Regulatory elements in the FBP1 promoter respond differently to glucose-dependent signals in Saccharomyces cerevisiae

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In Saccharomyces cerevisiae expression of the fructose-1,6-bisphosphatase-encoding gene, FBP1, is controlled by glucose through the upstream activating sequences UAS1 and UAS2 and the upstream repressing sequence URS1 in its promoter. We have studied the regulation of the proteins that could bind to these elements. We have investigated the role of the putative transcription factors Cat8 and Sip4 in the formation of specific DNA–protein complexes with UAS1 and UAS2, and in the expression of UAS1-lacZ and UAS2-lacZ. The expression of CAT8-lacZ and SIP4-lacZ has been also measured in mig1, tup1 or hxx2 mutants, partially refractory to catabolite repression. We conclude that there is no strict correlation between Cat8 and Sip4 expression or in vitro formation of DNA–protein complexes and expression of UAS1-lacZ and UAS2-lacZ. The URS1 element binds the regulatory protein Mig1, which blocks transcription by recruiting the proteins Cyc8 and Tup1. The pattern of complexes of URS1 with nuclear extracts was dependent on the carbon source and on Cyc8, but not on Tup1; it was also affected by the protein kinase Snf1 and by the exportin Msn5. The repression caused by URS1 in a fusion gene was dependent on Mig1, Cyc8 and Tup1, and on the carbon source in the medium; in a snf1 strain the repression observed was independent of the carbon source. Expression of Mig1 could occur in the absence of Snf1 and was moderately sensitive to glucose. We present data showing that different elements of the regulatory system controlling FBP1 responded differently to the concentration of glucose in the medium.

Key words: catabolite repression, fructose-1,6-bisphosphatase, signalling, transcription factor, yeast.

INTRODUCTION

In Saccharomyces cerevisiae the rate of transcription of a great variety of genes is modified in response to changes in the carbon source available to the yeast [1–3]. The alterations in transcription are mediated by the interplay of activators and repressors that interact with distinct regions in the promoters of the regulated genes [4].

A number of genes, encoding enzymes required for the growth of yeast on gluconeogenic carbon sources, share a common upstream activating sequence (UAS) in their promoters. This UAS has been called carbon-source-responsive element (CSRE), due to its ability to direct carbon-source-regulated expression of fusion genes [5–7]. The FBP1 gene, which encodes fructose-1,6-bisphosphatase, is unique among the gluconeogenic genes in possessing, in addition to a CSRE (UAS2), a second element, UAS1, which includes a sequence similar to CSRE but which does not bind the same nuclear proteins [6,8]. The protein(s) binding UAS1 have not been yet identified, but they appear to be repressed by glucose [6,8]. In the case of the CSRE, two zinc-cluster proteins, Cat8 and Sip4, are able to bind the DNA [9,10].

In addition to the two UAS elements, the FBP1 promoter contains an upstream repressing sequence (URS) able to bind the regulatory protein Mig1 [11,12]. Relief from inhibition by Mig1, which takes place in the absence of glucose, is dependent on the protein kinase Snf1 [13,14], which is also required for the operation of both UAS1 and UAS2 [6,7].

The changes in the operativity of the regulatory factors that bind to the different control elements in the FBP1 promoter should depend on a signal-transduction pathway which responds to the presence of glucose. Although it has been shown that cAMP is able to repress the expression of the FBP1 gene, this signal is redundant with other control mechanisms [15]. The glucose sensors Snf3 and Rgt2 are also dispensable for catabolite repression of FBP1 [16].

To investigate further the mode of regulation of UAS1, UAS2 and URS1, we have analysed the capacity of the corresponding DNA sequences to form DNA–protein complexes with nuclear proteins from different mutants, and measured in parallel the levels of expression of UAS1-lacZ, UAS2-lacZ and CYC1-URS1-lacZ (where CYC1 encodes cytochrome c) in the same mutants and under different metabolic conditions. We have also examined different factors which may affect the expression of Cat8, Sip4 and Mig1.

We conclude that there is no strict correlation between the expression of the genes encoding proteins which bind the regulatory elements UAS1, UAS2 or URS1 and that of reporter genes under the control of these elements. This is likely to be due to the fact that both the amount and the activity of the corresponding proteins are subject to control by glucose.

EXPERIMENTAL

Yeast strains and growth conditions

Yeast strains used in this study are listed in Table 1. The yeasts were grown at 30 °C in YPD (1 % yeast extract/2 % peptone/2 % glucose) or in Difco yeast nitrogen base (YNB) with the carbon source(s) indicated in each case and collected at the exponential phase of growth (2–3 mg of wet weight/ml). When the yeasts were grown in the presence of 0.2 % glucose they were collected when there was still glucose present in the medium (4–7 mM). Unless indicated otherwise, to obtain derepressed cells yeasts

Abbreviations used: CSRE, carbon-source-responsive element; URS, upstream repressing sequence; UAS, upstream activating sequence; YNB, yeast nitrogen base.
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grown on glucose were washed twice with distilled water, suspended at 20 mg/ml in YP (1 \% yeast extract/2 \% peptone) containing 2 \% ethanol and incubated overnight at 30 °C. In some cases the yeasts were grown in a synthetic complete medium [17] with the carbon sources indicated in the corresponding Figure.

Plasmids

Plasmid pJJ11b is a centromeric plasmid with the lacZ gene under the control of the FBPl promoter [6]. Plasmids containing UAS1-lacZ and UAS2-lacZ fusion genes have been described previously [8], as well as pDG225 containing a CAT8-lacZ fusion gene [7] and pSIP4lacZ [18]. Plasmid pLG669-ZS, containing a CYC1-lacZ fusion gene, was derived from pLG669-Z [19] by removal of a Smal–SmaI fragment of 1.4 kb. Plasmid pOV31 contains the URS1 sequence (−184 to −201) from the FBPl promoter inserted in the Smal site of pLG669-ZS.

pDSB [20] was used to disrupt the CYC8 gene in a W303-1A strain, yielding strain CJM207. pTUP1::HIS3 was constructed by cloning a 1 kb Smal–SalI fragment from Ydp-H [21] into the Stul and SalI sites from plasmid pFW47 [22]. This plasmid was digested with PvuII and the corresponding 2.2 kb fragment was used to disrupt the TUP1 gene in a W303-1A strain, yielding strain LOZ021.

Cloning procedures were performed according to standard protocols. Yeast cells were transformed using the lithium acetate method.

The different mutants used, mig1, tup1, hxx2, snf1, cat8 and sip4, are deletion mutants with part of the open reading frame deleted. The exact constructions are described in the references given in Table 1.

Northern-blot analysis

Total RNA was extracted as described in [23] using the Gibco TR1zol reagent. The RNA samples were heated at 65 °C for 15 min, fractionated on 1.5 \% agarose gels containing 2.2 M formaldehyde and transferred to a nylon membrane. The membrane was stained with 0.02 \% Methylene Blue in 0.3 M sodium acetate, pH 4.3, and then washed to water to visualize the rRNAs. The membrane was destained with 1 \% SDS and hybridization was performed in a buffer containing 50 mM Na\_2HPO\_4 (pH 7.2), 1 mM EDTA, 1 \% BSA and 7 \% SDS. As probe we used a 0.56 kb StuI–StyI fragment of the Mig1 gene, from position +820 to +1381. The probe was labelled as in [24] using the Amersham Pharmacia Biotech labelling kit. To quantify the RNA the gels were autoradiographed, scanned, and the area and intensity of the bands measured with the appropriate software (NIH image program).

Table 1 Yeast strains used in this study

<table>
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<th>Strain</th>
<th>Relevant genotype</th>
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<tr>
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<tr>
<td>S288C</td>
<td>MAA ura3 gal2 mal CUP1</td>
<td>[49]</td>
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Preparation of extracts and enzymic tests

Yeast extracts were prepared in 20 mM imidazole buffer, pH 7, by shaking with glass beads; for \( \beta \)-galactosidase assays the centrifugation step was omitted. \( \beta \)-Galactosidase was assayed as in [25], with the samples being centrifuged before reading. Fructose-1,6-bisphosphatase was tested spectrophotometrically as described in [26]. Protein was determined in the crude extracts using the bicinchoninic acid (BCA) protein assay (Pierce), with BSA as a standard.

Preparation of yeast nuclear extracts and band-shift assays

Nuclear extracts were obtained as described in [27]. Band-shift assays were performed as described previously, using oligonucleotides OL1 and OL2, which correspond to UAS2\_FBP1 and UAS1\_FBP1, respectively [6], or oligonucleotide UR1, which includes a fragment (−201 to −184) from the FBPl promoter:

5′-tcgacttcccctcaacttaattag-3′

3′-GAAGGGGTGGTATATAATcagct-5′

RESULTS

Cat8 and Sip4 play different roles in the regulation of UAS1 and UAS2

It has been reported previously that the transcription factor Cat8 is required for the formation of DNA–protein complexes with CSRE elements [28,29] and for transcription directed by such elements [30]. Sip4, a \( \alpha \) zinc-cluster protein related to Cat8, may also contribute to transcriptional activation by CSRE [10]. To examine whether Cat8 and/or Sip4 are required for the formation of DNA–protein complexes between UAS1 or UAS2 from FBPl and nuclear proteins, we performed band-shift experiments. The nuclear extracts were prepared either from wild-type cells or from cat8 or sip4 mutants grown on glucose or derepressed overnight in YP/ethanol. As shown in Figure 1, no specific complexes were observed when extracts from repressed cells were used. With derepressed cells, complexes with UAS1 were still formed with extracts from cat8 or sip4 mutants, although in the case of the sip4 mutant the amount of the low-mobility complex was strongly reduced. In contrast, the complexes formed with the UAS2 element were completely dependent on both Cat8 and Sip4.

To study whether the formation of complexes in vitro reflects the operativity of the UASs in vivo, we tested the expression of the fusion genes FBPl-lacZ, UAS1-lacZ and UAS2-lacZ in cat8 and sip4 mutants. As shown in Figure 2, under derepressed conditions, the lack of Sip4 had no significant effect on the
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**Figure 1** Effect of the *cat8* and *sip4* mutations on the DNA–protein complexes formed with UAS sequences from FBP1

Nuclear protein extracts were prepared from wild-type, *cat8* or *sip4* strains grown on YPD (Glu) or derepressed in YP/ethanol (Et). (A) The oligonucleotide corresponding to UAS1<sub>FBP1</sub> was used as probe, and 10 μg of nuclear proteins was added to the samples in lanes 2–13. (B) The oligonucleotide corresponding to UAS2<sub>FBP1</sub> was used as probe and 40 μg of nuclear protein was added to the samples in lanes 2–13. Where indicated, a 100-fold excess of competitor DNA was used. See the Experimental section for further details. Specific complexes are indicated by arrows.

**Figure 2** Effect of deletions in the *SIP4* or *CAT8* genes on the expression of fusion genes

Yeasts with different mutations (wild type, black bars; *sip4*, grey bars; *cat8*, white bars), transformed with plasmids containing the fusion genes indicated, were grown in YNB/glucose or derepressed in YP/ethanol as described in the Experimental section (which also contains a description of the plasmids and recipient strains used). Data are means ± S.D. from at least three independent experiments.

**Figure 3** Expression of the fusion genes *CAT8-lacZ* and *SIP4-lacZ* in different mutants and during growth on different carbon sources

Yeasts were grown in YNB/glucose (black bars) or YNB/galactose (grey bars) or derepressed in YP/ethanol (white bars) as described in the Experimental section. Data are means ± S.D. from at least three independent experiments.

transcription driven by the *FBP1* promoter or by UAS1 and, surprisingly, had a strong activating effect on the transcription directed by UAS2. On the other hand, in *cat8* mutants, the expression of *FBP1-lacZ* and UAS2-*lacZ* was strongly repressed. Expression of UAS1-*lacZ* was significant, but reached only 20% of that observed in a wild-type yeast. These results show that, while lack of Cat8 has a strong effect on transcription directed by either UAS1 or UAS2, Sip4 is dispensable for transcription directed by these UASs. They suggest also that different
proteins may bind to the UASs in vivo and in vitro (see the Discussion).

**Expression of CAT8 and SIP4 is regulated in different ways**

Since both CAT8 and SIP4 are repressed during growth on glucose [7,18,28], it may be expected that their expression is controlled by similar mechanisms. Repression of CAT8 by glucose has been reported to be markedly relieved in a mig1 mutant and only slightly so in a hxx2 (hexokinase 2) mutant [7,28]. As shown in Figure 3, neither the hxx2 nor the mig1 mutations could relieve glucose repression of SIP4-lacZ; in a tup1 mutant a weak increase in expression was seen in repressed cells. In our conditions, both the hxx2 and the mig1 mutations relieved significantly the repression of CAT8-lacZ, the effect being stronger for hxx2. Nevertheless, even in this case the expression reached in glucose was less than 5% of that achieved under derepressing conditions. On the other hand, in a tup1 mutant glucose repressed CAT8-lacZ only about 3-fold, as could be expected from the report that CAT8-lacZ is only slightly sensitive to glucose repression in a mig1mig2 mutant [31]. We also tested the expression of the fusion genes CAT8-lacZ and SIP4-lacZ during growth on galactose, a carbon source less repressing than glucose but able to block FBP1 expression [32]. As shown in Figure 3, while the expression of SIP4-lacZ was only moderately increased under these conditions, CAT8-lacZ expression was much higher than during growth on glucose, although it still remained significantly repressed.

**The capacity of URS1 to bind nuclear proteins and to repress transcription depends on the carbon source**

A sequence in the promoter of FBP1, which corresponds to the consensus for binding the repressor Mig1 [12], has been shown in footprinting experiments to bind nuclear proteins [11]. To ascertain if the formation of DNA–protein complexes with this URS1 sequence was regulated by the carbon source, we conducted band-shift experiments with an oligonucleotide with the corresponding sequence and nuclear extracts from repressed and derepressed yeast cells. As shown in Figure 4 (lanes 3 and 4), the pattern of DNA–protein complexes was the same in both cases. However, since the preparation of nuclear extracts involves a 1 h incubation of the repressed yeast cells in a medium devoid of glucose, the previous result could be due to an experimental artifact. When we performed the band-shifts using nuclear extracts from repressed cells prepared by a procedure which included glucose in all the solutions used, the pattern of DNA–protein complexes was completely modified (Figure 4, lane 5). The two bands of low mobility disappeared and two bands of high mobility, barely visible with extracts from derepressed yeast, became stronger.

The multiplicity of bands observed could be related with the fact that Mig1 acts as a complex with the Cyc8 and Tup1 proteins [13,33]. We observed that the absence of Tup1 in the band-shifts did not produce significant changes in the pattern of bands (Figure 4, lanes 6 and 7), but the lack of Cyc8 caused a very strong increase in the amount of the two complexes of higher mobility and complete disappearance of the complexes of lower mobility (Figure 4, lanes 8 and 9). In addition, in this case, the pattern of complexes formed was little affected by the presence or absence of glucose. These results suggest that the binding of Mig1 alone does not depend on the glucose in the medium, but that complexes including Cyc8 show a different behaviour when extracts of repressed or derepressed cells are used. It can be noted that a single specific DNA–protein complex of different mobility is still formed when extracts from a mig1 mutant are used (Figure 4, lane 2). This complex is also ob-

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Figure 4  Band-shift assays with a URS sequence from the FBP1 promoter

Nuclear extracts were prepared from different yeast strains grown on YP/glucose (Glu) or derepressed in YP/ethanol (Et). Where indicated, 2% glucose was added to all the solutions used for the preparation of the nuclear extracts. In lanes 1–17 the URS1 oligonucleotide described in the Experimental section was used as a probe, and 20 μg of the corresponding nuclear protein was added to samples in lanes 2–9, 11–12 and 14–17. In lanes 15 and 17 a 100-fold excess of unlabelled oligonucleotide was added to check the specificity of the interaction. Electrophoresis was performed using a polyacrylamide gel at 11%. Specific complexes are indicated with arrows.

<table>
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<th></th>
<th>mig1</th>
<th>wt</th>
<th>tup1</th>
<th>cyc8</th>
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Regulatory elements in *FBP1* from *Saccharomyces cerevisiae*

Figure 5 Expression of the fusion genes *CYC1-lacZ* and *CYC1-URS1-lacZ* in different media and in different mutants

The yeasts, transformed with pLG669-ZS or pOV31, were grown in YNB/glucose (black bars) or YNB/galactose (grey bars) or derepressed in YP/ethanol (white bars) as described in the Experimental section. Data are means ± S.D. from at least three independent experiments.

served with extracts from a *mig1mig2* double mutant (results not shown).

Upon removal of glucose from the medium, Mig1 is rapidly phosphorylated in a process that requires the protein kinase Snf1 [13,34]. We observed that when nuclear extracts from a *snf1* mutant derepressed in ethanol were incubated with URS1, the pattern of DNA–protein complexes formed was similar to that found with extracts from wild-type repressed cells (Figure 4, lanes 11 and 12). Therefore the modification in the structure of the URS1–protein complexes, observed when cells are maintained in the absence of glucose, is strictly dependent on Snf1. We have tried to establish whether some differences in the mobility of the complexes could be related with different phosphorylation states, and for this we looked at the effect of treating the nuclear extracts with alkaline phosphatase. There were, unfortunately, technical problems with this approach. Extracts from derepressed cells incubated at 30 °C or 37 °C, in the absence of added phosphatase, yielded the same pattern of DNA–protein complexes than extracts from repressed cells. We could not block a potential endogenous phosphatase with a mixture of inhibitors (EDTA, F−, PO43−). Other possible inhibitors tested (pyrophosphate and EGTA) interfered with the band-shift experiments.

Since it has been reported that phosphorylated Mig1 is taken out of the nucleus by the exportin Msn5 [35], we hypothesized that binding to Msn5 caused a shift in the mobility of the DNA–protein complexes. As shown in Figure 4 (lanes 14–17), this shift is indeed suppressed when extracts from a *msn5* strain are used.

To evaluate the *in vivo* behaviour of the URS1 sequence from the *FBP1* promoter, we inserted this sequence in a fusion gene *CYC1-lacZ*. The insertion caused a large decrease in the expression of this gene when the yeast was grown on glucose, but had no effect when the yeast was grown on galactose or derepressed in ethanol (Figure 5). Repression was completely relieved in a *mig1* mutant, a result which indicates that the protein other than Mig1 which may bind URS *in vitro* (Figure 4, lane 2) does not act as a repressor *in vivo*; repression during growth on glucose was also absent in *cyc8* or *tup1* mutants. On the other hand, in a *snf1* mutant the degree of repression was decreased to around 2-fold but became independent of the carbon source (Figure 5). Although the different mutations also affect the expression of *CYC1-lacZ*, it is clear that in the fusion gene URS1 had a negative effect in the presence of glucose and did not operate as an activator in any of the conditions tested.

**Mig1 mRNA levels are moderately regulated by the carbon source**

Since it was reported that the Mig1 mRNA level is not increased in cells grown on glucose [36], it has been assumed that *MIG1* expression is constitutive. However, there is recent evidence for some control of *MIG1* expression by the carbon source: a *MIG1-lacZ* fusion was repressed over 10-fold by 4% glucose, and this repression depended on Mig1 itself, together with the related repressor Mig2 [37]. As the only function described for Mig1
Repression process cannot account for them. There is a decrease in the Mig1 mRNA levels, in both glucose caused a decrease in the amount of Mig1 mRNA present. However, in a glycerol medium, the addition of as little as 0.05 % glucose caused a decrease in the amount of Mig1 mRNA present. It has been suggested that Snf1 is required for derepression of Mig1 expression [34]. We found, however, significant mRNA levels in a snf1 mutant (Figure 6B), a result consistent with the observation that repression of Mig1 depends on Mig1 and Mig2 [37].

Because no fluorescence could be detected in cells of a snf1 mutant carrying a GFP-MIG1 fusion (where GFP is green fluorescent protein), it has been suggested that Snf1 is required for MIG1 expression [34]. We found, however, significant mRNA levels in a snf1 strain grown in glucose (Figure 6C). It can be noted that when cells grown on YPD are derepressed in YP ethanol, there is a decrease in the Mig1 mRNA levels, in both the presence and absence of Snf1. This observation, together with the other results shown, indicates that control of the Mig1 mRNA levels is complex and that a straightforward catabolite-repression process cannot account for them.

### UAS1, UAS2 and URS1 respond differently to the concentration of glucose

Among the large number of genes from *S. cerevisiae* regulated by glucose some are able to respond to low concentrations of glucose, while others require high concentrations to be turned on or off. For instance HXT2, HXT3 or HXT4, which encode glucose transporters, and SUC2, which encodes invertase, are induced by glucose in the range of 0.05−0.1 %, while the glucose transporter gene HXT1 requires 2 % glucose to be transcribed [38,39], and genes such as ICL1, ACS1 or MLS1, which encode proteins required under gluconeogenic conditions and which contain CSRE elements in their promoters, are fully derepressed at 0.2 % glucose but are not transcribed at 2 % glucose [29,38,40,41]. We did not detect fructose-1,6-bisphosphatase activity in yeast growing on 0.2 % glucose, but this could be related to the capacity of glucose to trigger the proteolytic degradation of the enzyme [42]. However, under our experimental conditions (strains with W303 background growing on YNB with 0.2 % glucose), there was also no expression of the fusion genes FBP1-lacZ or UAS2-lacZ and only a limited expression of UAS1-lacZ (Table 2). To see if induction by a gluconeogenic carbon source is required in addition to the relief from glucose repression, we examined the expression of FBP1-lacZ, UAS1-lacZ and UAS2-lacZ in yeasts growing on 0.2 % glucose, in the presence of ethanol and glycerol (Table 2).

### DISCUSSION

We have studied different factors that affect the activity of the regulatory sites UAS1, UAS2 and URS1 of the FBP1 gene from *S. cerevisiae*. We have shown that the capacity of URS1 to repress transcription requires a high concentration of glucose in the medium (1−2 %), whereas expression directed by UAS1 or UAS2 is strongly decreased at 0.2 % glucose. Thus the mechanisms which relay the glucose signal to UAS1, UAS2 and URS1 appear to have different sensitivities to glucose. Since expression of MIG1 is partially repressed by 4 % glucose, we wondered whether some protein alternative to Mig1 may be binding to URS1 in these conditions. This does not appear to be the case, since we found that URS1 did not cause any repression at 4 % glucose in a mig1 background (results not shown).

### Table 2 Influence of a low concentration of glucose on the derepression of fusion genes

<table>
<thead>
<tr>
<th>Fusion gene</th>
<th>0.2 % Glu</th>
<th>1 % Glu</th>
<th>2 % Glu</th>
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<td>FBP1-lacZ</td>
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<td>UAS1-lacZ</td>
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<td>56 ± 2</td>
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<td>13 ± 1</td>
<td>29 ± 1</td>
<td>270 ± 50</td>
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<td>CAT8-lacZ</td>
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<td>24 ± 8</td>
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<td>SIP4-lacZ</td>
<td>15 ± 6</td>
<td>20 ± 8</td>
<td>29 ± 5</td>
<td>1096 ± 250</td>
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### Table 3 Effect of the concentration of glucose in repression mediated by URS1<sub>app</sub>

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<td>CYC1-lacZ</td>
<td>860 ± 60</td>
<td>460 ± 85</td>
<td>480 ± 105</td>
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<tr>
<td>CYC1-URS1-lacZ</td>
<td>670 ± 90</td>
<td>210 ± 30</td>
<td>96 ± 22</td>
<td>100 ± 20</td>
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Strain W303-1A was transformed with the reporter plasmids pGL669-25 or pV031 (see the Experimental section) and grown in YNB medium with glucose at the concentrations indicated. The yeasts were collected during the exponential phase of growth. Data are the means ± S.D. from at least four experiments. Glu, glucose.
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Figure 7 Model for the regulation of FBP1 expression by the carbon source

Active proteins are shown as circles and inactive proteins as rectangles. Grey arrows indicate facilitation of a protein conformational change. For details see the text.

Extracts from a sip4 mutant (Figure 1) appears contradictory with the high expression of the fusion gene UAS2-lacZ in this background (Figure 2). A possible interpretation is that different proteins (Cat8, Sip4 and perhaps another one, depending on both Cat8 and Sip4) are able to bind UAS2 in vitro, but that Cat8, at normal levels of expression, does not bind UAS2 in vitro. Since Sip4 is not synthesized in the absence of Cat8 [10], neither cat8 nor sip4 extracts would allow the formation of DNA–protein complexes in vitro. On the other hand, the capacity of Sip4 (or an unidentified protein depending on Sip4) to activate transcription would be relatively low, and its absence, or down-regulation, in a sip4 mutant would allow the function of a different transcription factor (Cat8?) able to activate UAS2 more strongly.

In wild-type strains growing on galactose or low glucose (or in hxxk2 mutants growing on high glucose), Snf1 is still able to inactivate Mig1 (Figure 7). However, in low glucose CAT8 expression is low (Table 2) and on galactose CAT8 is only partially derepressed (Figure 3). Under neither set of conditions is FBP1 expressed, and there is only a slight derepression of SIP4 during growth on galactose; it is therefore likely that in the presence of sugars Cat8 cannot be phosphorylated, a process required for its activity [43].

We observed that the operativity of URS1 in a fusion gene was dependent on the concentration of glucose (Table 3). The low level of repression, about 20%, observed at 0.2%, glucose is not due to lack of expression of MIG1, since this gene is well expressed in media with low glucose (Figure 6). It is also unlikely that at low glucose Mig1 is mislocalized, since at 0.1%, glucose most of the protein is in the nucleus [34]. It would seem therefore that an elevated glucose concentration is required for full activity of the Mig1 protein.

At high glucose Snf1 is inactive, Mig1 is in its active state and represses fully the expression of CAT8 (Figure 7). The proteins that activate the transcription of SIP4 and FBP1 are absent and the Mig1 complex binds URS1 and represses transcription. FBP1 is therefore completely turned off. In some mutants such as hxxk2, mig1 or tup1, there is some expression of CAT8 in high glucose, but glucose appears to interfere with the phosphorylation of Cat8 [43] and there is therefore no expression of FBP1.

When we examined the effect of glucose on the formation of DNA–protein complexes with the URS1 element, we observed several strong complexes (Mig1-dependent) with nuclear extracts from both repressed and derepressed cells (Figure 4). Since Mig1 leaves the nucleus upon removal of glucose from the medium [34], only small amounts of Mig1 should be expected in nuclear extracts from derepressed cells. The abundance of URS1-binding proteins (and therefore presumably of Mig1) in these extracts is unlikely to be due to contamination by cytoplasmic material, as the extracts contained only small amounts of cytoplasmic proteins (results not shown). We hypothesize that when Mig1 exits from the nucleus it does not become free in the cytoplasm, but remains associated with some particulate fraction which co-sediments with the nucleus. Although expression of MIG1 is controlled by the carbon source (Figure 6 and [37]), regulation of Mig1 occurs...
mainly through a phosphorylation catalysed by the protein kinase Snf1, in the absence of glucose [13,14,34,35,44,45]. Phosphorylation of Mig1 does not impair its binding to DNA, since strong DNA–protein complexes are formed with nuclear extracts from derepressed cells (Figure 4).

We interpret the multiplicity of URS1–protein complexes as follows. The pattern of two main bands found with extracts from a cyc8 mutant may correspond to the binding of a single Mig1 molecule and to that of a Mig1 dimer. When Cyc8 is present it follows. The pattern of two main bands found with extracts from derepressed cells (Figure 4).

The change in mobility is too marked to be accounted for simply by the phosphorylation of Mig1, but the phosphorylated Mig1 may be able to bind an additional protein, such as the Mig1 exportin, Msn5. The data with the cyc8 mutant suggest that this binding depends also on the presence of Cyc8. On the other hand, none of the DNA–protein complexes formed in vitro appear to depend on Tup1.

The patterns we obtain in the band-shifts are different from those reported by Wu and Trumbly [46]. These differences may be related to the experimental conditions prevailing in the previous report: whole-cell extract instead of nuclear extracts, glucose not included during the preparation of extracts from repressed cells, and the Mig1 binding site used, taken from SUC2, different from the Mig1 binding site from the FBP1 gene.

We have shown that control through glucose of the different regulatory elements in the FBP1 promoter takes place through different mechanisms. Different concentrations of glucose may activate different signalling pathways [47]. These pathways, however, have not been yet worked out. A factor that has been neglected up to now, but which is likely to be important in transmitting the signal of the presence of glucose, is the intracellular concentration of some intermediary metabolite(s).

The picture that emerges from our results and those of others is that the control of transcription of FBP1 is a complex process, involving the interaction of a multiplicity of elements. Although some of them have been identified, others remain to be understood.

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