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A yeast homologue of the bovine lens fibre MIP gene family complements the growth defect of a *Saccharomyces cerevisiae* mutant on fermentable sugars but not its defect in glucose-induced RAS-mediated cAMP signalling

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Recently a new family of membrane proteins comprising the bovine lens fibre major intrinsic protein, soybean nodulin-26 protein and the *Escherichia coli* glycerol facilitator has been described [M.E.Baker and M.H.Saier, Jr (1990) *Cell*, 60, 185-186]. These proteins have six putative membrane spanning domains and one (probably intracellular) intermembrane fragment is particularly well conserved. We have identified a new member of this family in the yeast *Saccharomyces cerevisiae*. It also possesses the six transmembrane domains and the highly conserved intermembrane sequence. In contrast to the other three proteins which are all ~280 amino acids long, the yeast protein has an N-terminal extension of ~250 amino acids, which contains a string of 17 asparagine residues and a C-terminal extension of ~150 amino acids. The gene, which we called *FPS1* (for *fdp1* suppressor), suppresses in single copy the growth defect on fermentable sugars of the yeast *fdp1* mutant but it is not allelic to *FDP1*. The deficiency of the *fdp1* mutant in glucose-induced RAS-mediated cAMP signalling and in rapid glucose-induced changes in the activity of certain enzymes was not restored. Deletion of *FPS1* does not cause any of the phenotypic deficiencies of the *fdp1* mutant.

Key words: *FPS1* gene/membrane spanning proteins/MIP gene family/RAS/*Saccharomyces cerevisiae*/signal transduction

Introduction

In a recent paper, M.E.Baker and M.H.Saier, Jr (1990) have pointed out the existence of a new gene family encoding proteins from evolutionarily very distinct organisms: the major intrinsic protein of bovine lens fibre, soybean nodulin-26 protein and *Escherichia coli* glycerol facilitator. All three genes code for membrane proteins with six putative membrane spanning domains. They show weak overall homology but in some places the homology is very striking, such as in one particular intermembrane loop. We have isolated a gene of the yeast *Saccharomyces cerevisiae* which clearly belongs to the same family. It was isolated as a suppressor of the growth defect on fermentable sugars of the yeast *fdp1* mutant.

The *fdp1* mutant was isolated by van de Poll *et al.* (1974). It shows an unusual and complicated phenotype both at the physiological and biochemical level (van de Poll *et al.*, 1974; Gancedo and Schwerzmann, 1976; van de Poll and Schamhart, 1977; Schamhart *et al.*, 1977; Banuelos and Fraenkel, 1982). The mutant is unable to grow on fermentable sugars but it is not deficient in any of the enzymes of glycolysis. It lacks the rapid inactivation of fructose-1,6-bisphosphatase which occurs in wild type strains upon transfer to a medium with a fermentable sugar, although this is not responsible for the growth defect on such media (Banuelos and Fraenkel, 1982). In addition, it shows high protein kinase activity when grown on non-fermentable carbon sources. The high protein kinase activity causes a striking change in the regulatory properties of glycogen synthase (van de Poll and Schamhart, 1977), causes low trehalose-6-phosphate synthase activity (Charlab *et al.*, 1985) and high trehalase activity (M.Beullens and L.Van Aelst, unpublished results), which results in very low trehalose levels. Addition of fermentable sugars to cells of the *fdp1* mutant causes rapid depletion of ATP and hyperaccumulation of sugar phosphates. Hence, it was suggested that this mutant was deficient in a hypothetical feedback-inhibition system of glycolysis on sugar transport (van de Poll and Schamhart, 1977).

Glucose induces a rapid, transient cAMP increase in yeast cells transferred from derepressive (growth on non-fermentable carbon sources) to repressive conditions (growth on fermentable carbon sources) (recent reviews: Thevelein, 1988, 1991). The yeast RAS proteins and the RAS activating protein, CDC25, are essential components of the signal transduction pathway leading from glucose to cAMP (Mbonyi *et al.*, 1988; Munder and Küntzel, 1989; Van Aelst *et al.*, 1990, 1991). Knowledge about the upstream part of the pathway is still limited: the affinity of the glucose receptor is relatively low (apparent K_m: 15-20 mM; Beullens *et al.*, 1988), as far as sugar metabolism is concerned, only sugar kinase activity, but no further metabolism of glucose, is required for induction of the cAMP signal and the pathway appears to contain a glucose-repressible protein (Beullens *et al.*, 1988; Argüelles *et al.*, 1990; Mbonyi *et al.*, 1990; Van Aelst *et al.*, 1991). Recently, several mutants have been identified which are deficient in induction of the cAMP signal by glucose, one of which is the *fdp1* mutant. The results with the other mutants show that the deficiency in cAMP signalling in the *fdp1* mutant cannot be responsible for the

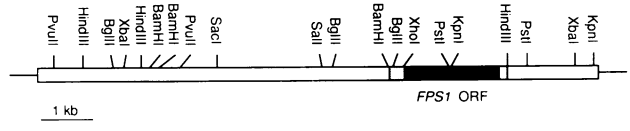


Fig. 1. Restriction map of the DNA insert in plasmid LVA4009/*FPS1*. The *Bam*HI-*Hin*dIII fragment present in YCp*FPS1Bam*HI-*Hin*dIII is shaded. The open reading frame encoding the *FPS1* gene is shown in black.

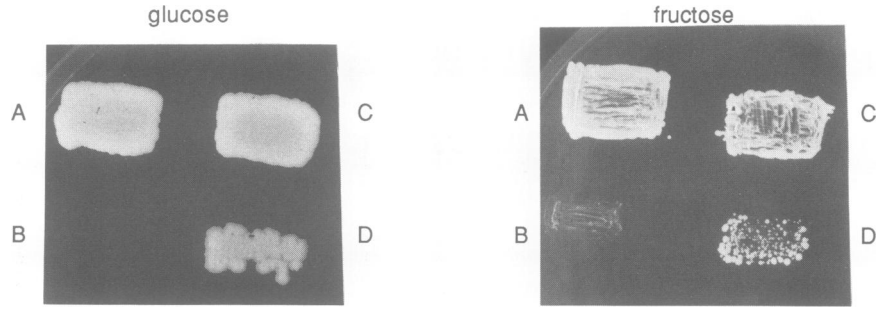


Fig. 2. Growth of the *fdp1* mutant (strain LVA1531) with and without *FPS1*-containing plasmids on rich medium with either glucose or fructose. (**A**) *FDP1*; (**B**) *fdp1*; (**C**) *fdp1* + pLVA4009/*FPS1* (*FPS1* on complete, original insert); (**D**) *fdp1* + YCp*FPS1Bam*HI−*Hin*dIII (*FPS1* on 2.6 kb *Bam*HI−*Hin*dIII fragment).

growth defect nor even for the absence of the glucose-induced changes in enzyme activity (M.Vanhalewyn, L.Van Aelst and K.Mbonyi, unpublished results). In the present paper we show that the *FPS1* gene only suppresses the growth defect on fermentable carbon sources of the *fdp1* mutant and none of the known regulatory defects of the mutant: the absence of induction by fermentable carbon sources of RAS-mediated cAMP signalling and of fructose-1, 6-bisphosphatase inactivation is not suppressed and the very low level of trehalose is not enhanced.

Results and discussion

Isolation and characterization of FPS1, a suppressor of the growth defect caused by the fdp1 mutation in yeast

Screening of a yeast gene library in the single copy vector pCS19 (Sengstag and Hinnen, 1987) resulted in isolation of a clone (pLVA4009/*FPS1*) which suppressed the growth defect of the *fdp1* mutant (strain LVA1531) on glucose and to a lesser extent on fructose. Although the transformant grew well on glucose the suppression was also not complete. The strain showed a longer lag phase when grown on glucose in liquid culture compared with the corresponding wild type strain, with or without the pLVA4009/*FPS1* plasmid (results not shown). After subcloning (restriction map of pLVA4009/*FPS1* is shown in Figure 1) a 2.6 kb *Bam*HI−*Hin*dIII fragment was obtained which still suppressed the growth deficiency on glucose but to a much lesser extent the growth deficiency on fructose (Figure 2).

The 2.6 kb fragment contained an open reading frame of 2007 bases. We called this putative gene '*FPS1*', for '*fdp1* suppressor'. Northern blot analysis revealed a mRNA length of ~2.3 kb, in accordance with the length of the open reading frame (Figure 3). The 5′ non-coding region contains TATA-like motifs at positions −91 and −147 (Mellor, 1989). Downstream of the TGA stop codon at position 2008 there are several other stop codons. Downstream of the third stop codon there is a sequence, TAG . . . TAGT . . . TTT at positions 2108, 2120 and 2125, which fits perfectly with the consensus sequence for transcription termination in yeast (TAG . . . TAGT/TATGT . . . TTT, Zaret and Sherman, 1982). The nucleotide and deduced amino acid sequence of the *FPS1* gene is shown in Figure 4. The predicted protein contains 669 amino acids and has an estimated molecular weight of ~73 959 daltons. The codon bias index (Bennetzen and Hall, 1982) of *FPS1* is 0.143, which means that codon usage is little biased. This and the weak signal in Northern blot analysis, indicate that *FPS1* is not a highly expressed gene.

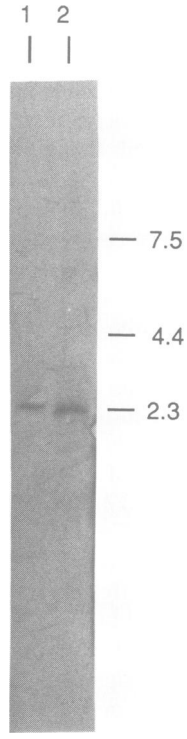
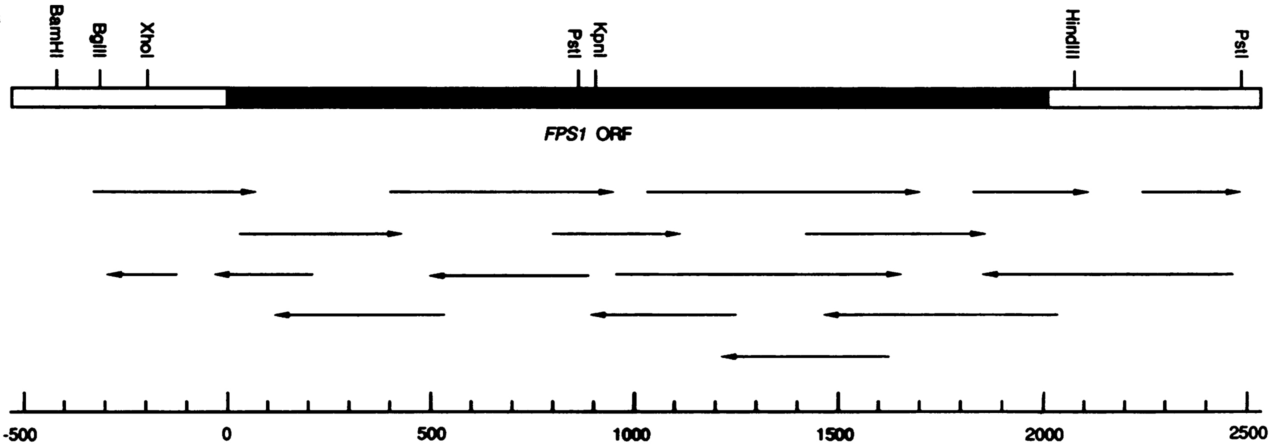


Fig. 3. Northern blot analysis of *FPS1* expression. The *Bam*HI−*Hin*dIII fragment was used as probe. The messenger detected has an estimated length of ±2.3 kb. Lane 1: 5 μg and lane 2: 10 μg of poly(A)$^+$-enriched RNA.

Screening of the EMBL/GenBank databases revealed three proteins with significant homology to the predicted FPS1 amino acid sequence: the *E.coli* glycerol facilitator, soybean nodulin-26 and bovine lens fibre major intrinsic protein (MIP). These three proteins have only recently been reported to comprise a new family of related proteins (Baker and Saier, 1990). All three proteins, however, are ~280 amino acids long while the predicted FPS1 sequence is 669 amino acids. The protein most similar to FPS1 is the glycerol facilitator: between amino acids 250 and 530 of the FPS1 protein the sequence identity with the entire glycerol facilitator is ~30%, sequence similarity is ~60% with some gaps. A short peptide of 13 amino acids is particularly well

a



b

-330
TTAAAATGCT
-320 -280
TCAGAGATGTTTCTTTAATGTGCCGTCCAACAAAGGTATCTTCTGTAGCTTCCTCTATTTTCGATCAGATCTCATAGTGA
-240 -200
GAAGGCGCAATTCAGTAGTTAAAAGCGGGGAACAGTGTGAATCCGGAGACGGCAAGATTGCCCGGCCCTTTTTGCGGAAA
-160 -120
AGATAAAACAAGATATATTGCACTTTTTCCACCAAGAAAAACAGGAAGTGGATTAAAAAATCAACAAAGTATAACGCCTA
-80 -40
TTGTCCCAATAAGCGTCGGTTGTTCTTCTTTATTATTTTACCAAGTACGCTCGAGGGTACATTCTAATGCATTAAAAGAC

1/1 31/11
ATG AGT AAT CCT CAA AAA GCT CTA AAC GAC TTT CTG TCC AGT GAA TCT GTT CAT ACA CAT
Met ser asn pro gln lys ala leu asn asp phe leu ser ser glu ser val his thr his
61/21 91/31
GAT AGT TCT AGG AAA CAA TCT AAT AAG CAG TCA TCC GAC GAA GGA CGC TCT TCA TCA CAA
asp ser ser arg lys gln ser asn lys gln ser ser asp glu gly arg ser ser ser gln
121/41 151/51
CCT TCA CAT CAT CAC TCT GGT GGT ACT AAC AAC AAT AAT AAC AAT AAT AAT AAT AAT AAT
pro ser his his his ser gly gly thr asn asn asn asn asn asn asn asn asn asn asn
181/61 211/71
AAC AGT AAC AAC AAC AAC AAC GGC AAC GAT GGG GGA AAT GAT GAC GAC TAT GAT TAT GAA
asn ser asn asn asn asn asn gly asn asp gly gly asn asp asp asp tyr asp tyr glu
241/81 271/91
ATG CAA GAT TAT AGA CCT TCT CCG CAA AGT GCG CGG CCT ACT CCC ACG TAT GTT CCA CAA
met gln asp tyr arg pro ser pro gln ser ala arg pro thr pro thr tyr val pro gln
301/101 331/111
TAT TCT GTA GAA AGT GGG ACT GCT TTC CCG ATT CAA GAG GTT ATT CCT AGC GCA TAC ATT
tyr ser val glu ser gly thr ala phe pro ile gln glu val ile pro ser ala tyr ile
361/121 391/131
AAC ACA CAA GAT ATA AAC CAT AAA GAT AAC GGT CCG CCG AGT GCA AGC AGT AAT AGA GCA
asn thr gln asp ile asn his lys asp asn gly pro pro ser ala ser ser asn arg ala
421/141 451/151
TTC AGG CCT AGA GGG CAG ACC ACA GTG TCG GCC AAC GTG CTT AAC ATT GAA GAT TTT TAC
phe arg pro arg gly gln thr thr val ser ala asn val leu asn ile glu asp phe tyr
481/161 511/171
AAA AAT GCA GAC GAT GCG CAT ACC ATC CCG GAG TCA CAT TTA TCG AGA AGG AGA AGT AGG
lys asn ala asp asp ala his thr ile pro glu ser his leu ser arg arg arg ser arg
541/181 571/191
TCG AGG GCT ACG AGT AAT GCT GGG CAC AGT GCC AAT ACA GGC GCC ACG AAT GGC AGG ACT
ser arg ala thr ser asn ala gly his ser ala asn thr gly ala thr asn gly arg thr
601/201 631/211
ACT GGT GCC CAA ACT AAT ATG GAA AGC AAT GAA TCA CCA CGT AAC GTC CCC ATT ATG GTG
thr gly ala gln thr asn met glu ser asn glu ser pro arg asn val pro ile met val
661/221 691/231
AAG CCA AAG ACA TTA TAC CAG AAC CCT CAA ACA CCT ACA GTC TTG CCC TCC ACA TAC CAT
lys pro lys thr leu tyr gln asn pro gln thr pro thr val leu pro ser thr tyr his
721/241 751/251
CCA ATT AAT AAA TGG TCT TCC GTC AAA AAC ACT TAT TTG AAG GAA TTT TTA GCC GAG TTT
pro ile asn lys trp ser ser val lys asn thr tyr leu lys glu phe leu ala glu phe
781/261 811/271
ATG GGA ACA ATG GTT ATG ATT ATT TTC GGT AGT GCT GTT GTT TGT CAG GTC AAT GTT GCT
met gly thr met val met ile ile phe gly ser ala val val cys gln val asn val ala
841/281 871/291
GGG AAA ATA CAG CAG GAC AAT TTC AAC GTG GCT TTG GAT AAC CTT AAC GTT ACC GGG TCT
gly lys ile gln gln asp asn phe asn val ala leu asp asn leu asn val thr gly ser
901/301 931/311
TCT GCA GAA ACG ATA GAC GCT ATG AAG AGT TTA ACA TCC TTG GTT TCA TCC GTT GCG GGC
ser ala glu thr ile asp ala met lys ser leu thr ser leu val ser ser val ala gly
961/321 991/331
GGT ACC TTT GAT GAT GTG GCA TTG GGC TGG GCT GCT GCC GTG GTG ATG GGC TAT TTC TGC
gly thr phe asp asp val ala leu gly trp ala ala ala val val met gly tyr phe cys
1021/341 1051/351
GCT GGT GGT AGT GCC ATC TCA GGT GCT CAT TTG AAT CCG TCT ATT ACA TTA GCC AAT TTG
ala gly gly ser ala ile ser gly ala his leu asn pro ser ile thr leu ala asn leu
1081/361 1111/371
GTG TAT AGA GGT TTT CCC CTG AAG AAA GTT CCT TAT TAC TTT GCT GGA CAA TTG ATC GGT
val tyr arg gly phe pro leu lys lys val pro tyr tyr phe ala gly gln leu ile gly
1141/381 1171/391
GCC TTC ACA GGC GCT TTG ATC TTG TTT ATT TGG TAC AAA AGG GTG TTA CAA GAG GCA TAT
ala phe thr gly ala leu ile leu phe ile trp tyr lys arg val leu gln glu ala tyr

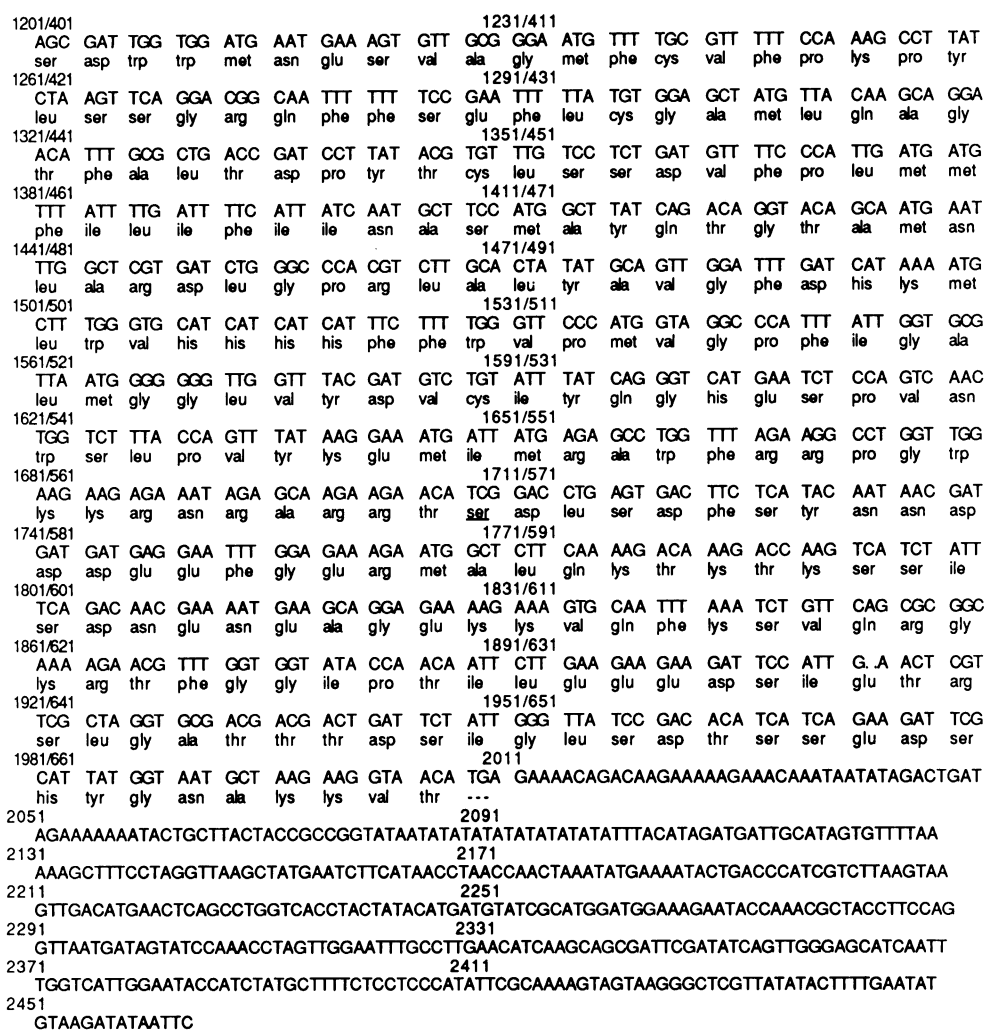
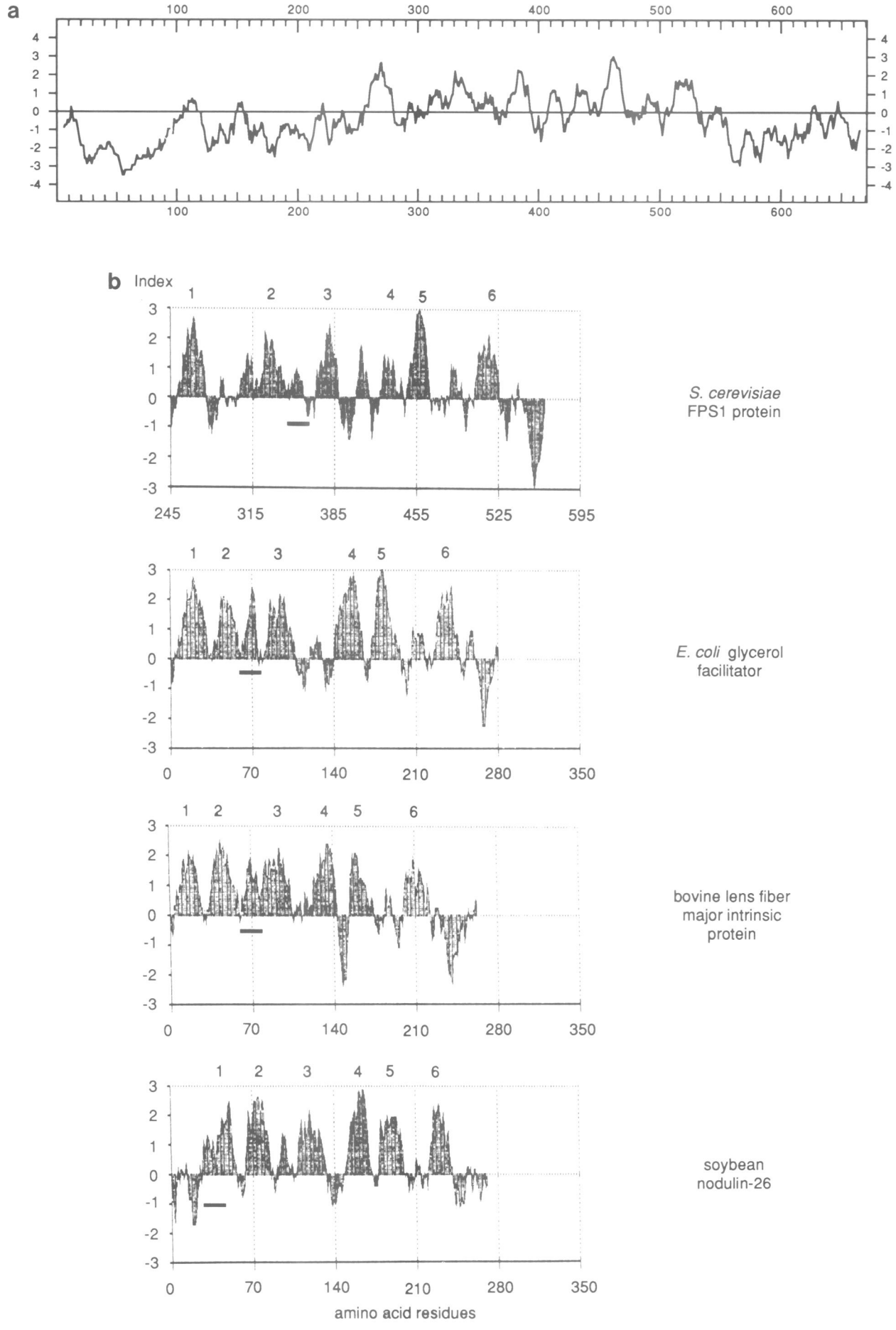


Fig. 4. Nucleotide sequence of the *FPS1* gene and the surrounding DNA region. (**a**) Sequencing strategy. (**b**) Nucleotide sequence. The A of the first 5′ ATG in the ORF was given the number +1. The string of 17 asparagine residues (starting at amino acid 50) and the two potential sites for cAMP-dependent protein phosphorylation (at amino acids 179 and 570) are underlined once. These sequence data are available from EMBL/GenBank/DDBJ under accession number X54157.

conserved between all four proteins and this sequence is shown in Figure 5c. The similarity between the four proteins becomes much more significant when the hydrophobicity plots (Kyte and Doolittle, 1982) are compared (Figure 5a and b). All four proteins are predicted to have six putative membrane spanning domains. The prediction for transmembrane domain 4 of FPS1 is somewhat doubtful. Interestingly, even the arrangement of the putative transmembrane domains is very similar: the first two transmembrane domains are very close together (except in FPS1), the two small intermembrane domains flank the transmembrane domain 3, the transmembrane domains 4 and 5 are again very close together and separated by another small intermembrane domain from transmembrane domain 6. While the FPS1 protein has an N-terminal extension of ~250 amino acids and a C-terminal extension of ~150 amino acids (Figure 5a) the other three proteins consist almost entirely of the described arrangement of the transmembrane and intermembrane domains. The most highly conserved 13 amino acid peptide is located in three of the four proteins in the intermembrane domain between transmembrane domains 2 and 3; in nodulin-26 this sequence is found in front of the first transmembrane domain. Thus, if the ends of the proteins are on the cytoplasmic side of the membrane this peptide would also be in the cytoplasm. It is tempting to speculate about possible functions of this short peptide in protein interactions or as an acceptor or binding site for small molecules.

Other interesting features of the FPS1 sequence are two possible sites of phosphorylation by cAMP-dependent protein kinase at positions 179 and 570 and a perfect leucine zipper with four leucine residues starting at position 437. Leucine zippers, first identified in transcription factors (White and Weber, 1989), but also found e.g. in sugar transporters (Kruckeberg and Bisson, 1990), are probably involved in subunit dimerization. The leucine zipper of FPS1 is located in the transmembrane domains 4 and 5 and therefore could trigger dimerization within the membrane. However, the three proteins similar to FPS1 do not have such a leucine zipper.

Unfortunately, the exact function of any of these similar proteins has not been elucidated yet. The localization of the proteins, MIP in gap junctions, nodulin-26 in the peribacteroid membrane and the glycerol facilitator in the plasma membrane, could indicate that all are involved in the transport of small molecules. All the proteins are clearly not


a
100
200
300
400
500
600
b
Index
1
2
3
4
5
6
S. cerevisiae
FPS1 protein
E. coli glycerol
facilitator
bovine lens fiber
major intrinsic
protein
soybean
nodulin-26
amino acid residues

C

		Highly conserved sequence													Position with respect to the transmembrane domains
FPS1	(346)	ILE	SER	GLY	ALA	HIS	LEU	ASN	PRO	SER	ILE	THR	LEU	ALA	(358)	TMD 2 - Intermembrane - TMD 3
GLPF	(62)	VAL	SER	GLY	ALA	HIS	LEU	ASN	PRO	ALA	VAL	THR	ILE	ALA	(74)	TMD 2 - Intermembrane- TMD 3
MIP	(62)	ILE	SER	GLY	ALA	HIS	VAL	ASN	PRO	ALA	VAL	THR	PHE	ALA	(74)	TMD 2 - Intermembrane- TMD 3
NOD	(25)	ILE	SER	GLY	GLY	HIS	PHE	ASN	PRO	ALA	VAL	THR	ILE	ALA	(37)	Intermembrane- TMD 1
Consensus		ILE	SER	GLY	ALA	HIS	Phob.	ASN	PRO	ALA	VAL	THR	Phob.	ALA		

Fig. 5. (**a**) Hydropathy profile of the FPS1 protein. The profile was calculated according to Kyte and Doolittle (1982). (**b**) Alignment of the putative transmembrane domains of the bovine lens fibre major intrinsic protein, the soybean nodulin-26 protein, the *E.coli* glycerol facilitator and the FPS1 protein. The six putative transmembrane domains are numbered from 1 to 6. The position of the particularly well conserved intermembrane sequence is indicated with a bar. (**c**) Alignment of the particularly well conserved intermembrane sequence of the four proteins in the gene family: the *S.cerevisiae* FPS1 protein, the *E.coli* glycerol facilitator (GLPF), bovine lens fibre major intrinsic protein (MIP) and the soybean nodulin-26 protein (NOD). (Phob. = hydrophobic).

homologous to known sugar transport proteins or to any other known transport proteins (Baldwin and Henderson, 1989; Bisson *et al.*, 1987; Celenza *et al.*, 1988; Cheng and Michels, 1989; Szkutnicka *et al.*, 1989). The long N-terminal and C-terminal extensions of the FPS1 protein could indicate that FPS1 has distinct additional functions. These parts of the protein have no significant homology to any protein in the databases with the exception of a string of 17 asparagine residues present in the N-terminal extension. A similar string of 11 asparagine residues was found before in the SCH9 protein (Toda *et al.*, 1988). This appears to be a novel structural element in protein sequences. Polyglutamine strings have been found in several transcription factors and may have a function in transcriptional activation (Courey and Tjian, 1988). In analogy, polyasparagine strings could play a role in possible protein–protein interactions.

An *fps1* deletion mutant was constructed by homologous recombination. A 0.9 kb *Xho*I–*Pst*I fragment was excised from the *FPS1* gene and replaced with the *LEU2* gene on a 3.3 kb *Xho*I–*Pst*I fragment. The deletion was confirmed by Southern blotting (not shown). The deletion mutant grew on fermentable sugars and did not show any of the phenotypic deficiencies of the *fdp1* mutant. In crosses the *fps1*Δ and *fdp1* mutations behaved as two unlinked genes. *Fps1*Δ *fdp1* double mutants obtained from such a cross had a phenotype indistinguishable from that of the *fdp1* mutant. Thus *FPS1* is different from *FDP1*. Moreover, deletion of *FPS1* in an *fdp1* strain did not change the *fdp1* phenotype, supporting the conclusion that *FPS1* is not allelic with *FDP1*. This is remarkable because *FPS1* was isolated on a single copy vector. It could point to a strong dosage effect of the FPS1 protein on *fdp1*-induced malfunction. Alternatively the *FPS1* gene in the strain used to construct the pCS19 gene bank might have been a mutant gene which does not produce a phenotypic effect in wild type cells but fortuitously suppresses the growth deficiency of the *fdp1* mutant.

Southern blot analysis using *FPS1* as a probe shows that there is no second copy of *FPS1* in the haploid yeast genome. However, at reduced stringency, some additional weak bands appeared, indicating that there are one or more genes with homology to *FPS1* in yeast (Figure 6).

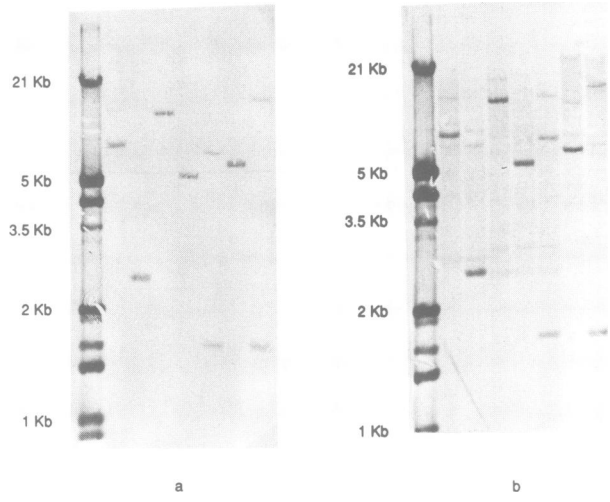


Fig. 6. Southern blot analysis of genomic yeast DNA digested with different restriction enzymes and hybridized with the 2.6 kb *Bam*HI–*Hin*dIII fragment comprising the entire coding region of *FPS1* (Figure 1). (**a**) High stringency hybridization, (**b**) low stringency hybridization. Digests from left to right: 1. *Hin*dIII (number of expected fragments: 1/size: 7.2 kb), 2. *Hin*dIII/*Bam*HI (1/2.6 kb), 3. *Eco*RI (1/>9.5 kb), 4. *Eco*RI/*Bam*HI (1/>4.2 kb), 5. *Eco*RI/*Pst*I (2/6.5 kb; 1.5 kb), 6. *Bam*HI (1/>4.2 kb), 7. *Pst*I (2/>8 kb, 1.5 kb). Additional bands at reduced stringency and also at high stringency in the *Hin*dIII digest indicate the existence of one or more genes with partial homology.

Suppression by FPS1 of phenotypic defects caused by the fdp1 mutation

Addition of fermentable sugars to cells of the *fdp1* mutant causes excessive sugar phosphate formation, especially of fructose-1,6-bisphosphate. Sugar phosphate continues to accumulate for several hours in the presence of glucose or fructose (van de Poll and Schamhart, 1977; Banuelos and Fraenkel, 1982, Figures 7 and 8) and this is probably the reason for the lethality caused by fermentable sugars. In the presence of the *FPS1* suppressor the sugar phosphate level in *fdp1* cells also rises to much higher levels than in wild type cells (Figures 7 and 8). However, the effect is only transient and, as opposed to *fdp1* cells without suppressor, after ~20 min the sugar phosphate level starts to drop again (Figure 8). This probably explains why the presence of the *FPS1* suppressor restores growth of *fdp1* cells. The partial hyperaccumulation of fructose-1,6-bisphosphate was observed with the *FPS1* suppressor on both the small (YCp*FPS1Bam*HI–*Hin*dIII) and the complete insert (pLVA4009/*FPS1*). The dramatic effect of glucose on sugar phosphate formation in the *fdp1* mutant and its prevention by the *FPS1* suppressor is shown in Figure 7 by means of *in vivo* ^{31}P-NMR spectra taken before and after addition of

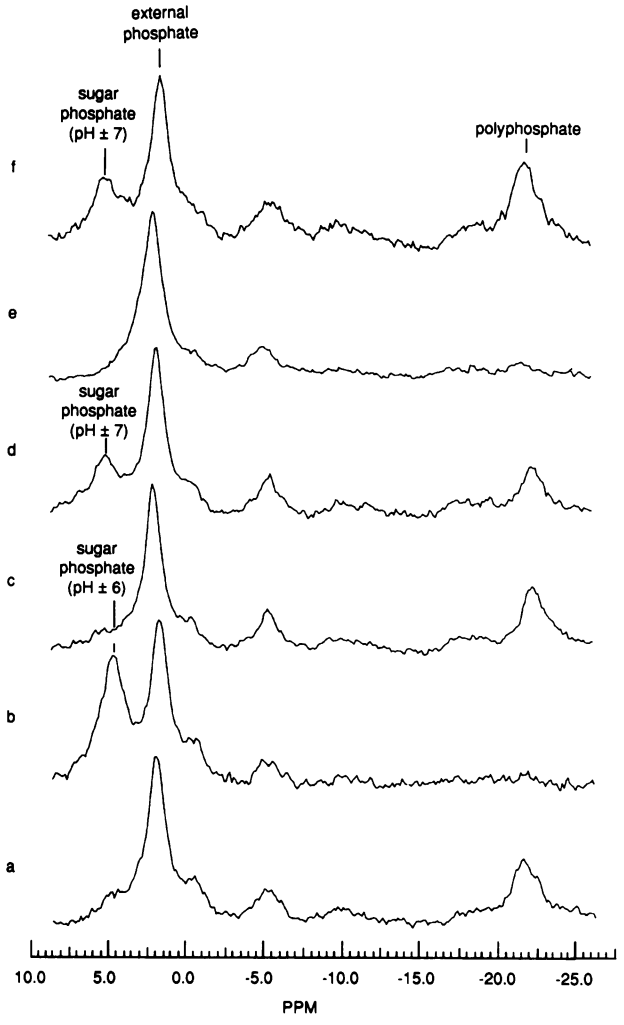


Fig. 7. *In vivo* ^{31}P-NMR spectra before and after addition of glucose to *fdp1* cells with or without the *fdp1* suppressor. Glycerol-grown *fdp1* cells were incubated with 200 mM glucose in the presence of 20 mM phosphate in 50 mM MES buffer pH 6. The cells were continuously bubbled with O_2. Before addition of glucose (**a**), a prominent polyphosphate peak is present. 45 min after addition of glucose (**b**) the polyphosphate has disappeared while a very large sugar phosphate peak is apparent at a position reflecting an acidic internal pH (±6). Glycerol-grown *fdp1* cells containing the *FPS1* suppressor on the complete insert (pLVA4009/*FPS1*) before (**c**) and 45 min after (**d**) addition of glucose. Polyphosphate breakdown is less extensive and the much smaller sugar phosphate peak appears at a position indicating a more neutral intracellular pH (±7). Glucose-grown *fdp1* cells containing the *FPS1* suppressor on the complete insert (pLVA4009/*FPS1*) (**e**: before, **f**: 45 min after addition of glucose) showed a similar behaviour except that polyphosphate was no longer broken down but accumulated.

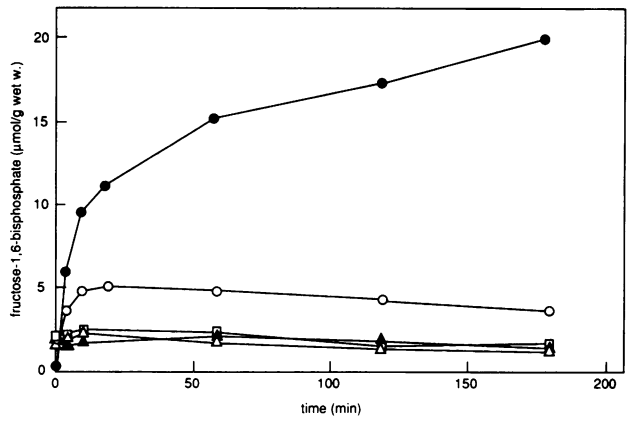


Fig. 8. Fructose-1,6-bisphosphate level after addition of glucose to wild type (w.t.) and *fdp1* cells with or without the *FPS1* suppressor and to the *fps1*Δ strain. ●, *fdp1*; ○ *fdpl* + YCp*FPS1Bam*HI−*Hin*dIII; △, w.t.; ▲, w.t. + YCp*FPS1Bam*HI−*Hin*dIII; □, LVAS-1 (fps1 :: LEU2). (Similar results were obtained with the *FPS1* suppressor on the complete insert (pLVA4009/*FPS1*): not shown.)

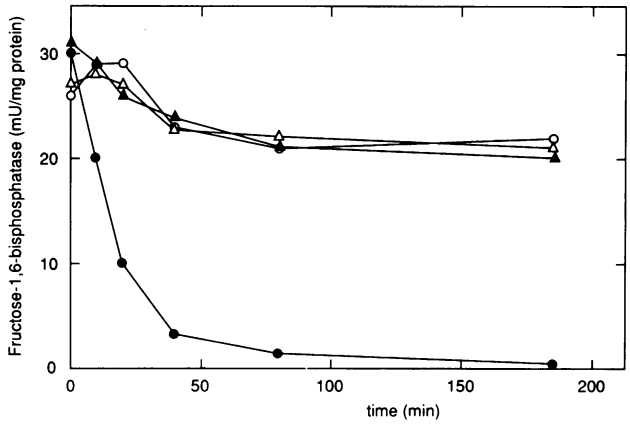


Fig. 9. Glucose-induced inactivation of fructose-1,6-bisphosphatase in the wild type strain (●), the *fdp1* mutant (○) and the *fdp1* mutant containing a centromeric plasmid with the *FPS1* suppressor on the complete insert (pLVA4009/*FPS1*) (▲) or on the *Bam*HI−*Hin*dIII 2.6 kb fragment (△).

glucose. Direct measurements of the different sugar phosphates in cell extracts using biochemical determination methods have confirmed the results obtained by *in vivo* ^{31}P-NMR spectroscopy (Figure 8). They also showed that fructose-1,6-bisphosphate is the major component responsible for sugar phosphate hyperaccumulation (Figure 8).

The phenotype of the *fdp1* mutant is more severe on fructose than on glucose. In fact, partial revertants have been isolated which grow on glucose but not on fructose (van de Poll and Schamhart, 1977). The transient hyperaccumulation of sugar phosphate in *fdp1* cells with the *FPS1* suppressor resembles the transient hyperaccumulation of sugar phosphate which is observed in partial revertants of the *fdp1* mutant. Revertants that grow on glucose but not on fructose show continuous accumulation of sugar phosphate when given fructose but only transient accumulation when given glucose (unpublished results).

Of particular interest is also the difference in intracellular pH observed in *fdp1* cells without and with the *FPS1* suppressor. The *fdp1* mutant displays strong intracellular acidification upon glucose addition. This can be inferred from the position of the sugar phosphate peak which is clearly shifted towards a more acidic pH compared with the peak in wild type cells (Figure 7). When the *FPS1* suppressor is present, however, the sugar phosphate peak reflects a more neutral pH. Differences in pH optima of the enzymes of the first and the second part of the glycolytic pathway might offer a partial explanation for the continuous hyperaccumulation of sugar phosphate in the *fdp1* mutant. Hence, the strong intracellular pH drop might be a major cause of lethality. Because the presence of the *FPS1* suppressor shifts the

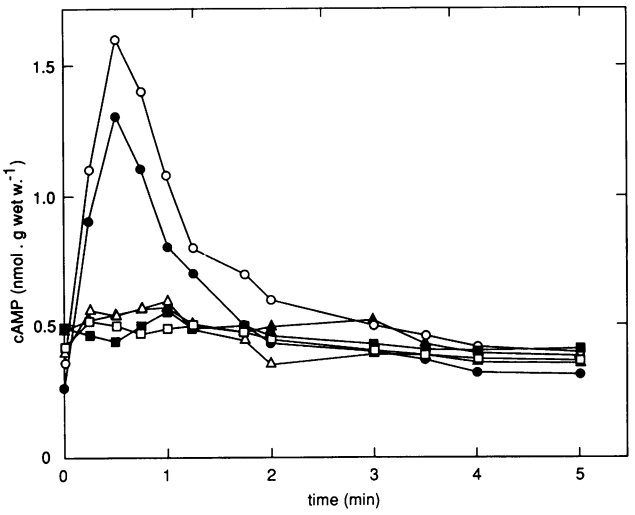


Fig. 10. The glucose-induced cAMP signal in the wild type strain, the *fdp1* mutant and the *fdp1* mutant containing the *FPS1* suppressor on single copy and multi-copy vectors with different insert lengths. ●, w.t. + pLVA4009/*FPS1*; ○, w.t. + YEp*FPS1Bam*HI−*Hin*dIII; ▲, *fdp1*; △, *fdp1* + pLVA4009/*FPS1*; ■, *fdp1* + YEp*FPS1 Bam*HI−*Hin*dIII; □, *fdp1* + YCp*FPS1Bam*HI−*Hin*dIII.

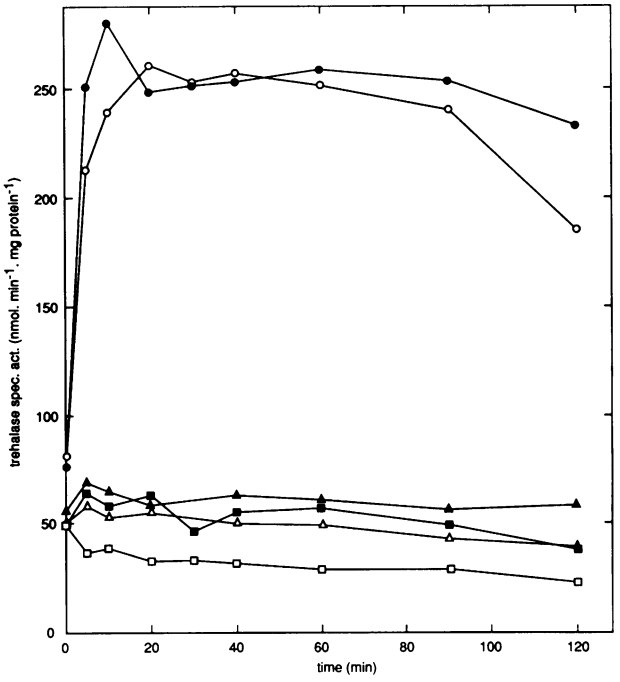


Fig. 11. Glucose- and fructose-induced activation of trehalase in the wild type strain, the *fdp1* mutant and the *fdp1* mutant containing the *FPS1* suppressor. ●,▲,■, glucose; ○,△,□, fructose. ●,○, w.t. + pLVA4009/*FPS1*; ▲,△, *fdp1*; ■,□, *fdp1* + pLVA4009/*FPS1*.

intracellular pH back to a normal value without on the other hand completely suppressing sugar phosphate hyper-accumulation, one could speculate that the FPS1 protein acts as a proton channel or pump, causing faster exit of protons from the cell.

Lack of suppression of the regulatory defects in fdp1 cells

The *fdp1* mutant shows a number of regulatory defects for which a biochemical connection with the inability to grow on fermentable sugars is unclear. The *FPS1* suppressor was not able to suppress either on a single copy or on a multi-copy vector the deficiencies in glucose-induced inactivation of fructose-1,6-bisphosphatase (Figure 9), glucose-induced cAMP signalling (Figure 10) or glucose-induced activation of trehalase (Figure 11). Glucose-induced inactivation consists of two processes: a rapid reversible inactivation process, which is mediated by cAMP-dependent protein phosphorylation and a slower irreversible inactivation process, which is mediated by proteolysis (Mazon *et al.*, 1982; Holzer, 1984). The proteolytic inactivation process does not depend on the phosphorylation process (Rose *et al.*, 1988). Our results demonstrate that the FDP1 protein is required for both processes (Figure 9). Glucose-induced cAMP signalling is mediated by the CDC25-RAS-adenyl cyclase signalling pathway (Mbonyi *et al.*, 1988, 1990; Munder and Küntzel, 1989; Van Aelst *et al.*, 1990, 1991). However, recent results with a mutant that lacks all cAMP responses but displays a normal basal cAMP level, have indicated that glucose-induced cAMP signalling may not be required for glucose-induced inactivation of fructose-1,6-bisphosphatase nor for glucose-induced activation of trehalase (unpublished results). The FDP1 protein therefore appears to have a function upstream in the glucose-induced cascade of regulatory events and this at a point which is common to several glucose-induced signalling pathways.

A regulatory deficiency of the *fdp1* mutant which is particularly difficult to link to the growth defect on fermentable carbon sources is the high protein kinase activity present when *fdp1* cells are grown on non-fermentable carbon sources (van de Poll and Schamhart, 1977). One of the consequences of this high protein kinase activity is a very low trehalose level. Presence of the *FPS1* suppressor has no restoring effect on the very low trehalose level in *fdp1* cells (Table I). Hence, of all the regulatory deficiencies in the *fdp1* mutant which we have checked, none was restored by the *FPS1* suppressor. These results make it likely that of all the (known and unknown) regulatory defects that are caused by the *fdp1* mutation none is restored by the *FPS1* suppressor. The suppression of the growth deficiency by *FPS1* might be a fortuitous event. The reason for this effect is at present unclear, although it might be related to the suppression of glucose-induced intracellular acidification in the *fdp1* mutant. The discovery of the *FPS1* suppressor creates the very interesting perspective of studying other possible deficiencies in glucose-induced regulatory effects in the *fdp1* mutant, in particular long-term glucose-induced effects for which growth on glucose is a prerequisite.

Table I. Trehalose level in glycerol-grown cells of the wild type strain, the *fdp1* mutant and the *fdp1* mutant containing the *FPS1* suppressor on single copy and multi-copy vectors with different insert lengths

	Trehalose (μg/mg wet weight)
FDP1 (strain SP1)	11.3
fdp1 (strain LVA1531)	0.76
fdp1 (strain MV6807)	0.50
fdp1 (strain LVA1531) + pLVA4009/*FPS1*	1.20
fdp1 (strain LVA1531) + YEp*FPS1Bam*HI−*Hin*dIII	1.10
fdp1 (strain LVA1531) + YCp*FPS1Bgl*II−*Xba*I	1.12
fdp1 (strain LVA1531) + YEp*FPS1Sal*I−*Hin*dIII	0.89
fdp1 (strain LVA1531) + YCp*FPS1Bam*HI−*Hin*dIII	0.90

Conclusion

The *FDP1* gene product seems to play a role very early in sugar metabolism and in several glucose-induced signalling pathways, e.g. the RAS pathway leading to activation of adenyl cyclase and the pathway leading to proteolytic inactivation of fructose-1,6-bisphosphatase. The FDP1 protein could play a role as a sensor for the availability of fermentable sugar, controlling both the initiation of glycolysis and the activation of signalling pathways. These are clearly two distinct functions since the FPS1 gene product complements only the growth defect on fermentable sugars of the *fdp1* mutant, but not the defects in the activation of the signalling pathways. The regulatory defects in the *fdp1* mutant are clearly not a consequence of the growth defect. The cause of the growth defect of the *fdp1* mutant appears to be situated at the level of sugar transport/sugar phosphorylation. Hence, it was not surprising to identify a putative membrane protein as a suppressor of the growth defect. FPS1 and FDP1 might share a common or related function in the regulation of early sugar metabolism, or alternatively FPS1 might restore growth by suppressing intracellular acidification. It remains difficult to interpret why a second copy of *FPS1* is sufficient to complement at least partially the growth defect of the *fdp1* mutant on glucose and fructose.

Materials and methods

Strains and media

The following yeast strains were used: LVA1531 (*MATα ura3 his3 lys2 fdp1*) and MV6807 (*Matα ura3 his3 lys2 ade8 trp1 leu2 fdp1*). These strains were obtained by successive backcrosses of the *fdp1* mutation present in strain DFY334 (*MATα lys2 MAL6 fdp1*), kindly supplied by D.Fraenkel (Harvard University), into the genetic background of strain SP1 (*MATa leu2 his3 ura3 trp1 ade8 can1*), kindly supplied by M.Wigler, Cold Spring Harbor Laboratory. Construction of *fps1Δ* in the SP1 strain (LVAS-1 *Mata fps1 :: LEU2 leu2 his3 ura3 trp1 ade8 can1*) has been described in the Results. *E.coli* strain HB101 was used as bacterial host for plasmid DNA proliferation. Rich media used were YPD, YPGlycerol or YPGalactose. Minimal media were as specified by Sherman *et al.* (1986).

Nucleic acid manipulations

Preparation and manipulation of nucleic acids and transformation into yeast or *E.coli* were done using standard procedures (Sambrook *et al.*, 1989; Sherman *et al.*, 1986). Northern blot analysis was done using poly(A) enriched RNA preparations. The DNA probe was labelled by nick translation using [α-^{35}S]dATP. Southern blot analysis was essentially performed following the instructions of the Boehringer Mannheim digoxigenin DNA labelling and detection kit. To achieve conditions of reduced stringency, hybridization was started for 12 h at 68°C and then the temperature was reduced to 45°C for another 6 h. The high stringency wash (2 × 15 min in 0.1 × SSC, 0.1% SDS at 68°C) was omitted.

Cloning and characterization of the FPS1 gene

The yeast genomic library of Sengstag and Hinnen (1987; kindly provided by C.Sengstag) consists of ±5000 independent *E.coli* clones maintained separately in microtitre plates. These clones were pooled into 13 sublibraries with 384 clones each. Plasmid DNA from these sublibraries was transformed independently into yeast strain LVA1531. Transformants were isolated on synthetic medium lacking uracil and with galactose as carbon source. Glucose positive clones were identified after replica plating onto the same synthetic medium with glucose instead of galactose as carbon source and then tested for plasmid instability. The plasmid complementing the growth defect of the *fdp1* mutant on glucose was isolated twice from different sublibraries. Fragments of the insert were subcloned into vectors described by Gietz and Sugino (1988).

DNA sequencing

DNA fragments used for sequencing were subcloned into the vectors M13mp18 or M13mp19 (Vieira and Messing, 1982). Sequencing was carried out by the chain-termination method (Sanger *et al.*, 1977) using the T7 DNA polymerase based sequencing kit of Pharmacia-LKB. Sequences were analysed using the DNASIS/PROSIS software package (Hitachi).

Biochemical determinations and ^{31}P-NMR spectroscopy

Determination of cAMP levels and specific activity of trehalase were performed as described previously (Thevelein *et al.*, 1983, 1987). Fructose-1,6-bisphosphatase activity was measured according to Gancedo and Gancedo (1971). Fructose-1,6-bisphosphate was determined enzymatically using standard methods. For the biochemical determinations the cells were incubated in MES/KOH buffer as described before (Thevelein *et al.*, 1987). Sugars were always added in a concentration of 100 mM. *In vivo* ^{31}P-NMR spectroscopy was performed as described previously (Thevelein *et al.*, 1987) except that for each spectrum 104 scans (1 min) were accumulated.

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Note added in proof

While this paper was being prepared for publication, a new review on the MIP family of membrane proteins has appeared, in which several newly discovered members of the family have been described (Pao,G.M., Wu,L.-F., Johnson,K.D., Höfte,H., Chrispeels,M.J., Sweet,G., Sandal,N.N. and Saier,M.H.Jr (1990) *Mol. Microbiol.*, **5**, 33–37).