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Regions in the promoter of the yeast *FBP1* gene implicated in catabolite repression may bind the product of the regulatory gene *MIG1*

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We have identified in the promoter of the yeast *FBP1* gene two sites able to bind nuclear proteins. These sites have a nucleotide sequence strongly similar to that of sites which bind the regulatory protein MIG1 in the promoters of *GAL4* and *SUC2*. Deletions performed in the *FBP1* promoter showed that one of the sites contributes to catabolite repression of this gene. In the same promoter, another region was identified with a strong effect on the catabolite repression of *FBP1*. In this region a sequence similar to the consensus for the binding site of the MIG1 protein was also present.

Catabolite repression; Fructose-1,6-bisphosphatase; *MIG1*; *FBP1*; *Saccharomyces cerevisiae*

1. INTRODUCTION

A great variety of enzymes involved in the utilization of carbon sources alternative to glucose are subject to catabolite repression in *Saccharomyces cerevisiae*. The available evidence indicates that the molecular mechanisms underlying catabolite repression are different for different genes [1,2]. Therefore to understand catabolite repression it is important to investigate a variety of genes which may be controlled in different ways. We have undertaken the study of the *FBP1* gene, which codes for the gluconeogenic enzyme fructose-1,6-bisphosphatase. To establish the function of the different regions of the promoter of this gene, we examined the capacity of fragments of the promoter to bind nuclear proteins and studied how deletions in the promoter affect the expression of the gene. We have identified regions which appear to be involved in the repression of transcription by glucose and which contain sequences strongly similar to those known to bind the product of the regulatory gene *MIG1* in other promoters.

2. MATERIALS AND METHODS

2.1. *Strains*

S. cerevisiae CJM 088 (MATα, ura3, can^R) was from our collection and *S. cerevisiae* ABYS1, (MATa, pra1, prb1, prc1, cps1, ade), deficient in vacuolar proteases, was provided by D.H. Wolf (Stuttgart, Germany). *Escherichia coli* HB101 and TG1 were used for the plasmid manipulations.

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2.2. *Media and growth conditions*

Yeasts were grown on Difco yeast nitrogen base supplemented with 2% glucose. Repressed cells were obtained by harvesting them at the end of the exponential phase of growth (ca. 3 mg yeast wet wt/ml). Derepressed cells were prepared by overnight incubation in 1% yeast extract, 1% peptone, and 2% ethanol at a cell density of 20 mg/ml.

2.3. *Transformations*

Competent cells were prepared, stored and transformed by standard techniques [3]. Yeast transformations were performed according to Ito et al. [4].

2.4. *Preparation of yeast nuclear extracts*

Nuclear extracts were obtained from strain ABYS1 as described in [5] using cells collected during the stationary phase of growth.

2.5. *Bandshift and DNase I footprint assays*

For bandshift experiments, protein–nucleic acid complexes were allowed to form in 10 mM Tris-HCl, pH 7.5, 5 mM $MgCl_2$, 2 mM EDTA, 10 mM 2-mercaptoethanol, 1 mM PMSF, 50 mM NaCl, and 12.5% glycerol (v/v). In a volume of 20 μl, nuclear extract (1–4 μl containing 10–40 μg protein) was preincubated with poly dI-dC (1–2 μg) for 15 min in ice. The DNA probe, labelled with the Klenow fragment of DNA polymerase I as described [3], was then added (0.2–0.6 ng, ca 20 000 cpm) and incubation was continued for 30 min at room temperature. Electrophoresis was performed on 4% polyacrylamide gels in Tris-borate, pH 7.6, [3] at 12.5 V/cm for 2.5 h at 4°C. Footprint assays were performed as described [6] but with the incubation conditions of the bandshift experiments.

2.6. *Construction of deletions in the promoter*

Unidirectional 5' deletions in the *FBP1* promoter were performed in the pJJ11 plasmid (Fig. 1) using the exonuclease III/mung bean nuclease system [7]. We used as resistant end in the exonuclease III digestion the *Xho*I site filled in with thionucleotides [8] and as susceptible end the *Sal*I site. At the ends of each deletion we inserted a *Bam*HI linker. The constructions were sequenced by the dideoxy chain-termination method [9] using Sequenase (USB, Cleveland, USA). To obtain 3' unidirectional deletions in the *FBP1* promoter, plasmid pJJ12 was constructed as follows: the *Eco*RI–*Eco*RV fragment from *FBP1* (cf. Fig. 1) was modified at the *Eco*RV junction by

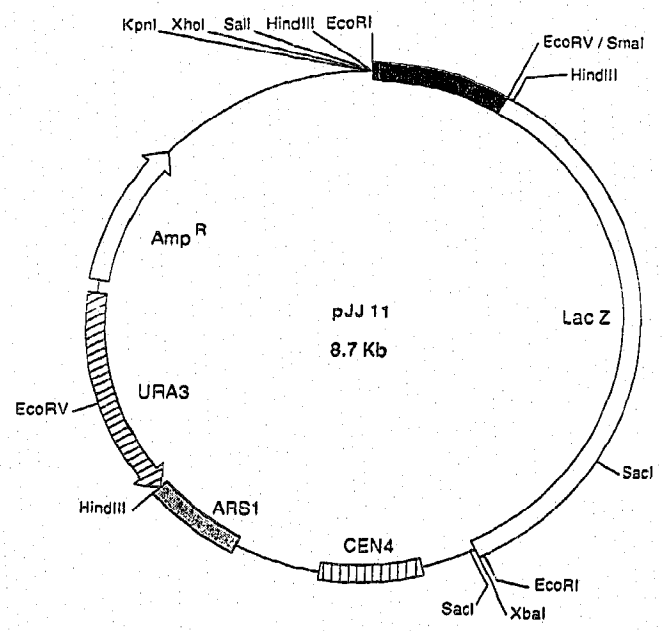


Fig. 1. Structure of the pJJ11 plasmid. The 540 bp *Eco*RI–*Eco*RV fragment containing the *FBP1* promoter from plasmid pRG6 [14] was fused in phase with a 3 kb *Sma*I–*Xba*I fragment carrying the *LacZ* gene. This fragment was originated from YEp 353 [15] and slightly modified so that in the polylinker region only a *Hin*dIII site remains and at the other end *Eco*RI and *Xba*I sites have been introduced. The 5.1 kb *Xba*I–*Eco*RI fragment corresponds to the pUN85 plasmid [10].

addition of a *Bam*HI site and inserted into the *Eco*RI–*Bam*HI site of plasmid pUN75 [10]. The deletions were performed as described above, digesting first pJJ12 with *Sac*I and *Xba*I and inserting *Bam*HI linkers at the end of each deletion. To construct internal deletions, *Kpn*I–*Bam*HI fragments from 3' deleted derivatives from pJJ12 were ligated to 5' deleted pJJ11 derivatives digested with *Kpn*I and *Bam*HI.

2.7. *Assay of β-galactosidase*

Extracts were prepared as described [11] with 20 mM imidazole, pH 7, and the enzyme assayed as in [12]. Protein was determined by the method of Lowry et al. [13].

3. RESULTS

A schematic structure for the *FBP1* promoter is shown in Fig. 2. When the fragments *Eco*RI–*Hin*dIII or *Hin*dIII–*Sau*3A were incubated with a nuclear extract a specific band-shifting was observed (Fig. 3). The precise sequences which bind the nuclear proteins were identified by DNase I footprinting experiments. As shown in Fig. 4, two clear footprinting regions are apparent, centered around the positions −192 and about −425, respectively. These regions define the sites A and B whose sequences are shown in Fig. 4.

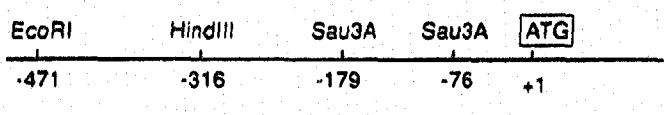


Fig. 2. Structure of the promoter of the *FBP1* gene. Location of the restriction sites is indicated relative to the first ATG of the coding sequence [16].

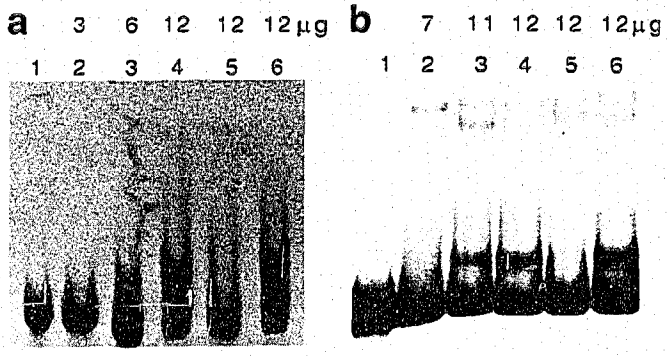


Fig. 3. Protein–DNA complex formation on regions of the *FBP1* promoter. Bandshift assays were performed as described in section 2. The fragments of the *FBP1* promoter used as probes were: panel (a) *Hin*dIII–*Sau*3A (−316 to −179 relative to the ATG); (b) *Eco*RI–*Hin*dIII (−479 to −316). In each panel, samples in lane 1 were incubated without added protein and samples in lanes 2 to 6 were incubated with the amount of nuclear protein indicated. Lanes 5 and 6 show the results of a competition experiment with a 50-fold molar excess of the unlabelled fragment used as probe (lane 5) or of an unrelated DNA fragment of similar length (lane 6).

To determine the role of the binding sites A and B in the expression of the *FBP1* gene, we deleted them and analyzed the activity of the modified promoters using an in-frame gene fusion between the *FBP1* promoter and the *LacZ* gene (Fig. 1). The expression of β-galactosidase directed by the different constructions in conditions of repression and derepression is shown in Fig. 5. Elimination of site A (compare the pairs pJJ11 and pF1550 and pJJ 1158 and pJJ 1121) had a moderate effect on glucose repression as shown by the 3-fold decrease in the ratio of β-galactosidase activity in derepressed and repressed conditions. Elimination of site B (compare pJJ116 and pJJ1144) had no effect on the extent of repression. However, elimination of the B site decreased 4-fold the expression of the gene in derepressed cells. Interestingly, the greatest effect on catabolite repression was observed when the region −480 to −438 of the promoter was eliminated (compare pJJ11 and pJJ116). This indicates the existence of another important regulatory site, site C (see section 4).

4. DISCUSSION

In this study we have identified two sites in the *FBP1* promoter that bind nuclear proteins. Site A participates in catabolite repression since its elimination decreases the extent of repression by glucose although the decrease is only 3-fold. Site B behaves as an upstream activating sequence since its deletion decreases markedly the expression of *FBP1*. These sites have sequences similar to that of sites which bind the regulatory protein MIG1 [17] (see Fig. 6). Based on the sequence of *MIG1* and that of the genes coding for certain mammalian early growth response proteins, Nehlin and Ronne [18]

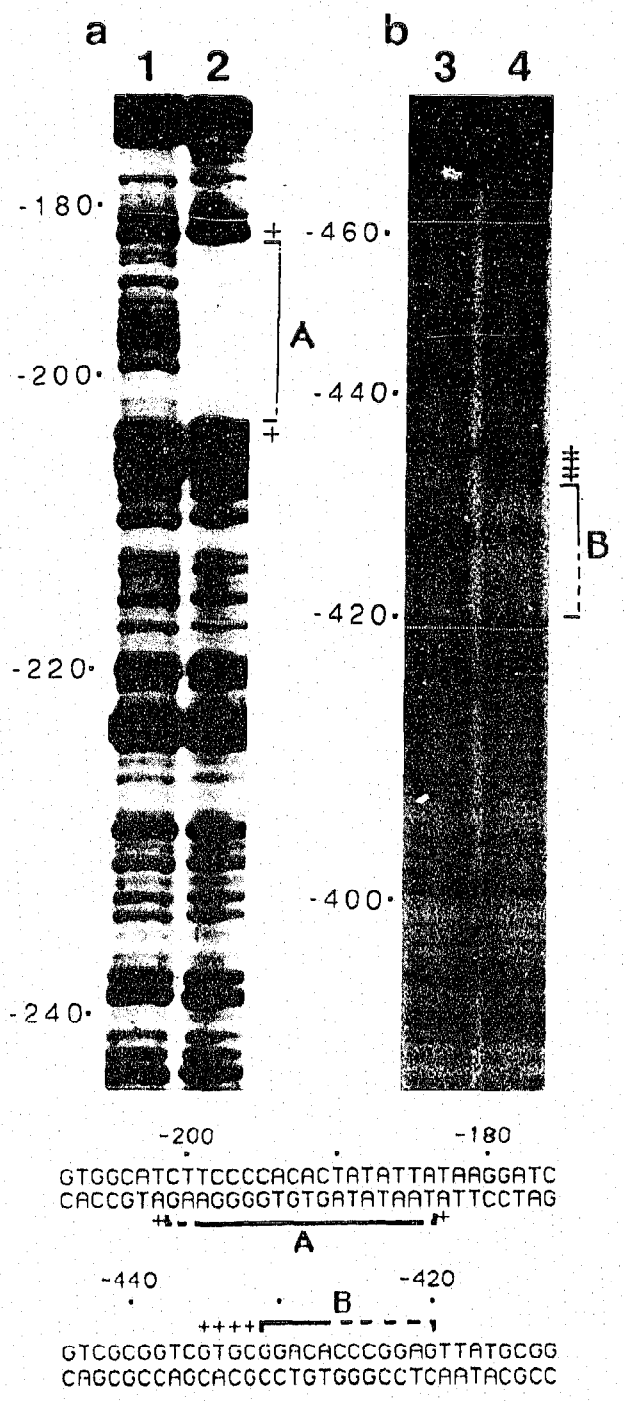
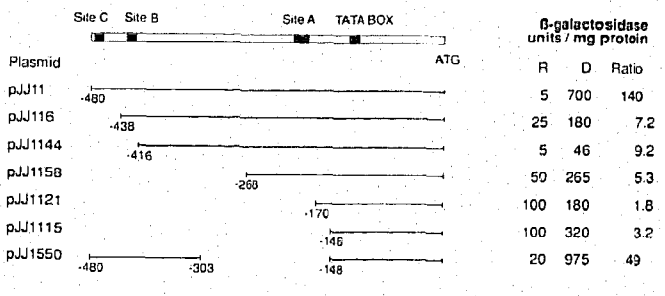


Fig. 4. DNase I footprint analysis of the DNA–protein complexes. The DNA probes were as follows: (a) non-coding strand from −316 to −179 labelled at the *Hin*dIII site; (b) coding strand from −479 to −316 labelled at the *Hin*dIII site. The sample in lane 1 was incubated without added protein and that in lane 2 with 1.4 mg/ml of nuclear protein. The lanes 3 and 4 correspond respectively to free and protein-bound DNA separated in a non-denaturing 4% acrylamide gel after incubation with 1 mg/ml of nuclear protein and DNase treatment (see section 2 for details). Protected and hypersensitive bases are shown as brackets and + signs respectively.



Plasmid	β-galactosidase units / mg protein R	D	Ratio
pJJ11	5	700	140
pJJ116	25	180	7.2
pJJ1144	5	46	9.2
pJJ1158	50	265	5.3
pJJ1121	100	180	1.8
pJJ1115	100	320	3.2
pJJ1550	20	975	49

Fig. 5. Deletions in the *FBP1* promoter and effect on the expression of a fused *LacZ* gene. Deletions were performed as described in section 2. The end point of the deletions is indicated with relation to the first ATG in the coding sequence of *FBP1*. *S. cerevisiae* CJM088 was transformed with the different plasmids and β-galactosidase activity was determined in repressing (R) and derepressing (D) conditions (see section 2).

have proposed that the MIG1 protein may act as a repressor or as an activator depending on the conditions. This appears to happen in the case of *FBP1*.

Examination of the region −480 to −438 in the *FBP1* promoter reveals the existence of another site, site C, with a sequence similar to the MIG1 binding sites (see Fig. 6). Full catabolite repression of *FBP1* requires the −480 to −438 region pointing to a role for site C in the process. However in our footprinting assays, no protection of this sequence was observed. At least 2 explanations may be offered for this fact. One is that this site is very close to the *Eco*RI site, a region that is not well resolved in the gels. The other one is that the site C does not bind the regulatory protein but is necessary for the binding at site B.

Up to now, two other genes with two binding sites for the MIG1 protein have been described. In the case of *GAL4* [17] the sites appear also to play different roles: deletion of one of them relieves the gene from glucose repression while deletion of the other has only a limited effect. Since the data are only available as repression ratios it is not possible to ascertain how the removal of the individual sites affects the level of transcription.

In the case of *SUC2* [18] removal of one binding site [19] has no effect on glucose repression and precise removal of the other one has not been carried out. However the role of MIG1 in catabolite repression of *SUC2*

FBP1 Site A	TCCCCAcAcTAT
FBP1 Site B	caCCCGGAgTTa
FBP1 Site C	aCCCCGGAggTg
Consensus	TCCCCRGATTNT

Fig. 6. Comparison between the sequences of sites A, B and C of the *FBP1* promoter and the consensus sequence described for the binding of the *MIG1* product [17]. Bases which differ from the consensus are written in lower case.

is clear, as strains with a disruption of the MIG1 gene are derepressed for invertase [18].

From the data available it appears that the sequences that could bind the MIG1 protein behave generally as upstream repressing sequences. Upstream repressing sequences have been looked for in genes subject to catabolite repression but without much success. A possible reason for that could be that whenever these sequences exist they are so intertwined with the upstream activating sequences that it is not possible to delete them without interfering with the transcription of the gene. In fact in the promoter of *SUC2*, an heptamer motif which activates transcription [20] is very close to one binding site for the MIG1 protein [18]. Upstream repressing sequences which mediate repression by glucose in the gene *GAL1* have been detected only in an indirect way [21]. Interestingly in two of them, sequences similar to the consensus sequence for binding the MIG1 protein are found.

The *FBP1* promoter appears to be the first clearcut case where deletion of a fragment from the promoter (see pJJ1121 in Fig. 5) nearly abolishes glucose repression while allowing a significant level of expression.

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