluted to an optical density of approximately 0.5 at 546 nm (OD_{546}) and cultivated for another 6 h before assay. One unit of β -galactosidase activity is defined as 1 nmol 4-methylumbelliferone per hour per milliliter per OD_{546} unit. The given values are means of at least four independent cultures. The standard deviations of the means were less than 20%.

Enrichment of the d(CT) repeat-specific DNA-binding activity

Yeast strain RH1381 ($MAT\alpha$ $aro3-2$ $gcn4-101$ $ura3-52$) was cultivated in YEPD complete medium (Rose et al. 1990) to an optical density of about 2 OD_{546} units, harvested by centrifugation and washed three times with potassium-phosphate buffer [50 mM potassium phosphate, pH 7.6, 0.1 mM phenylmethylsulfonyl fluoride (PMSF), 0.1 mM EDTA, 1 mM dithiothreitol (DTT)]. Yeast cells (32 g wet weight) were suspended in 40 ml of potassium phosphate buffer, disrupted in a French Press (Aminco, Silver Spring, Md., USA) and the cell debris was removed by centrifugation. Proteins of the supernatant were precipitated by adding an $(NH_4)_2SO_4$ solution to 30% saturation. The protein precipitate was removed by centrifugation and the supernatant was further precipitated to 50% $(NH_4)_2SO_4$ saturation and centrifuged. After thorough dialysis against buffer P50 [50 mM potassium phosphate, pH 7.6, 1 M $(NH_4)_2SO_4$, 0.1 mM PMSF, 0.1 mM EDTA, 1 mM DTT], the precipitated proteins were applied to an ethylamino-Sepharose column (Jennissen 1978) equilibrated with buffer P50 and fractionated with a linear 1–0.005 M $(NH_4)_2SO_4$ gradient. Aliquots of fractions obtained were analyzed for binding activity using the band-shift assay. Active fractions were pooled, precipitated with $(NH_4)_2SO_4$ and dialyzed against single-stranded DNA (ssDNA) buffer (20 mM TRIS-HCl, pH 7.6, 50 mM NaCl, 0.1 mM EDTA, 1 mM β -mercaptoethanol). Proteins were then chromatographed on an ssDNA-cellulose column (Pharmacia, Uppsala, Sweden) using a linear gradient of 0.05–1.4 M NaCl. Fractions containing proteins active in the band-shift assay were dialyzed against MonoQ buffer (20 mM TRIS-HCl, pH 7.6, 20 mM NaCl, 0.1 mM PMSF, 0.1 mM EDTA, 1 mM DTT). Pooled fractions were applied to a MonoQ HR5/5 column (Pharmacia, Uppsala, Sweden) and after extensive washing with MonoQ buffer, the proteins were eluted with a linear 20–500 mM NaCl gradient. This resulted in purified, active d(CT) repeat-specific DNA-binding activity, which was eluted at about 0.3 M NaCl.

Purification of Fas1p

Fas1p was purified according to the procedure described by Schweizer et al. (1986).

Microsequencing

Peptide microsequencing was performed at the MIT Biopolymers Laboratory (Cambridge, Mass., USA). Peptides were partially digested with endopeptidase LYS-C. The amino acid sequence was determined on an Applied Biosystems Model 477 microsequencer with an on-line Model 120 PTH Amino Acid Analyzer.

Southwestern hybridization

Proteins were separated on an SDS-polyacrylamide gel, electroblotted onto a nitrocellulose membrane and hybridized against ^{32}P -

labeled $HIS7$ promoter fragment in the presence of 1 mg/ml poly(dIdC) (Boehringer-Mannheim, Mannheim, Germany). Hybridization conditions were according to Singh et al. (1988). Membranes were subjected to autoradiography for 12–24 h.

Gel-retardation experiments

Conditions for gel-retardation experiments with crude yeast extracts and enriched d(CT) repeat-specific DNA-binding activity were the same. Yeast crude extracts were prepared according to Arndt et al. (1987). Tests for protein binding to DNA were carried out in binding buffer (7.5 mM TRIS-HCl, pH 7.6, 37.5 mM NaCl, 0.075 mM EDTA, 2 mM DTT) in a final volume of 20 μ l. Proteins were incubated with 2 μ g of poly(dIdC) (Boehringer-Mannheim, Mannheim, Germany) and the ^{32}P -labeled DNA fragments for 15 min at 4°C, after which the assay mixture was immediately loaded onto a 3.5% native polyacrylamide gel in 50 mM TRIS-borate, pH 8.3, 12.5 mM EDTA for electrophoretic separation of free and protein-bound DNA. Gels were dried and subjected to autoradiography for 3–12 h. For competition assays, the protein was preincubated with an excess of appropriate competitor DNA and 2 μ g of poly(dIdC). After 5 min, the assay mixture was treated as above.

DNase I-protection experiments

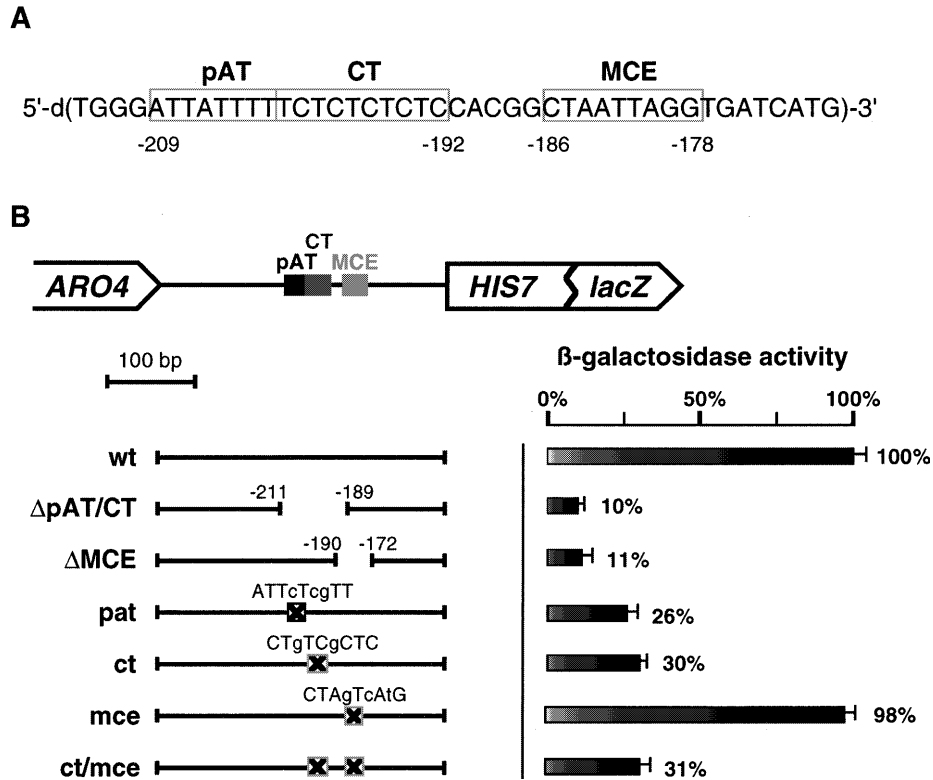
Following 15 min of incubation of enriched d(CT) repeat-specific DNA-binding activity with incubation buffer [7.5 mM TRIS-HCl, pH 7.6, 0.075 mM EDTA, 37.5 mM NaCl, 1 mM $MgCl_2$, 0.5 mM $CaCl_2$, 2.5 mM DTT, 0.05 mg/ml poly(dIdC)] with the 5' end-labeled $HIS7$ promoter fragment in a final volume of 40 μ l on ice, 11.25 U of DNase I (FPLCpure, Pharmacia, Uppsala, Sweden) was added to the reaction mixture. After exactly 90 s, the action of the enzyme was stopped by adding 160 μ l of stop mix (192 mM sodium acetate, 32 mM EDTA, 0.14% SDS, 1 mg/ml tRNA). The DNA fragments were subsequently extracted with phenol and methylene chloride, precipitated with ethanol, and separated on a 6% polyacrylamide gel under denaturing conditions.

Results

A polyd(A/T) stretch and a d(CT) repeat are essential for Gcn4p-independent basal $HIS7$ expression

Under environmental conditions of amino acid starvation and adenine starvation, $HIS7$ expression is activated by the transcription factors Gcn4p and Bas1/2p, respectively (Springer et al. 1996). In this study, Gcn4p-independent basal $HIS7$ expression was investigated. All analyzed strains had a $gcn4-101$ genetic background to avoid interference with the general control of amino acid biosynthesis in yeast. $HIS7-lacZ$ fusions with the wild-type or various mutated $HIS7$ promoter constructs were integrated (single copy) at the genomic $HIS7$ locus by gene replacement and $HIS7$ expression was monitored by determining β -galactosidase activities (Fig. 1).

The 23 bp sequence at position –211/–189 relative to the $HIS7$ translational start codon contained a 9 bp polyd(A/T) stretch and a d(CT) repeat of 10 bp. Deletion of this sequence resulted in the $\Delta pAT/CT$ $HIS7$ promoter allele and reduced $HIS7$ expression roughly tenfold (Fig. 1). The adjacent 19 bp sequence element at



position $-190/-172$ comprises a putative MCE. Deletion of this element resulted in the Δ MCE *HIS7* promoter allele and reduced *HIS7* activity to 11% of wild-type activity. The strong effect of both deletions on *HIS7* expression prompted us to analyze this sequence element in more detail.

Three point mutations (in lowercase), changing the polyd(A/T) stretch at position $-209/-201$ from wild-type 5'-d(ATTATTTTT)-3' to 5'-d(ATTcTcgTT)-3' (Fig. 1, pat), reduced *HIS7* expression to 26% compared with wild-type activity. Two point mutations within the 10 bp d(CT) repeat at position $-201/-192$, changing the wild-type sequence 5'-d(CTCTCTCTC)-3' to 5'-d(CTgTCgCTC)-3' (Fig. 1, ct), had a similar effect, resulting in 30% of *HIS7* wild-type activity. However, *HIS7* promoter activity was not affected by point mutations within the putative MCE at position $-186/-178$ (Fig. 1, mce), where the sequence 5'-d(CTAATTAGG)-3' was changed to 5'-d(CTAgTcAtG)-3', resulting in a low-affinity Mcm1p-binding site (Passmore et al. 1989). The combination of mutations in the d(CT) repeat and the putative MCE (promoter allele ct/mce) showed the same effect as the single mutation in the d(CT) repeat (Fig. 1). Therefore only the sequence element within the *HIS7* promoter containing the polyd(A/T) stretch and the d(CT) repeat seems to be important for the Gcn4p-independent basal level of *HIS7* expression.

Fig. 1A, B A polyd(A/T) stretch and a d(CT) repeat are part of the Gcn4p-independent basal *HIS7* promoter. **A** The *HIS7* promoter sequence between positions -213 and -170 is shown. The polyd(A/T) stretch (pAT), the d(CT) repeat sequence (CT) and the putative Mcm1p control element (MCE) are indicated. **B** *HIS7* transcription directed by wild-type and mutated versions of the *HIS7* promoter was monitored in a *Saccharomyces cerevisiae* strain with a *gcn4-101* background to avoid any interference with the general control of amino acid biosynthesis. Strains were cultivated in minimal medium supplemented with the recommended amounts of appropriate vitamins. Specific β -galactosidase activities of strains carrying the indicated translational *HIS7-lacZ* fusions integrated at the genomic *HIS7* locus are shown. The *HIS7-lacZ* activity driven by the wild-type promoter was 15 U and was set as 100%. Values are calculated from at least five independent measurements. Standard deviation bars are indicated. Yeast strains not carrying an *Escherichia coli lacZ* gene showed no detectable β -galactosidase activity (data not shown).

An abundant DNA-binding activity binds to the d(C/T) repeat within the *HIS7* promoter

No yeast DNA-binding protein with a sequence specificity matching a consensus within the sequence of the *HIS7* promoter between positions -211 and -192 has been described in the EMBL database. Therefore we analyzed yeast extracts for putative DNA-binding activities for this sequence element. Gel-retardation experiments were performed with crude cell extracts derived from the yeast strain RH1381 with a *gcn4-101* genetic background. DNA fragments carrying the same set of point mutations as tested in the expression studies were used as 32 P-labeled *HIS7* promoter probes. DNA-protein complexes were visualized as retarded species using the wild-type (wt) promoter as control. Similar

complexes were found for *HIS7* promoter alleles carrying mutations in the polyd(A/T) stretch (*pat*) or in the putative MCE (*mce*). By contrast, only a weak retarded band was obtained using as binding template a *HIS7* promoter fragment mutated in the d(CT) repeat (*ct*) or in both the d(CT) repeat and the MCE (*ct/mce*) (Fig. 2). These results suggest that there is an abundant yeast protein that specifically binds to the d(CT) repeat at position -201/-192 within the *HIS7* promoter.

Identification and specificity of the enriched d(CT) repeat-specific DNA-binding activity

The d(CT) repeat-binding activity was enriched from crude cell extracts of a yeast strain with a *gcn4-101* genetic background as described in Materials and methods. Each step in the enrichment process was visualized by SDS-polyacrylamide gel electrophoresis by staining the proteins with Coomassie Brilliant Blue (Fig. 3A). The DNA-binding activity was enriched approximately 800-fold with a 3.4% yield (Table 1).

Because the final pool of enriched DNA-binding activity contained two major high molecular weight polypeptides (Fig. 3A), southwestern hybridization experiments were performed to identify the d(CT) repeat-specific DNA-binding activity (Fig. 3B). A blot of a polyacrylamide gel of the enriched DNA-binding activity was hybridized with a 200 bp ³²P-labeled *HIS7* promoter fragment. Autoradiography revealed a single signal corresponding to a polypeptide with an apparent molecular weight of about 130 kDa (Fig. 3B). Use of a ³²P-labeled *HIS7* promoter fragment mutated in the d(CT) repeat as a DNA probe did not result in hybridization (data not shown).

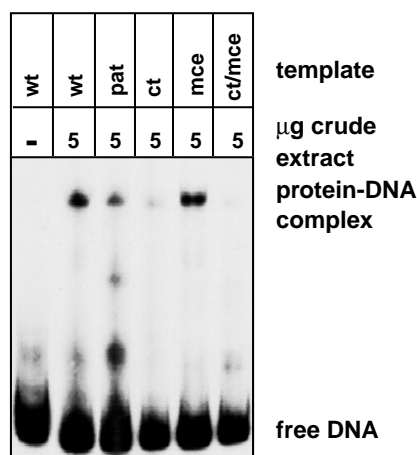


Fig. 2 In vitro binding of the d(CT) repeat-specific DNA-binding activity to the *HIS7* promoter. A gel-retardation experiment was used to demonstrate in vitro binding of the d(CT) repeat-specific DNA-binding activity to the *HIS7* promoter. The DNA fragments were either wild type (*wt*) or mutated in the polyd(A/T) stretch (*pat*), the d(CT) repeat (*ct*), the Mcm1p control element (*mce*) or in both the d(CT) repeat and the Mcm1p control element (*ct/mce*)

Table 1 Purification of the d(CT) repeat-binding activity

Purification step	Protein content (µg)	Specific activity (µg ⁻¹) ^a	Yield (%)	Enrichment (fold)
Crude extract	2000	0.04	100	1
(NH ₄) ₂ SO ₄ precipitate	789	0.048	47.5	1.2
Ethylamino-Sepharose	19.8	0.23	5.8	5.8
ssDNA-cellulose	1.82	2.17	4.9	54
MONO-Q	0.11	32	3.4	800

^aThe specific activity was calculated as the reciprocal of the amount of protein used in each gel-retardation experiment that led to a retarded band of approximately the same intensity

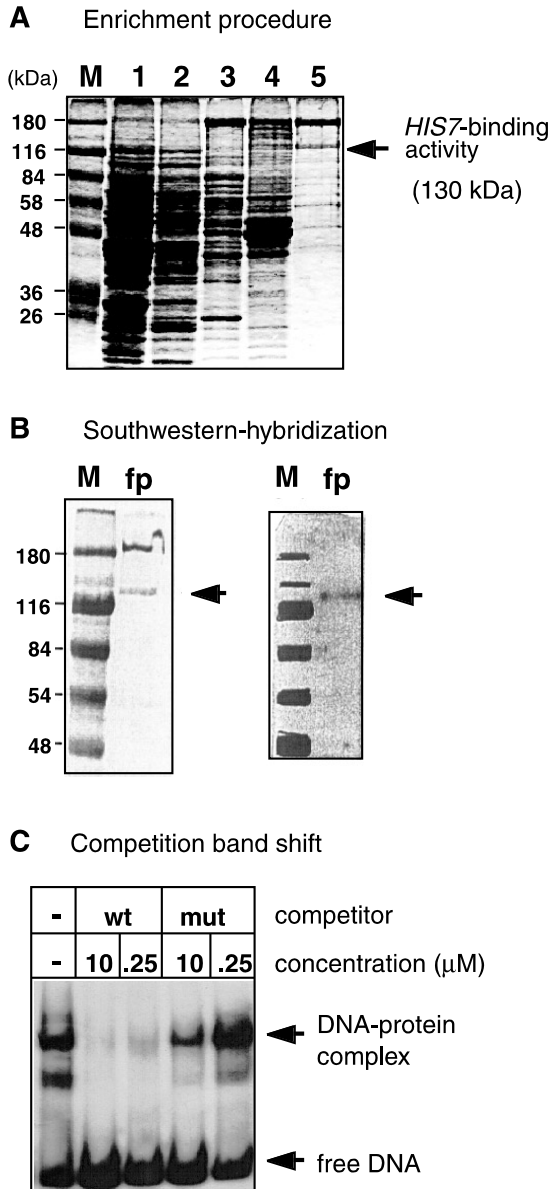
The specificity of the enriched protein fraction for binding to the d(CT) repeat within the *HIS7* promoter was analyzed by competitor gel-retardation experiments. A ³²P-labeled *HIS7* promoter fragment was used as DNA template. Annealed oligonucleotides of 45 bp in length comprising both strands of the *HIS7* promoter at positions -211 to -172 were used as specific nonlabeled competitor DNA. Even at low concentrations (250 nM) of wild-type competitor DNA, no retarded band could be detected. By contrast, a retarded band was still visible when high amounts (10 mM) of competitor DNA mutated in the d(CT) repeat were used (Fig. 3c). Therefore, the final protein preparation containing enriched DNA-binding activity includes a protein that specifically binds to the d(CT) element at position -201/-192 within the *HIS7* promoter.

The d(CT) repeat-specific DNA-binding activity of the *HIS7* promoter is Abf1p

An SDS-polyacrylamide gel slice containing the 130 kDa polypeptide identified as the d(CT) repeat-specific DNA-binding activity was subjected to protein microsequencing. After partial digestion of the 130 kDa peptide, the amino acid sequence of two of the obtained polypeptides was determined. The two peptides revealed the amino acid sequences KIEPYHSHPL and KLLHQYYGDD, respectively. A BLAST analysis of these sequences was performed on the Non-redundant GenBank CDS translations + PDB + SwissProt + SPupdate + PIR 196 024 databases.

The first peptide, KIEPYHSHPL, gave a match of 10/10 (100%) identities to the *S. cerevisiae* Abf1p (= BAF1p = GF1p = OBF1p = SUFp = TAFp = SBF-Bp) (EMBL, locus SCBAF1, accession no. X16385). The yeast Abf1p is an essential, abundant, sequence-specific DNA-binding protein. It binds to the promoters of many genes as well as to functionally diverse elements such as transcriptional silencers, origins of DNA replication [autonomously replicating sequences (ARSs)], centromeres and telomeres, suggesting that it regulates a number of important nuclear processes (Silve et al. 1992).

The second peptide, KLLHQYYGDD, matched the *S. cerevisiae* Fas1p (EMBL, locus SCFAS1, accession no. X03977).



Gel-retardation experiments with Abf1p-specific, nonlabeled competitor DNA were performed to test whether yeast Abf1p binds to the d(CT) repeat at position $-201/-192$ within the *HIS7* promoter. Two different versions of a DNA fragment covering 200 bp of the *HIS7* promoter region from position -296 to -96 were applied as 32 P-labeled DNA probes. The probes were either wild type (wt) or mutated in the d(CT) repeat (ct). The same point mutations were used as in the expression studies. Incubation of the wild-type DNA probe with the final protein preparation containing enriched DNA-binding activity revealed one major retarded band, which was not present when a DNA probe mutated in the polyd(CT) repeat was used. This band also specifically disappeared upon addition of a synthetic DNA fragment corresponding to element B of ARS1 (ARS1B) (Sweder et al. 1988), which contains an Abf1p-binding

site. An analogous fragment carrying two point mutations in the Abf1p-binding site (ars1B) (Dorsman et al. 1990) did not compete for binding (Fig. 4). These results suggest that the abundant multifunctional yeast protein Abf1p binds to the poly d(CT) repeat at position $-201/-192$ of the *HIS7* promoter in vitro.

The other microsequenced peptide matched the *S. cerevisiae* Fas1p. In gel-retardation experiments with purified Fas1p protein, no *HIS7* promoter-specific Fas1p complex could be detected (data not shown). All constructed Δ *fas1* strains revealed the same *HIS7* activity as their corresponding *FAS1* strain (data not shown). We therefore conclude that Abf1p and not Fas1p is involved in transcription of the *HIS7* gene.

Abf1p protects 17 bp on the *HIS7* promoter

Since the polyd(CT) repeat within the *HIS7* promoter does not resemble a consensus sequence for Abf1p, DNase I-protection assays were performed to reveal the exact binding site of Abf1p within the *HIS7* promoter. Abf1p binding to the *HIS7* promoter protects 17 bp at positions -198 to -182 relative to the translational start of the *HIS7* gene, including the polyd(CT) repeat sequence from cleavage, by DNase I. Protection of the coding strand resulted in a hypersensitive band directly adjacent to the protected region (Fig. 5A). No protection was observed when a DNA probe with a point mutation in the polyd(CT) repeat was used (data not shown). Thus, this result is in agreement with the gel-retardation experiments using the same DNA probe point-mutated in the polyd(CT) repeat (Fig. 4, lane 1).

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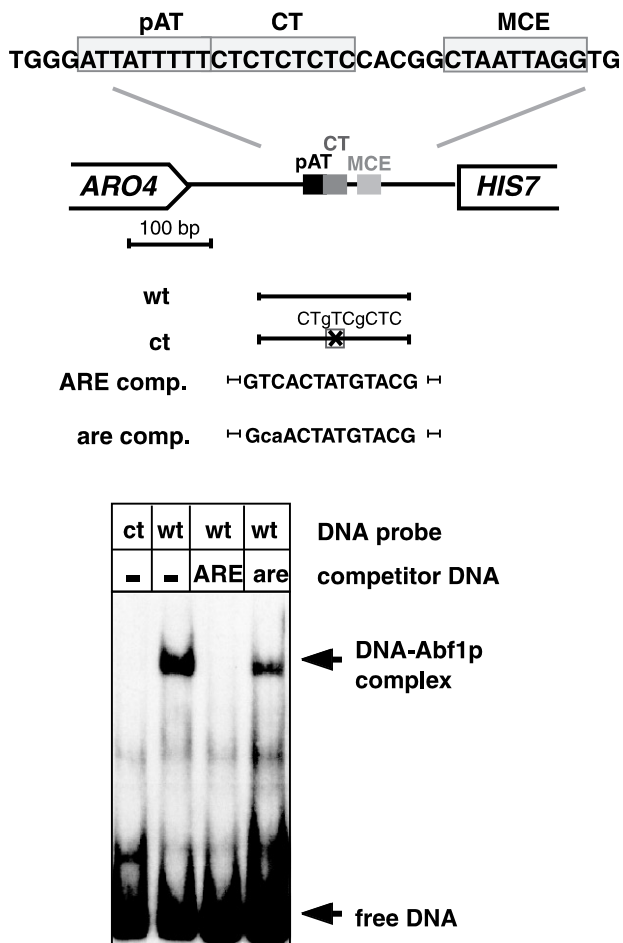


Fig. 4 In vitro binding of Abf1p to the *HIS7* promoter was demonstrated by gel retardation with Abf1p-specific competitor DNA. The final pool of enriched DNA-binding activity (fp) was incubated with ^{32}P -labeled *HIS7* promoter fragments. The labeled fragments were either wild type (*wt*) or mutated in the d(CT) repeat (*ct*). For competition assays, unlabeled DNA corresponding to element B of the autonomous replicating sequence 1 in either the wild type (*ARE*) or a version specifically mutated in the Abf1p-binding site (*are*) was added to the binding reaction

N = A, C, G or T) at many sites in the yeast genome (Dorsman et al. 1990). A part (in uppercase) of the protected sequence 5'-d(CTCTCTCTCCACGGctaata)-3' within the *HIS7* promoter matches this consensus except for two deviations (italicized) (Fig. 5B). All previously described Abf1p-binding sites contain purine nucleotides at positions 1 and 4 (Della Seta et al. 1990). The polyd(CT) repeat within the *HIS7* promoter therefore represents a novel Abf1p-binding site with pyrimidine nucleotides at positions 1 and 4.

Abf1p bends the DNA upon binding to its target site within the *HIS7* promoter

Binding of the enriched d(CT) repeat-specific DNA-binding protein to the *HIS7* promoter caused the DNA to bend. This is consistent with the view that the en-

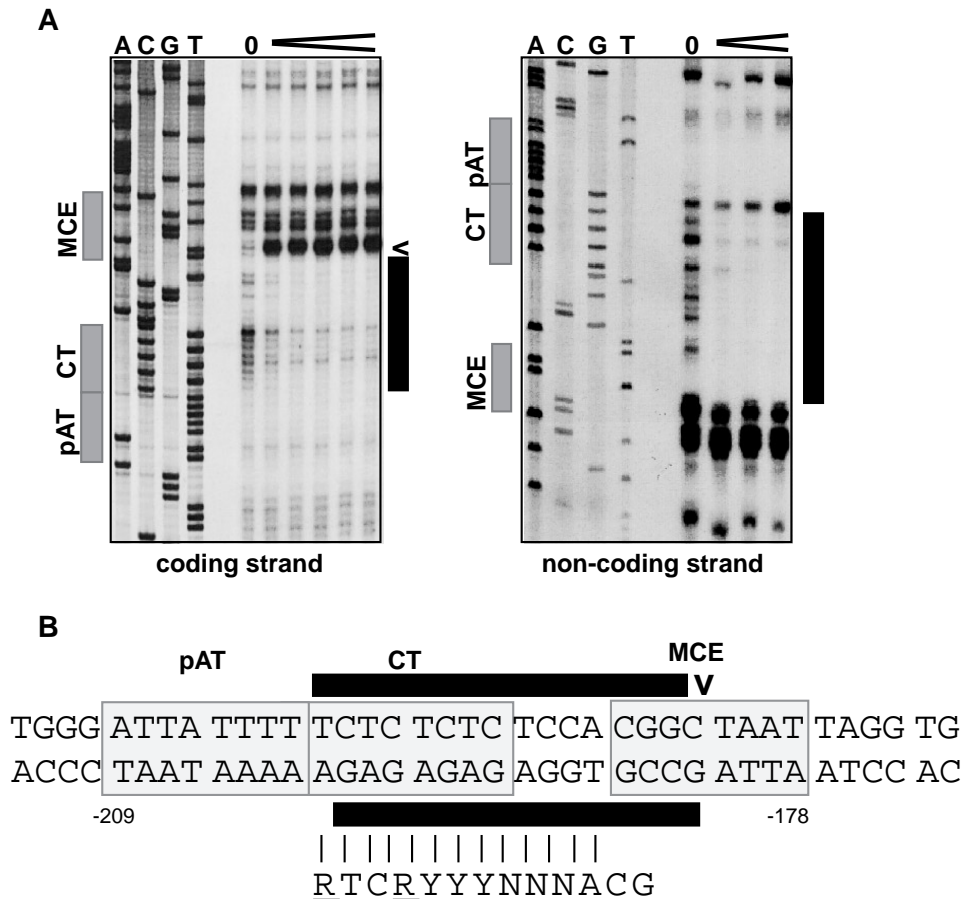
riched activity is indeed Abf1p, which has been reported to bend DNA (McBroom and Sadowski 1994). Protein-induced DNA bending was studied using a gel-retardation assay with linear, circularly permuted DNA substrates (Fig. 6A). This assay is based on the principle that bending of a DNA fragment near the middle reduces the end-to-end distance of the molecule. This results in a decrease in the electrophoretic mobility of the protein-DNA complex relative to a complex containing unbent DNA. In contrast, bending of a DNA fragment near the end will have little effect on the end-to-end distance and hence fragment mobility (Zinkel and Crothers 1987). As shown in Fig. 6B, there was a decrease in the electrophoretic mobility of the Abf1p-DNA complex when the Abf1p binding site was present near the middle of the DNA fragment (lane 3) as compared with when it was present near the end of the DNA fragment (lanes 1 and 5). The identical electrophoretic mobility of all free, unbound DNA fragments indicated that the bending of the DNA was induced by the DNA-binding protein and was not spontaneous.

The bend angle induced by a protein can be estimated by comparison of the ratio of the mobilities of the slowest- and fastest-migrating complexes ($\mu\text{M}/\mu\text{E}$) against the relative position of the binding element using the formula of Ferrari et al. (1992). The size of the Abf1p-induced bend in the *HIS7* promoter is approximately 34° . On determining the minima of the quadratic equation the bending center could be localized to the poly(A/T) stretch just upstream of the Abf1p-binding site within the *HIS7* promoter.

Discussion

By a combined deletion and mutation analysis of the *HIS7* promoter a d(CT) repeat element has been identified as essential for Gcn4p-independent basal transcription. The global regulatory factor Abf1p has been identified by its ability to bind to this d(CT) sequence within the *HIS7* promoter in vitro. Binding of Abf1p to its recognition element within the *HIS7* promoter causes a bending of the target DNA structure.

Binding excess studies have revealed the Abf1p consensus 5'-RTCryYYNNNACG-3', which is characterized by the presence of two conserved elements, separated by a region that is variable in sequence, but constant in length (Dorsman et al. 1990). The Abf1p-binding site within the *HIS7* promoter defined in this work deviates from this consensus in positions 1 and 4. All described Abf1p-binding sites consist of purine residues at positions 1 and 4, whereas the *HIS7* Abf1p-binding site carries pyrimidine residues at these positions and therefore represents a novel Abf1p-binding site. A point mutation changing the conserved position 3 to a G residue decreased Abf1p-binding activity. A similar mutation in the Abf1p-binding site of ARS1B had the same effect (Dorsman et al. 1990). The fact that Abf1p was purified as a result of its capacity to bind to a se-



quence element within the *HIS7* promoter, and that a point mutation known to decrease the binding affinity for Abf1p also decreased *HIS7* promoter activity, strongly supports the idea that Abf1p is involved in transcriptional activation of the *HIS7* gene.

Abf1p-dependent transcription is involved in transcriptional regulation of a number of seemingly unrelated yeast genes and hence represents a global regulatory mechanism in *S. cerevisiae*. Abf1p is a multifunctional DNA-binding protein present in great abundance in the cell and is indispensable for growth (Rhode et al. 1992). The protein participates in diverse nuclear processes, suggesting a global regulatory function. Abf1p-dependent processes include replication (Walker et al. 1990), transcriptional silencing (Diffley and Stillman 1988) and transcriptional activation (Buchman and Kornberg 1990). Abf1p has been implicated in activation of genes involved in amino acid biosynthesis (Martens and Brandl 1994; Künzler et al. 1995), carbon metabolism (Brindle et al. 1990), nitrogen metabolism (Kovari and Cooper 1991), oxidative metabolism (De Winde and Grivell 1992; Trawick et al. 1992) and signal transduction (Halfter et al. 1989). In addition, Abf1p is required for the transcription and translation machinery (Della Seta et al. 1990) and components of the cytoskeleton (Halfter et al. 1989). It has been suggested

Fig. 5A, B Abf1p protects 17 bp on the *HIS7* promoter. **A** DNase I-protection assay. A wild-type *HIS7* promoter fragment was 5'-³²P-labeled, incubated with the enriched Abf1p, treated with DNase I and run on a sequencing gel. The coding and the noncoding strand were analyzed. Lanes A, C, G and T represent the DNA sequence of the *HIS7* promoter of the coding and noncoding strands. Lane 0 shows *HIS7* promoter DNA treated with DNase I. The lanes with Abf1p are arranged according to increasing amounts of protein used. The polyd(A/T) stretch (*pAT*), the d(CT) repeat (*CT*) and the Mcm1p control element (*MCE*) are indicated. The *black box* indicates the Abf1p-protected region within the *HIS7* promoter. The *black arrowhead* shows the DNase I-hypersensitive region. **B** Schematic drawing of the Abf1p-protected region of the *HIS7* promoter. The polyd(A/T) stretch (*pAT*), the d(CT) repeat (*CT*) and the Mcm1p control element (*MCE*) are indicated by *stippled boxes*. The Abf1p-protected part of the *HIS7* promoter is marked with a *black box*. The DNase I-hypersensitive region is indicated with a *black arrowhead*. The consensus Abf1p-binding motif is shown below with the positions that differ *underlined*.

that this functional diversity of Abf1p is due to a general mechanism, such as nucleosome exclusion or DNA bending. Alternatively it has been suggested that Abf1p interacts with different proteins depending on the sequence context at the functional site (Doorenbosch et al. 1992; Rhode et al. 1992). The nucleosome exclusion hypothesis is consistent with the finding that Abf1p is not able to function as a transcriptional activator in vitro, whereas many of the known transcriptional activators, such as Gal4p, Gcn4p, and Rap1p, can

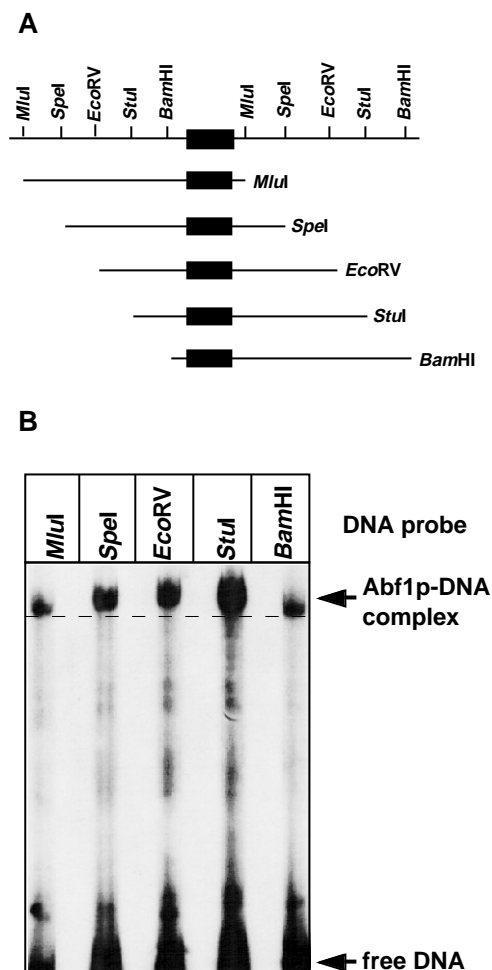


Fig. 6A, B Abf1p induces bending of the *HIS7* promoter. **A** The plasmid pME1400 derived from plasmid pBEND2 (Kim et al. 1989) was digested with the restriction endonucleases *MluI*, *SpeI*, *EcoRV*, *StuI* or *BamHI*, respectively, to obtain DNA fragments with the *HIS7* promoter element (*black box*) at various positions relative to the end of the DNA fragment. **B** The *MluI*, *SpeI*, *EcoRV*, *StuI* and *BamHI* DNA fragments were ^{32}P -labeled and assayed in a gel-retardation experiment. Only the relevant parts of the gel are shown. The relative mobilities of the complexes that were formed with Abf1p were plotted as a function of the relative position of the DNA molecular end for each substrate. Distances migrated were measured from the intact autoradiograph. Relative mobilities were calculated as the mobility of the complex divided by the mobility of the unbound substrate. The bending-angle was determined as 34° using the formula of Ferrari et al. (1992)

(Buchman and Kornberg 1990). This result may indicate that higher-order structures not present in *in vitro* assays are required for Abf1p function.

Polyd(A/T) stretches have been implicated in the promoter function of many protein-encoding genes. In contrast to other *cis*-acting elements, which represent binding sites for transcription factors, polyd(A/T) stretches seem to be involved in transcriptional regulation by virtue of their intrinsic structure. Polyd(A/T) affects nucleosome structure locally and increases the accessibility of transcription factors bound to nearby sequences (Iyer and Struhl 1995). For example, the constitutively expressed *DED1* gene contains two

Abf1p-binding sites, which play an important role in its activation. However, these two Abf1p-binding sites function only weakly on their own. They require a flanking polyd(A/T) stretch to become strong upstream activation sequences. This T-rich element acts synergistically with all other Abf1p-binding sites and with binding sites for other multifunctional yeast activators (Buchman and Kornberg 1990). The polyd(A/T) stretch within the *HIS7* promoter could play a similar role.

We have shown by circular permutation analysis that Abf1p binding to its target site within the *HIS7* promoter induces considerable DNA bending *in vitro*. This result is consistent with earlier findings concerning the *MATa* Abf1p-binding site (McBroom and Sadowski 1994). Abf1p shows a high degree of amino acid sequence similarity to the yeast protein Rap1p (Diffley and Stillman 1988). It has previously been observed that Rap1p also induces a large DNA bend, which is centered outside of the binding site (Gilson et al. 1993). From the point of view of protein function it is interesting to note that both Abf1p and Rap1p induce DNA bends. These multifunctional *trans*-acting factors have both been shown to function in various nuclear processes, such as DNA replication, telomere control and transcriptional activation and silencing (Doorenbosch et al. 1992). DNA bending could be involved in the activity of these proteins by facilitating the formation of specific nucleoprotein structures that are recognized by or formed with other proteins. Alternatively, DNA bending could play a role in organizing a particular chromatin structure by facilitating the phasing of nucleosomes adjacent to the Abf1p- or Rap1p-binding sites.

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