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Regulatory regions in the yeast *FBP1* and *PCK1* genes

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By deletion analysis of the fusion genes *FBP1-lacZ* and *PCK1-lacZ* we have identified a number of strong regulatory regions in the genes *FBP1* and *PCK1* which encode fructose-1,6-bisphosphatase and phosphoenolpyruvate carboxykinase. Lack of expression of β-galactosidase in fusions lacking sequences from the coding regions suggests the existence of downstream activating elements. Both promoters have several UAS and URS regions as well as sites implicated in catabolite repression. We have found in both genes consensus sequences for the binding of the same regulatory proteins, such as yAP1, MIG1 or the complex HAP2/HAP3/HAP4. Neither deletion nor overexpression of the *MIG1* gene affected the regulated expression of the *FBP1* or *PCK1* genes.

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

1. INTRODUCTION

The expression of many genes in yeast is repressed by glucose. Repression by glucose of the genes *FBP1* and *PCK1* which encode the gluconeogenic enzymes fructose-1,6-bisphosphatase (FbPase) and phosphoenolpyruvate carboxykinase (PEPCK) is particularly important because these enzymes antagonize the physiologically irreversible steps of glycolysis. The decrease in enzyme levels caused by glucose is parallel to a decrease in the concentration of the corresponding mRNAs [1–3] thus pointing to transcription as the main controlling step of glucose repression. To assess the role of different regions of the respective promoters on the transcription of the genes *FBP1* and *PCK1* we have performed a deletion analysis of *FBP1-lacZ* and *PCK1-lacZ* fusion genes and we have found that a number of regions in both genes might bind the same regulatory proteins.

2. MATERIALS AND METHODS

2.1. *Strains, growth conditions and enzyme assays*

Saccharomyces cerevisiae CJM 088 (MAT α *ura3 can*^{R}) was transformed with different plasmids according to Ito et al. [4]. *S. cerevisiae* strains H190 (MAT a *SUC2 ade2 his3 leu2 trp1 ura3 can*^{R} *mig1-δ2::LEU2*) and H368 (MAT a *SUC2 ade2 his3 leu2 trp1 ura3 can*^{R} *snf1-δ::HIS3 mig1-δ1::LEU2*) are W303-1A congenic and were kindly provided by Hans Ronne (Uppsala, Sweden), who provided also plasmids pHR81 and pMIG1 [5]. Conditions for repression and derepression and assay of β-galactosidase were as described [6]. FbPase and PEPCK were assayed as in [2] and [3]. *Escherichia coli* HB101 and TG1 were used for plasmid isolation.

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2.2. *Construction of gene fusions and deletions in the promoters*

DNA manipulations were done by standard procedures [7]. Gene fusions to *lacZ* for studying the role of coding sequences of *FBP1* and *PCK1* in gene expression were performed using a set of multicopy plasmids constructed by Myers et al. [8].

Deletions in the promoter of a *FBP1-lacZ* fusion gene were constructed using plasmids pJJ11 and pJJ12 and the procedure described earlier [6]. The pJJ13 plasmid contains the sequence coding for the first 109 amino acids of PEPCK fused in-frame with the *lacZ* coding sequence; it was constructed as follows: the 1.65 kb *Sal*I–*Pst*I fragment of the *PCK1* gene was taken from plasmid pMV2 [3] and fused to a 3 kb *Pst*I–*Xba*I fragment carrying the *lacZ* gene (this fragment was originated from YEp 354 [8] and slightly modified to introduce the *Xba*I site), the fusion was then inserted into the centromeric vector pUN85 [9]. Unidirectional 5′ deletions in the *PCK1* promoter were performed in the pJJ13 plasmid using the exonuclease III/mung bean nuclease system [10]. We used as resistant end in the exonuclease III digestion the *Xho*I site filled-in with thionucleotides [11] and as susceptible end the *Sal*I site. At the ends of each deletion we inserted a *Bam*HI linker. To obtain 3′ unidirectional deletions in the *PCK1* promoter, plasmid pJJ14 was constructed as follows: the 1.7 kb *Sal*I–*Bam*HI fragment from *PCK1*, taken from pMV2 [3] was inserted into the *Sal*I–*Bam*HI site of plasmid pUN75 [9]. The deletions in pJJ14 were performed as described above, using as resistant end the *Sac*I site and as susceptible end the *Xba*I site. *Bam*HI linkers were also inserted at the end of each deletion. To construct internal deletions, *Kpn*I–*Bam*HI fragments from 3′ deleted derivatives from pJJ14 were ligated to 5′ deleted pJJ13 derivatives digested with *Kpn*I and *Bam*HI.

Sequencing was done by the dideoxy chain-termination method [12] using Sequenase (USB, Cleveland, USA).

3. RESULTS AND DISCUSSION

While a sequence of 640 bp upstream of the first ATG of the coding sequence was available for the *FBP1* promoter [13], only about 340 bp of the 5′-non-coding region had been sequenced in the case of *PCK1* [14]. Since important regulatory sites are often found further upstream, we sequenced the *PCK1* promoter up to position −645 (Fig. 1).

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        CACT TGGCAGAGCC CCCACCCAGG CCTTGTCGGA AAAAATCGGA ATATCCCACA
          -640                                  -610
CGATCCACCG GAGAATATCG GACGCTGACG GGTGAATGGA GATCTGGATT TCCTAATTTG
          -580                                  -550
GATACATCTC TTTTCTTTTT TTGACTCACA ATAGGAAAAA ACCGAGCTTC CTTTCATCCG
          -520                                  -490
GCGCGGCTGT GTTCTACATA TCACTGAAGC TCCGGGTATT TTAAGTTATA CAAGGGAAAG
          -460                                  -430
ATGCCGGCTA GACTAGCAAG TTTTAGGCTG CTTAACATTA TGGATAGGCG GATAAAGGGC
          -400                                  -370
CCAAACAGGA TTGTAAAGCT TAGACGCTTC TGGTTGGACA ATGGTACGTT TGTGTATTAA
          -340                                  -310
GTAAGGCTTG GCTGGGGATA GCAACATTGG GCAGAGTATA GAAGACCACA AAAAAAAGGT
          -280                                  -250
ATATAAGGGC AGAGAAGTCT TTGTAATGTG TGTAACTTCT CTTCCATGTG TAATCAGTAT
          -220                                  -190
TTCTACTTAC TTCTTAAATA TACAGAAGTA AGACAGATAA CCAACAGGCT TTCCCAGATA
          -160                                  -130
TACATATATA TCTTTATTTC AGCTTAAACA ATAATTATAT TTGTTTAACT CAAAAATAAA
          -100                                   -70
AAAAAAAAAC CAAACTCACG CAACTAATTA TTCCATAATA AAATAACAAC ATGTCCCCTT
           -40                                   -10
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Fig. 1. Sequence of the *PCK1* promoter. Consensus sequences for the binding of regulatory proteins are underlined and are discussed in the text.

To assess the role of different regions of the *FBP1* and *PCK1* genes on glucose-regulated transcription, in-frame fusions of different truncated versions of the promoters of the *FBP1* and *PCK1* genes with the *E. coli lacZ* gene were constructed and β-galactosidase levels were measured in conditions of repression and derepression.

The expression of β-galactosidase from fusion genes containing promoters with different 3′ ends is shown in Table 1. A *FBP1* fusion gene carrying the entire promoter region did not express β-galactosidase; however expression was observed when the first 57 bp of the coding sequence were included in the fusion. This expression was regulated by glucose. A similar phenomenon occurred for the *PCK1* gene, but in this case the length of the coding sequence necessary to allow transcription was greater, expression being observed only when the fusion contained the first 327 bp of the coding region. These results suggest that downstream activating sequences are necessary for transcription to take place.

Table 1

Expression of different *FBP1-lacZ* and *PCK1-lacZ* gene fusions

Fusion	bp from *FBP1* or *PCK1* coding region	β-galactosidase (mU/mg protein)	
		Glucose	Ethanol
FBP1-lacZ	0	<1	<1
	57	25	11,000
PCK1-lacZ	0	<1	<1
	210	<1	<1
	327	55	26,000

Fusions were performed by fusing in-frame with the *lacZ* gene of the multicopy vectors described in [8] truncated *FBP1* or *PCK1* promoters starting at positions −480 and −550, respectively, and with different lengths of coding sequence. *S. cerevisiae* CJM088 was transformed with the different plasmids and β-galactosidase activity was determined in repressing (glucose) and derepressing (ethanol) conditions [6].

To study the effect of upstream and internal deletions in the promoters on gene expression, it is important to avoid interferences due to differences in copy numbers, therefore centromeric plasmids were used for these experiments. Fig. 2 illustrates the results with the *FBP1* promoter. A deletion from −480 to −438 caused a marked decrease in expression, indicating the presence of an upstream activating sequence. Simultaneously, the degree of repression by glucose decreased strongly showing that a regulatory sequence is also located in this region. Although both sequences are drawn separately in the figure, it is possible that the same sequence performs both functions. The presence of another upstream activating sequence is indicated by the decrease in expression caused by a further deletion from −438 to −416. In this case the extent of glucose repression remained unchanged. The next two deletions up to position −369 had no effect but deletions from −369 to −317 and from −317 to −308 increased maximal expression 2- and 3-fold, respectively, indicating that different upstream repressor sequences have been removed. Successive deletions between −308 and −268 had not a marked

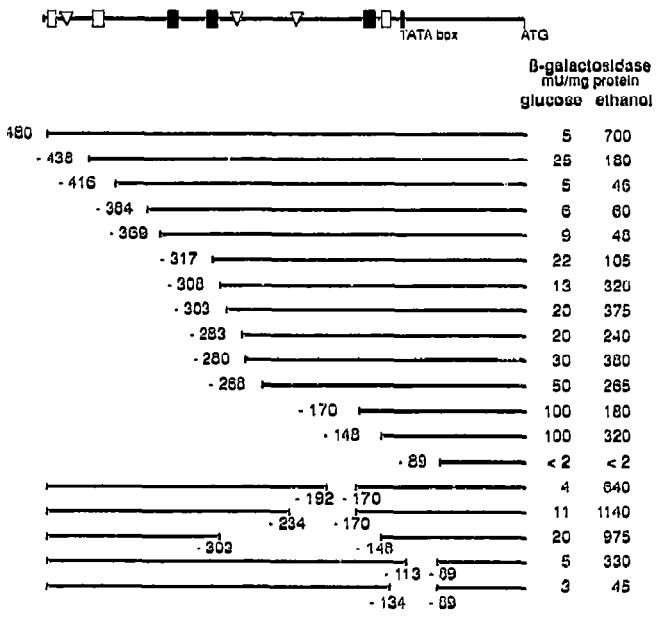

Fig. 2. Deletions in the *FBP1* promoter and their effect on the expression of a fused *lacZ* gene. Deletions were performed in the pJJ11 plasmid [6] as described in Section 2; their end points are indicated with relation to the first ATG in the coding sequence. Regulatory regions are indicated as (□) UAS, (■) URS, (▽) element affecting catabolite repression.

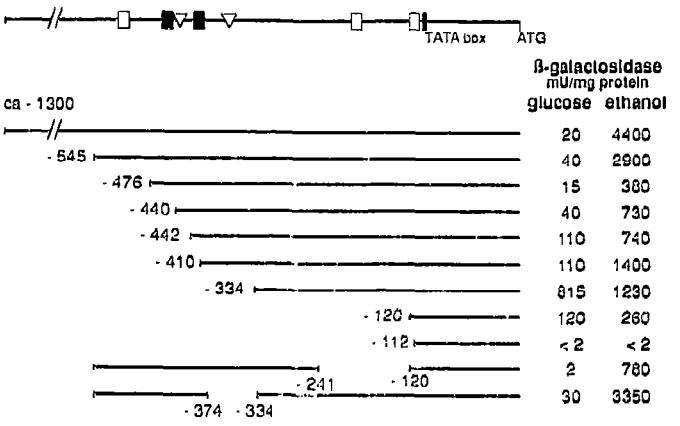

Fig. 3. Deletions in the *PCK1* promoter and their effect on the expression of a fused *lacZ* gene. Deletions were performed in the pJJ13 plasmid [6] as described in Section 2; their end points are indicated with relation to the first ATG in the coding sequence. Regulatory regions are indicated as (□) UAS, (■) URS, (▽) element affecting catabolite expression.

effect on β-galactosidase activity in derepressing conditions but increased progressively its activity in conditions of repression pointing to the existence of a site implicated in catabolite repression in this region. A further regulatory site should be present between positions −268 and −170 since removal of this region decreased glucose repression to less than 2-fold. Finally deletion from −170 to −148 allows to situate a third upstream repressing sequence. A truncated promoter with only 25 bp upstream of the TATA box was sufficient to direct transcription at about one-half the rate directed by the intact promoter suggesting that a strong upstream activating sequence is located within these 25 bp. It cannot be excluded, however, that transcription is driven by a downstream activating sequence in the *FBP1* gene. This sequence would not operate in the promoter extending only to position −89 due to removal of the TATA box.

The results of the internal deletions point to a redundant role for the regulatory sequences implicated in catabolite repression, since strong glucose repression was still observed when one or even two of these sequences were removed, as long as the sequence upstream of −303 was present. A deletion between positions −113 and −89, downstream of the TATA box decreased expression only 2-fold; when the deletion included the canonical TATA box, expression decreased by about 7-fold but it was not completely abolished; it is likely that non-standard TATA boxes are able to drive regulated transcription at a slow rate.

The *PCK1* promoter shows also a high degree of complexity (Fig. 3). There is a strong upstream activating sequence between positions −545 and −476 followed by upstream repressing sequences between −476 and −440 and −422 and −410. Most interesting are sites related with glucose repression located between −440 and −422 and −410 and −334. Removal of this last site did not affect β-galactosidase activity in derepressing conditions but increased activity up to 8-fold in a glucose-grown yeast. Two further upstream activating sequences are located between −334 and −120 and just in front of the TATA box. Removal of the first one by an internal deletion decreased expression about 4-fold. The fact that the internal deletion −374, −334 had no effect on expression could suggest that the regulatory region between positions −410 and −334 is located upstream of −374 or, alternatively, that it plays a redundant role with the other regulatory sequence located further upstream.

Since FbPase and PEPCK are regulated in parallel it could be expected that the promoters of the corresponding genes would have similar regulatory elements. Although no obvious homologies exist between the sequences of the *FBP1* and *PCK1* promoters, a detailed study shows the presence of a number of sites which could bind the same regulatory proteins. Sequences resembling the binding sequence for protein yAP1 [15] are found in two of the UAS regions of *FBP1* and in one UAS region of *PCK1* (Fig. 4A); there is also a CCAAT box at positions −264 (in the non-coding strand) and −266 in the *PCK1* and *FBP1* promoters respectively and consensus regions for binding the regulatory protein complex HAP2/HAP3/HAP4 [16] are found in both genes (Fig. 4B). Although there are in both promoters binding regions for the general transcription factor GRF1 [17] (Fig. 4C), two of the elements which could bind GRF1 are situated far upstream in the promoters

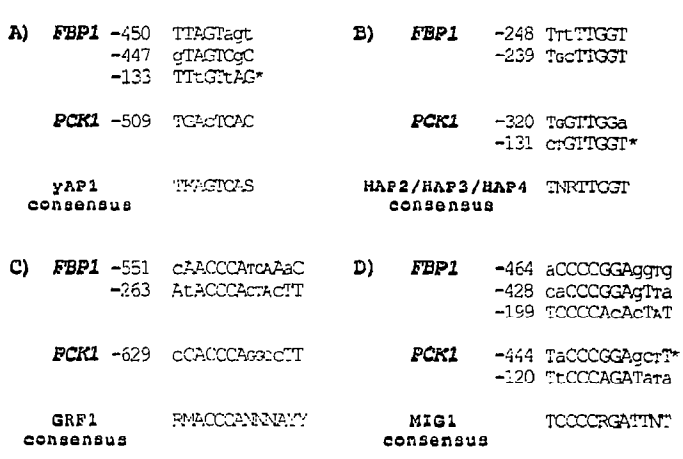

Fig. 4. Comparison between sequences in the *FBP1* and *PCK1* promoters and consensus sequences described for the binding of different regulatory proteins: yAp1 [15], HAP2/HAP3/HAP4 [16], GRF1 [17] and MIG1 [19]. K stands for G or T; M for A or C; R for A or G; S for C or G, and Y for C or T; N represents any base. Bases which differ from the consensus are written in lower case, those in N positions are in small capitals. Numbers refer to the first base of the shown sequence. * The sequence corresponds to the non-coding strand.

and appear to play at best a marginal role: in the case of the *PCK1* fusion gene the removal of the GRF1 binding site only slightly decreased β-galactosidase expression (compare the first two constructions of Fig. 3), while the elimination of a similar site in the *FBP1* promoter did not change the amount of FbPase expressed (see Table I in Ref. [2]). It should be observed also that the GRF1 binding site operates in many cases together with a CTTCC motif [18] and such a motif is not present in the *FBP1* and *PCK1* promoters.

As shown in Fig. 4D binding regions for the MIG1 protein [19] can be found in the two genes and removal of these regions had marked effects on gene expression (see Figs. 2 and 3). Unexpectedly, regulation of the expression of *FBP1* and *PCK1* was not affected in a strain carrying a deletion of the *MIG1* gene and regulated expression of β-galactosidase from the fusion gene *FBP1-lacZ* was also independent of the presence of the MIG1 protein (Table II). Moreover we did not observe differences between the $MIG1^+$ and mig^- strains in the kinetics of derepression of FbPase and PEPCK in a rich medium with glucose upon glucose exhaustion. These results would suggest that MIG1 is redundant with some other repressory protein(s). The situation differs from that of other genes regulated by catabolite repression, such as *SUC2* or *GAL1*, for which glucose repression is strongly reduced by a deletion of *MIG1* although not totally eliminated [5,20]. While overexpression of *MIG1* has a marked repressing effect on the *SUC2* and *GAL* genes [5], we found that overexpression of *MIG1* driven by the pMIG1 plasmid [5] did not reduce significantly the derepressed levels of FbPase and PEPCK. In addition, while defects in the *CAT4* gene (allelic to *MIG1*, H. Ronne, personal communication) allowed the

Table II

Effect of MIG1 on the expression of *FBP1*, *PCK1* and the *FBP1-lacZ* fusion gene

Strains	FbPase		PEPCK		β-galactosidase	
	R	D	R	D	R	D
W303-1A/pJJ11 ($MIG1^+$)	<3	60	9	170	9	720
H190/pJJ11 ($mig1^-$)	<3	55	8	200	9	980

Enzyme levels were determined in repressing (R) and derepressing (D) conditions [6] in strains transformed with the pJJ11 plasmid. Activities are expressed in milliunits per mg protein.

expression of *SUC2* and *MAL* genes in a *cat1* background [21], we did not observe any FbPase or PEPCK derepression in strain H368 which has deletions in both the *MIG1* and *CAT1* (=*SNF1*) genes.

Since MIG1 has been described as a repressing protein it was surprising to find consensus binding sites for MIG1 in UAS elements of the *FBP1* and *PCK1* promoters. A possible explanation would be the existence of some activating protein(s) with a Zn-finger sequence similar to that of MIG1, and which could therefore bind to sequences related to those recognized by MIG1. This would also explain the paradox of the large effect observed on the expression of the fusion genes upon removal of MIG1 binding sequences and the absence of effect of a disruption in the *MIG1* gene.

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