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Transcriptional control of yeast phosphofructokinase gene expression

J. Heinisch, K. Vogelsang and C.P. Hollenberg

Institut für Mikrobiologie, Heinrich-Heine-Universität Düsseldorf, Universitätsstr. 1, Geb.: 26.12, D-4000 Düsseldorf 1, Germany

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We here provide the complete nucleotide sequences of the 5'-non-coding regions of the yeast phosphofructokinase genes, *PFK1* and *PFK2*. *lacZ* fusions of the *PFK1* and *PFK2* promoters were constructed and a deletion analysis was performed. In contrast to other glycolytic gene promoters, no strong regulatory elements could be found. However, we detected moderate UAS and URS functions. In general, the effects on expression upon deletion of these regions were more pronounced on media containing ethanol than on those containing glucose as carbon sources. Overexpression of either one of the *PFK* genes led to a decreased enzymatic activity in a wild-type background but did not affect transcription from the promoters.

Promotor; *Saccharomyces cerevisiae*; Glycolytic gene

1. INTRODUCTION

Yeast phosphofructokinase (PFK) is an octameric enzyme, composed of 4α- and 4β-subunits [1], encoded by the genes *PFK1* and *PFK2*, respectively. Both genes have been cloned and their open reading frames have been sequenced [2,3].

From the data available on PFK, only the heterooctameric enzyme appears to be fully catalytically active in yeast. This implicates that the cell has to produce approximately equal amounts of each subunit at any time. A simple way to achieve that would be a coordinate control of the transcription of *PFK1* and *PFK2*. Furthermore, as PFK accounts for about 1% of the total soluble protein of a yeast cell [4], the genes are strongly transcribed. The transcriptional regulation of other glycolytic gene promoters has been extensively studied. Most of them contain a consensus binding site for RAP1, a protein implicated in transcriptional activation of glycolytic and other well expressed genes [5], as well as in transcriptional silencing at the *HML* and *HMR* loci [6]. One or more consensus binding sites for this protein have been found in the promoters of yeast *TDH1* [5], *PGK1* [5], *GPM1* [7], *ENO1* [8], *PYK1* [9], *PDC1* [10], and *ADH1* [11]. Binding of RAP1 is also likely in the promoter region of *TPI1* [12]. The degree of transcriptional activation seems to be dependent on the context of each promoter [13]. In the cases of *PGK1* [13] and *ENO2* [8], another multifunctional protein, ABF1, implicated in transcriptional activation or silencing [14,15] was shown to bind adjacent to or overlapping with the RAP1 binding sites. A consensus sequence for binding of this factor was also detected in the *PYK1* promoter [13]. How these factors interact with each other and with other transcriptional regulators that do not bind DNA by themselves (i.e. GCR1 [4,16,17]) is not yet understood.

We here provide data on the nucleotide sequences of the yeast *PFK* promoters and a deletion analysis to assess the role of consensus sequences found. We also compare the promoter organizations to those of other glycolytic genes.

Correspondence address: J. Heinish, Institut für Mikrobiologie, Heinrich-Heine-Universität Düsseldorf, Universitätsstr. 1, Geb.: 26.12, D-4000 Düsseldorf 1, Germany. Fax: (49) (211) 311 5370.

2. EXPERIMENTAL

2.1. *Strains and media*

The yeast strain AMW-13C$^-$ (*MATa leu2-3, 112 his3-11,15trp1(FS) ura3(FS) can1*; where 'FS' designates frameshift mutations) was kindly provided by Malcolm Whiteway (Montreal, Canada) as a cir^0 strain and made cir$^+$ in this laboratory. Strain KHTD-5A (*MATa mig1::URA3 ura3-52 leu2-3, 112 trp1*) was a gift from Klaus Huse (Darmstadt, Germany) and used to introduce the *mig1* deletion into strains carrying *lacZ* fusions by standard genetic procedures [18]. Media and growth conditions were as described earlier [2].

For plasmid isolation the *E. coli* strain DH5αF' was used.

2.2. *Manipulations of DNA, sequencing and plasmids used*

Standard molecular procedures were applied in most steps [19]. For exonuclease III digestion, the kit of Pharmacia (Freiburg) was used, according to the instructions of the manufacturer. For sequencing, the kit of Pharmacia (Freiburg) was used, which is based on the dideoxy-chain-termination method of Sanger [20]. To determine the endpoints of promoter deletions, only short readings were necessary. Therefore, we added $MnCl_2$ to a final concentration of 5 mM to the sequencing reactions. Furthermore, 10% polyacrylamide sequencing gels were used with glass plates of 20 × 20 cm and spacers of 0.25 mm. Gels were run at a constant 15 W (750 V) for 1 h. As a sequencing primer, an 18-mer oligonucleotide homologous to a region 40 bp upstream of the polylinker region of the *lacZ* fusion vectors was used [21].

Fusions of the *PFK* promoters to the *lacZ* coding sequence were obtained in a set of integrative vectors constructed by Myers et al. [21], using suitable restriction sites (see Fig. 2).

Plasmid pKHD3 contains a deletion/substitution mutation in the *mig1* gene [22] and was kindly provided by Klaus Huse (Darmstadt). By restriction digestion with *Mlu*I and *Xba*I it yields a fragment

suitable for substitution of the genomic *MIG1* copy with the mutation containing a *URA3* marker [23].

2.3. *Enzymatic analysis*

β-galactosidase activities were determined according to published procedures [24] in crude extracts obtained as described previously [2]. Crude extracts were not subjected to centrifugation prior to the reaction, but afterwards. Protein was determined after a modification of the method of Zamenhoff [25].

3. RESULTS AND DISCUSSION

3.1. *Sequencing of the PFK1- and PFK2-promoters*

Published work [3] contains little information on the 5'-non-coding regions of the two *PFK* genes. The *PFK1* sequence was determined up to −260 bp relative to the ATG translation start codon and the *PFK2* sequence up to −420 bp. As important binding sites for regulatory proteins have been found further upstream in several glycolytic gene promoters [9,13,17], we have now sequenced both strands of the 5'-non-coding regions up to −961 bp and −1276 bp for *PFK1* and *PFK2*, respectively (Fig. 1). Both sequences are AT-rich. In fact, of the 500 bp preceding the ATG translation start codon, 74% and 68% are A- or T-residues in *PKF1* and *PKF2*, respectively. Several consensus sequences could be detected (if not stated otherwise, such sequences were taken from [26]; the positions in the *PFK* genes are numbered relative to the ATG translation start codon): overlapping ABF1 consensus binding sites are located at −930 bp on both strands in the *PFK1* promoter (Fig. 1A). Apart from that, no obvious homologies to any DNA motifs known to interact with regulatory proteins could be found in this promoter. In contrast, the *PFK2* promoter contains four such sequences: a sequence known to confer cell cycle regulation (5'-GCCACAC-GAAAA-3', the CCBF-box) is located at −838 bp. Recently, a new transcriptional repressor protein, MIG1, and its consensus DNA binding site has been described [22]. A sequence located at −658 bp in the *PFK2* promoter coincides with this consensus (5'-ATCCGGGG-3'). Finally, we found a consensus ABF1 binding site at −616 bp and a consensus RAP1 binding site at −530 bp in the same promoter (Fig. 1B).

3.2. *Deletion analysis*

To gain an insight into the role of these promoter elements for the transcription of the *PFK* genes, we performed a deletion analysis using in frame *lacZ* fusions. All deletions described below were integrated at the *LEU2* locus in strain AMW-13C$^+$. Only single copy integrations as confirmed by Southern analysis (data not shown) were used for further testing. First, we observed an influence of the fusion point of *lacZ* to *PFK1*. A fusion retaining 185 amino acids of the α-subunit showed similar β-galactosidase activities on media containing glucose as on media containing ethanol as carbon sources. This reflects the wild-type situation, where the heterooctameric PFK enzyme has the same specific activities on both media [4]. However, a fusion retaining only 13 amino acids showed 2- to 3-fold reduced β-galactosidase activities, with higher values on ethanol than on glucose (Fig. 2A). For *PFK2*, only a fusion retaining 162 amino acids of the β-subunit was tested. There, similar β-galactosidase activities were obtained on glucose and on ethanol media (Fig. 2C). *PGI1–lacZ* fusions have been reported to drastically increase in β-galactosidase activities when approaching stationary phase [27]. This was not observed in the *PFK–lacZ* fusions.

The *lacZ* fusions were then used to construct promoter deletions starting from the 5'-end using exonuclease III. Their effects on β-galactosidase expression are summarized in Fig. 2. A deletion of the 5' 36 bp in the *PFK1*-promoter, spanning the consensus ABF1 sequence, led to a 2-fold lower expression. Further deletion to −896 bp defined a putative UAS element, as the values were reduced. The effect was more pronounced on glucose than on ethanol media. This region is followed by an URS element, as deleting sequences upstream of −645 bp cause an increase in specific β-galactosidase activities that is more pronounced on ethanol media than on glucose. Deletions downstream from −400 bp then lead to a decrease in expression down to the level of detection at −41 bp (Fig. 2B). This seems to be related to the consecutive deletion of several T-rich elements in this region. Such sequences have been proposed to function in constitutive promoters (see [28] for a review). No known consensus binding sites for transcriptional regulators could be detected except for the ABF1 binding site mentioned above.

As there was a significant decrease in expression in the shorter *lacZ* fusion of *PFK1*, we tried to determine which sequence within the N-terminal part of the PKF-α-subunit is responsible for this effect. Therefore, deletions were introduced starting at the *lacZ* fusion point protruding into the *PFK* sequence (Fig. 2B). There, the drop in β-galactosidase activities on both carbon sources was observed between the deletions at +484 bp and +286 bp. The only homology to any consensus sequences in this region of the DNA is an intron-specific 'TAC-TAACA-box' at +257 bp. However, no consensus sequences for intron boundaries (see [29]) could be detected in the flanking sequences.

Some constructs with shorter and longer *lacZ* fusions of *PFK1* were checked by Western blot analysis, using polyclonal antibodies against β-galactosidase. The results correlated with the measured enzymatic activities in that the shorter fusions showed significantly weaker signals than the longer ones (not shown).

In deletions starting from the 5'-end of the *PFK2* promoter (Fig. 2C) less drastic effects on the β-galactosidase activities were observed than in the *PFK1* promoter. A region with moderate UAS functions is located between −521 and −381 bp. This is followed by a URS element, with more pronounced effects on ethanol than

***PFK1* promoter**

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  -955          -940          -920          -900          -880
GCATGCCTGGTTCAATCTACGGGTTACAGTCATCGTTGAAAACGATGGAAAAACATGTCGAGATTCCTCAATCCATACACCATTATAGTCCGTTTTATCA

  -855          -840          -820          -800          -780
GCTTCCACTAATTTTTTAAATCTCAGTTTCTTCTTGAAATTTAGCATCGTGCATGGGATAGCGGCTAGTAAAAAAGAAAATTAATATCTCATTAACAAAG

  -755          -740          -720          -700          -680
TTATTGTACATAATCCGGTACAATATTCTTCAATGTACTCTCTAATATCGAGCACACTGGCAATATTCATGCACACATTCGCCTAATGCTGACGAATGCT

  -655          -640          -620          -600          -580
TAATCAGTGCAATTACTGCCACCCTCTTGATATGTGGGCTAAATCCTTTAGGACCTGTAAAAAATGCAATCACGTTTTCACATTTTTTTTTTTCTTGCGG

  -555          -540          -520          -500          -480
AATTGCGGAATTTCCCAGTTGGCAGCGTTATCCGATTTGAGATCGACTTGCATCAACCTTTGAAAAATATAAGGATGAGAAAGTGAAATCGGTTTTTTT

  -455          -440          -420          -400          -380
TTCCATTGTCGTCATCAACATGATTTTTTAAATAAATAAATACGATTTTTTATTTTTTTCCCTTCTTTGTTTTTGTTTTGCTTATTCCCATCTTCATTA

  -355          -340          -320          -300          -280
TTAAATTCTTCCGCTCTTAATAAAGGAGTTTTTTTATTATCTTCTTGTGTAATCATCCTTTTTCTTTAATTTTCTTCCTTTTCTTTTTCTCTTTACTGGT

  -255          -240          -220          -200          -180
TTTTTTACTTCTTTATTCTCAACCATCTAAAGAATATTATTGCTTTCTACCAATAAAATCTGTTAATTCTATTTGGATTGTCGTCTACTCAAGTCTCGCC

  -155          -140          -120          -100           -80
TAGTAAATAAACGATAAACAAATTTGAAGTAAGAATAACAATATAGGGAGAGAAATTTTTCTATTTTTAATTTCGAAACAGGTACCAAAAAATCTAAGTT

   -55           -40           -20           +1            +20
CACTTTAGCACTATTTGGGAAAGCTTTTATATAAAAAATCTGAAACAAAATCATATCAAAGATGCAATCTCAAGATTCATGCTACGGTGTTGCATTCAGA

   +45           +60           +80          +100          +120
TCTATCATCACAAATGATGAAGCTTTATTCAAGAAGACCATTCACTTTTATCACACTCTAGGATTTGCAACTGTGAAAGATTTCAACAAATTCAAACATG

  +145          +160          +180          +200          +220
GTGAAAATAGCTTACTATCTTCAGGGACTTCCCAAGATTCCTTGAGAGAAGTTTGGTTAGAATCTTTCAAGTTGAGTGAGGTTGATGCTTCTGGGTTCCG

  +245          +260          +280          +300          +320
TATACCACAACAAGAAGCTACTAACAAGGCTCAAAGTCAAGGTGCTCTATTAAAGATTCGTTTAGTGATGTCTGCTCCAATCGATGAAACTTTCGACACC

  +345          +360          +380          +400          +420
AACGAAACCGCCACAATCACTTATTTCTCTACTGATTTGAACAAGATTGTCGAGAAATTCCCAAAACAAGCCGAAAAATTGTCCGATACCTTAGTGTTTT

  +445
TGAAAGATC
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***PFK2* promoter**

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 -1270          -1250          -1230          -1210          -1190
AGATCTGTTACTCTCCAGAAAATAGTCGTCATGATGGCCACCACTTCCGGCACCGCTTCTCATATCCTCCCCCTCTTCCTCCTGAATAGCCTTGTAATGG

 -1170          -1150          -1130          -1110          -1090
ATGGTTAGATGGGCAGATATGGGTCTATTGCTACTGCTACTCAGCATGGTTATATTCTGTTGTCTTCTTTACTTTAAGGCGAAAGGTCAGACAATATGCA

 -1070          -1050          -1030          -1010           -990
TATATATATATAGTCTTCGAATGCACGGCAAAAATGATATGAAGAGCTATGCTACAGTATATACTTCTTTGTTATTGCAATCAACGTGAGCCTTAACCAA

  -970           -950           -930           -910           -890
TGAGGAAGTAAGAAGGAAATAAGAAGTAAGACATAAAATAAAACTACAAAAAAGAAAGAAGATCCGCAAAAACCCAAGAACAGCATTGTAAGTACTACCA

  -870           -850           -830           -810           -790
TCTATATAGATCGAATATCCTACTAATATATAGTAACTGCCACACGAAAAACTGTGGGTGTAAAGACTATTCATAGCCATTCTCTGCTGCTTTGTTGCAT

  -770           -750           -730           -710           -690
CGTATCATCGCCCCGTTTCAGCCAGTCCCGCATACCCCCTTTGCAACGTTAACGTTACCGCTAGCGTTTACCATCTCCACGCTAAAAGGAAAACGAAGATT

  -670           -650           -630           -610           -590
AAGATAAAGTTGGGTAAAATCCGGGGTAAGAGGCAAGGGGGTAGAGAAAAAAAAACCGGAGTCATTATATACGATACCGTCCAGGGTAAGACAGTGATTT

  -570           -550           -530           -510           -490
CTAGCTTCCACTTTTTTCAATTTCTTTTTTTCGTTCCAAATGGCGTCCACCCGTACATCCGGAATCTGACGGCACAAGAGCCGATTAGTGGAAGCCACGG

  -470           -450           -430           -410           -390
TTACGTGATTGCGGTTTTTTTTTCCTACGTATAACGCTATGACGGTAGTTGAATGTTAAAAACGAAAACAGAGATATTGAATTGATCAATTTGATCAGTA

  -370           -350           -330           -310           -290
CGTATGTAATCTTTTGTTTGCCATGTTAAATCGGCCTTTTTTTCCATCCGGTCTTTATCTACCATTCATTTATTACATCGCAATGAAGTCTCTCGCTTCA

  -270           -250           -230           -210           -190
TGTTGTTGTTTTTTTTCCTGCCTTTTTTTTATCCGCTCTCATTCTTATTTTTCCTACTTGCATTTTATAAAACAATAATATTTTTTTCTTCTTTTTGTCC

  -170           -150           -130           -110            -90
TTTATTTATTACTTCCTTGATTAAAAGCTTCTTATCTTCGGTTTCATAAAGAAGAAAATTCCTTTTTTTTTTTTTTTTTCCTTCTTCTTGCTTAAAAGCC

   -70            -50            -30            -10     +1
TTTCTTATACCTCATTTGAACAATAGAACTAGATTTAGAGACTAGTTTAGCATTGGCCAAGAACTAACCATACGCAATG
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Fig. 1. Sequences of the promoters of the *PFK* genes. Consensus sequences for interactions with DNA binding proteins are underlined (see text for details). For clarity, the part of the *PFK1* coding region discussed in the text is included. The complete nucleotide sequences of both genes and their promoters are available in the EMBL database under the accession numbers: SCPFK1AA.EMNEW M26943 and SCPKF1AA.EMNEW M26944.

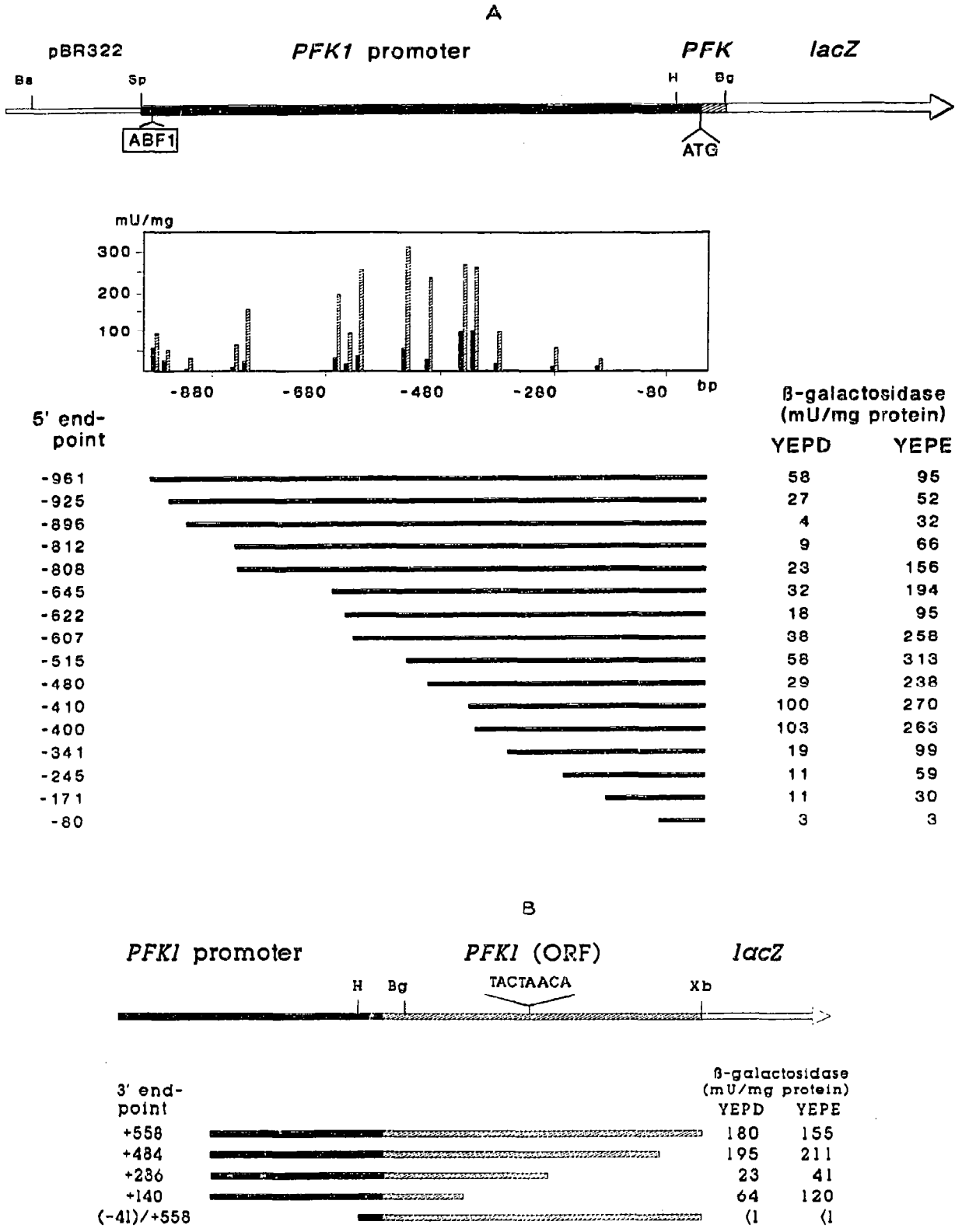
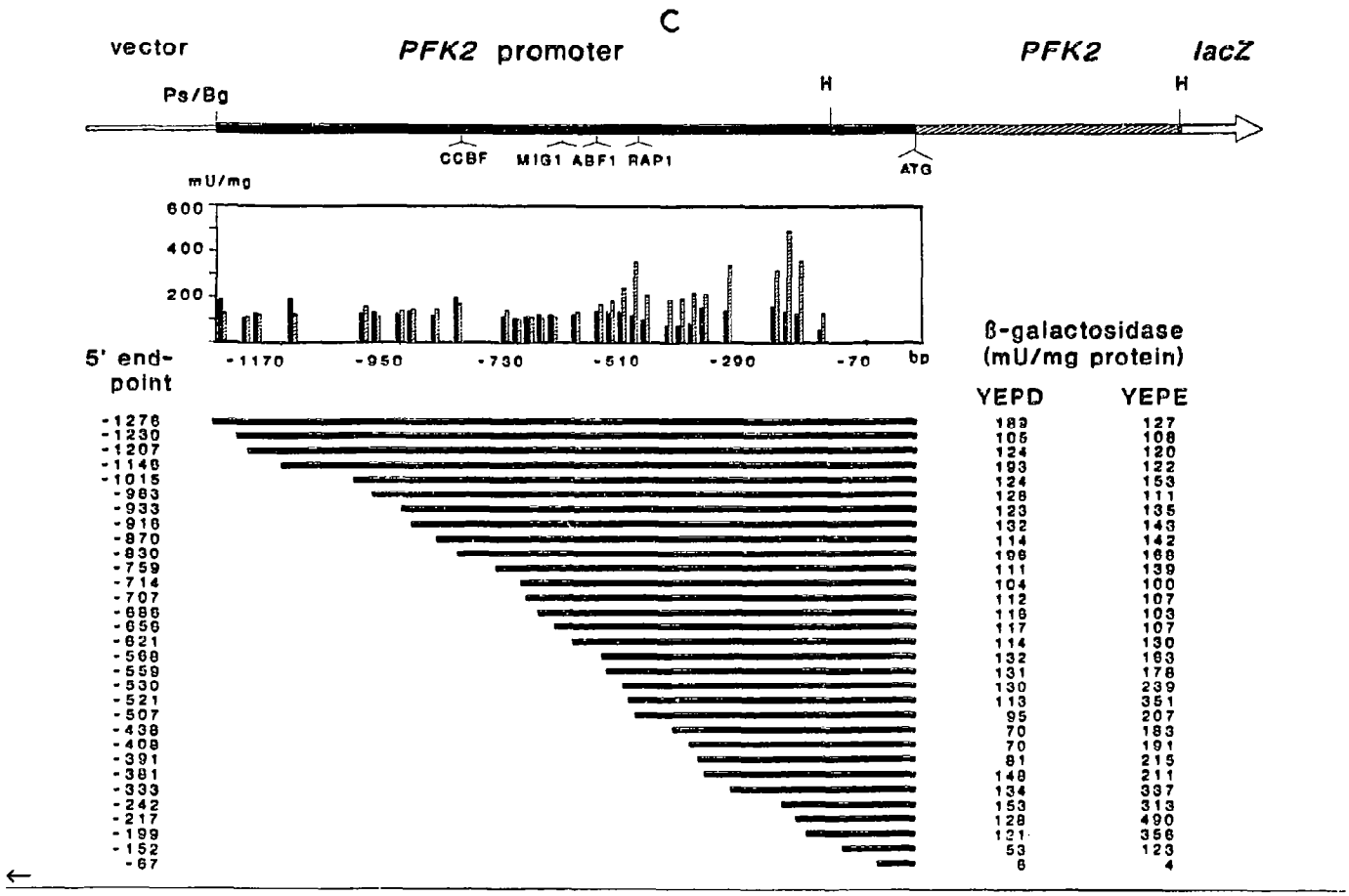


Fig. 2. Expression of different *PFK-lacZ* fusions. *PFK* promoter fragments are shown by closed bars, *PFK* coding sequences by hatched bars and the *lacZ* gene by an open arrow. Consensus DNA sequences are depicted below (see text and Fig. 1 for details). In-frame *lacZ* fusions were obtained in the multiple cloning site of the vectors described by Myers et al. [21] (details available upon request). Below the schematic representation of the fragment arrangements, the specific β-galactosidase activities are shown in relation to the deletion endpoints. Closed bars represent the activities on glucose media, hatched bars the ones on ethanol media. (A) Promoter deletions of *PFK1* starting from the 3'-end. (B) Internal in frame deletions of the *PFK1* coding regions in a *lacZ* fusion. (C) Promoter deletions of PFK2 starting from the 3'-end.



on glucose. Again, this region contains several AT-rich elements. However, not even 2-fold changes in specific activities were observed. Deletions into the putative TATA box at −69 bp then lead to a drastic decrease in specific activities. These results suggest that no strong regulatory elements are functioning in the *PFK2* promoter. The first moderate effects (increasing expression on ethanol) are observed, when the putative ABF1 binding site (at −616 bp) is deleted and they are further increased upon deletion of the RPG box (−530 bp, see Figs. 1B and 2C). However, the effects of these deletions are quite weak in comparison to similar changes in the promoters of *PGK1* [13] and *PYK1* [9]. It seems noteworthy, that PFK activity is almost unaffected by a mutation in *gcr1* [16]. This mutation leads to a downregulation of most glycolytic genes at the transcriptional level. It has been proposed, that the *GCR1* gene product acts via the proteins RAP1 of ABF1 [11,17]. This interpretation would agree with both these elements playing only a minor regulatory role in *PFK2* gene expression.

3.3. *Effect of mig1 deletions*

The original *lacZ*-fusions were also used in crosses with a strain carrying a deletion in the gene encoding the transcriptional repressor MIG1. Especially the *PFK2* promoter was expected to interact with this repressor, as it contains a consensus binding site (Fig. 1B). However, no correlation could be found between varying β-galactosidase activities in the segregants and the deletion *mig1::URA3* (not shown). To avoid the variability in the enzyme measurements which is likely to be due to the difference in genetic backgrounds of the segregants, *mig1* deletions were constructed in strains carrying the original *lacZ* fusions. Again, no effect of the MIG1 deficiency on the β-galactosidase activities could be detected (Table I).

3.4. *Overexpression of PFK genes in strains carrying lacZ fusions*

An overproduction of one of the PFK subunits in a wild-type yeast strain leads to a reduction in specific PFK activity ([2]; Table II). One explanation would be a common transcription factor present in limiting

Table I

β-Galactosidase activities in relation to *MIG1*

Promoter	Promoter fragment	β-Galactosidase (mU/mg protein) *MIG1*		*mig1::URA3*	
		YEPD	YEPE	YEPD	YEPE
PFK1	−961/+558	153	169	155	112
PFK1	−961/+ 42	40	91	54	101
PFK2	−1276/+478	213	143	228	132
PFK2	−152/+448	101	138	106	154

Table II

β-Galactosidase activities in relation to *PFK* copy number

Promoter fragment	Plasmid[a]	Specific activities (mU/mg protein)	
		phosphofructokinase	β-galactosidase
PFK1	none	284	147
(−961/+558)	pGSF1	89	100
	pGSF2	86	126
	pGSF-D1.2	2188	106
PFK2	none	254	129
(−1276/+448)	pGSF1	133	133
	pGSF2	80	189
	pGSF-D1.2	1561	111

(Cells were grown in synthetic media with glucose as carbon source, omitting uracil where necessary.)

[a]The plasmids used contain either *PFK1* (pGSF1), *PFK2* (pGSF2), or both genes simultaneously (pGSF-D1.2) in a derivative of pJDB207 [30]. The small *Hin*dIII fragment of this vector was replaced by the 1.1 kb *Hin*dIII fragment of YEp24 [30] carrying the *URA3* gene.

amounts that binds to the two promoter regions. This would be diluted out by one of the genes being carried on a high copy number vector. As a consequence less of the other subunit would be produced and the amount of enzymatically active heterooctamers would be reduced. To test this possibility, we transformed the strains carrying integrated *lacZ* fusions with multicopy plasmids carrying either one or both *PFK* genes (Table II). The β-galactosidase activities in all transformants were comparable to the untransformed strains. Thus, a titration effect of a transcriptional activator can be ruled out. One possible explanation for the observed decrease in specific PFK activities would be a disturbance in the assembly of the two subunits caused by the overexpression of one of them, which could lead to the formation of less active heterooctamers.

4. CONCLUSIONS

The data presented do not show any evidence for a coordinate control of *PFK1* and *PFK2* transcription. It thus seems likely, that equal amounts of the subunits in the cell are produced by an independent, constitutive, and high level expression of both genes. Regulatory elements found in the promoters of other glycolytic genes, although present, do not seem to play a major role in *PFK* gene expression.

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