Suppression of *pdc2* Regulating Pyruvate Decarboxylase Synthesis in Yeast

Soundarapandian Velmurugan, Zita Lobo and Pabitra K. Maitra[1]

Molecular Biology Unit, Tata Institute of Fundamental Research, Mumbai 400 005, India

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ABSTRACT

Mutants lacking pyruvate decarboxylase cannot grow on glucose. We have isolated three different complementation groups of extragenic suppressors that suppress mutations in *pdc2*, a regulatory locus required for the synthesis of the glycolytic enzyme pyruvate decarboxylase. The most frequent of these is a recessive mutation in the structural gene *PFK1* of the soluble phosphofructokinase. The other class *XSP18* (extragenic suppressor of *pdc2*) is a dominant temperature-sensitive suppressor that allows the cells to grow on glucose only at 30° but not at 36°. It also affects the normal induction of the glucose-inducible enolase 2, which can be rescued by providing a copy of wild-type *xsp18* in *trans*-heterozygotes. The pyruvate decarboxylase activity in the triple mutant *pdc2 pfk1 XSP18* is nearly equal to the sum of the activities in the two double mutants *pdc2 pfk1* and *pdc2 XSP18*, respectively. This implies that the two suppressors act through independent pathways or that there is no cooperativity between them. In the *pdc2 pfk1 XSP18* strain, *pfk1* suppresses the loss of induction of glucose-inducible enolase 2 brought about by *XSP18*, but fails to rescue temperature sensitivity. The third class (*xsp37*) supports the growth of the *pdc2* mutant on glucose but fails to support growth on gluconeogenic carbon sources. All the three suppressors suppress *pdc2*Δ as well and act on *PDC1* at the level of transcription.

IN the yeast *Saccharomyces cerevisiae* the glycolytic genes are highly expressed and constitute ~30% of soluble proteins (FRAENKEL 1982). Most of these enzymes are coordinately induced by glucose (MAITRA and LOBO 1971a), and induction is primarily at the level of transcription (MOORE *et al.* 1991). It was shown earlier in this laboratory that glucose 6-P is a likely inducer of glycolytic enzymes (MAITRA and LOBO 1971b). Although there has been considerable information about *trans*-acting factors that bind to the upstream sequence of glycolytic genes (HOLLAND *et al.* 1987; BUCHMAN *et al.* 1988a,b; BRINDLE *et al.* 1990; SANTANGELO and TORNOW 1990; BAKER 1991; HUIE *et al.* 1992; UEMURA and JIGAMI 1992), the exact mechanism by which glucose mediates these responses is far from clear.

The enzyme pyruvate decarboxylase catalyzes the conversion of pyruvate to acetaldehyde. It is induced ~10–20 fold by glucose (SCHMITT and ZIMMERMANN 1982). Three structural genes that code for this enzyme independently, *viz. PDC1, PDC5* and *PDC6,* have been identified (KELLERMANN *et al.* 1986; SCHAFF *et al.* 1989; SEEBOTH *et al.* 1990; HOHMANN 1991). However, in the wild-type, *PDC1* is the only gene that is transcribed. *PDC5* is expressed only when *PDC1* is deleted and *PDC6* is a weakly expressed gene in both alcohol and glucose (HOHMANN and CEDERBERG 1990; HOHMANN 1991a). The regulation of PDC activity is largely determined by changes in the mRNA levels of *PDC1* (SCHMITT *et al.* 1983). *PDC2* and *PDC3,* two additional genes required for the expression of pyruvate decarboxylase, have also been identified (SCHMITT *et al.* 1983; WRIGHT *et al.* 1989). Mutation in *PDC2* renders *S. cerevisiae* incapable of synthesizing pyruvate decarboxylase due to an acute inability to transcribe the structural gene *PDC1* (RAGHURAM *et al.* 1994). The *PDC2* locus was cloned and sequenced (HOHMANN 1993; RAGHURAM *et al.* 1994) and was shown to encode a transcriptional activator required for the expression of *PDC1* and *PDC5* (RAGHURAM *et al.* 1994). The *PDC1* promoter contains two distinct regions responsible for glucose- and ethanol-dependent regulation of pyruvate decarboxylase synthesis (KELLERMANN *et al.* 1988). An RPG box, containing upstream activation site, has been identified by promoter deletion analysis (BUTLER *et al.* 1988). Gel retardation and oligonucleotide competition experiments have shown the interaction of TUF factor with the RPG box (BUTLER *et al.* 1990). The TUF factor acts independently of the carbon source. Likewise, *PDC2* expression is largely independent of the presence of glucose (RAGHURAM *et al.* 1994).

The mechanism by which glucose brings about this high-level induction of PDC has not been worked out. The *gcr1* and *gcr2* mutations identified as general glycolytic regulators are involved in the regulation of several other glycolytic enzymes (CLIFTON *et al.* 1978; CLIFTON and FRAENKEL 1981; BAKER 1986; UEMURA and FRAENKEL 1990) without affecting the synthesis of pyruvate decarboxylase very much (unpublished observation from this laboratory). Gcr1p binds to many glycolytic enzyme promoters at CTTCC motif (BAKER 1991; HUIE

Corresponding author: Zita Lobo, Molecular Biology Unit, Tata Institute of Fundamental Research, Homi Bhabha Rd., Colaba, Mumbai-400 005, India. E-mail: zita@tifrvax.tifr.res.in

[1]*Present address:* Agharkar Research Institute, Pune 411 004, India.

TABLE 1

Strains used in this study

Strains	Genotype
EG103[a]	*MAT***a** *ura3 leu2 trp1*
Kn79[a]	*MAT*α *leu2 trp1*
pdc2 T17B	*MAT*α *pdc2-2 ura3 leu2 trp1*
pdc2Δ T14D	*MAT*α *pdc2Δ::TRP1 trp1 leu2 ura3*
pdc2 pfk1 T4A	*MAT*α *pdc2-2 pfk1-1*
pdc2 XSP18 T2D	*MAT*α *pdc2-2 XSP18 leu2 trp1*
pdc2 xsp37 T5B	*MAT*α *pdc2-2 xsp37 ura3*
pdc2 pfk1 XSP18 T7B	*MAT*α *pdc2-2 pfk1-1 XSP18 ura3 leu2 trp1*
pdc1 pdc2 pfk1 T2D	*MAT*α *pdc1-1 pdc2-2 pfk1-1 ura3 trp1*
pdc1 pdc2 XSP18 T2C	*MAT***a** *pdc1-1 pdc2-2 XSP18 ura3 leu2 trp1*
pdc2Δ pfk1	*MAT*α *pdc2Δ::URA3 pfk1-1 ura3 trp1*
pdc2Δ XSP18	*MAT*α *pdc2Δ::URA3 XSP18 ura3 leu2 trp1*
pdc2Δ xsp37	*MAT*α *pdc2Δ::URA3 xsp37 ura3 trp1*
HD56-5A pfk1Δ[b]	*MAT***a** *ura3 leu2 his3-15 MAL GAL SUC pfk1Δ::HIS3*
HD56-5A pfk2Δ[b]	*MAT*α *ura3 leu2 his3-15 MAL GAL SUC pfk2Δ::HIS3*
pdc2Δ pfk1Δ	*MAT*α *pdc2Δ::URA3 pfk1Δ::HIS3 ura3 his3-15 leu2*
pdc2Δ pfk2Δ	*MAT***a** *pdc2Δ::URA3 pfk2Δ::HIS3 ura3 his3-15 leu2*
pdc2 pfk1 pfk2 T14D	*MAT***a** *pdc2-2 pfk1-1 pfk2-1*
pdc2 pgi T2A	*MAT***a** *pdc2-2 pgi1-1 leu2 trp1*
PDC2 XSP18 T2B	*MAT*α *XSP18 ura3*
PDC2 xsp37 T4C	*MAT***a** *xsp37 ura3*

[a] Both the wild-type strains were obtained from B. HALL.

[b] *pfk1* and *pfk2* deletions were obtained from HEINISCH; however, their nomenclature has been revised according to GAYATRI and MAITRA (1991). All other strains are from this laboratory.

et al. 1992). It has been shown that Gcr1p and Gcr2p act as a complex, in which Gcr1p provides the DNA-binding function and Gcr2p the transcriptional activation function (UEMURA and JIGAMI 1992, 1995). The synthesis of PDC seems to be mediated through a unique regulatory mechanism with *PDC2* as a major player (RAGHURAM *et al.* 1994). The main goal of this study is to identify new genes involved in the regulation of pyruvate decarboxylase synthesis. As a step in this direction, we have isolated extragenic suppressors of *pdc2*. We have identified mutations in three genetic loci that can suppress the *pdc2* phenotype. Genetic characteristics of these suppressors have been examined.

MATERIALS AND METHODS

Yeast strains and media: Yeast strains are shown in Table 1. Yeast cultures were grown in YEP (0.3% yeast extract, 1% Bacto peptone) medium with indicated carbon source or synthetic complete (SC) medium (SHERMAN *et al.* 1986) containing amino acids. For auxotrophic marker selection the appropriate amino acid(s) was omitted. Sporulation medium was used as described in SHERMAN *et al.* (1986).

Escherichia coli: E. coli strains HB101 and DH5α (SAMBROOK *et al.* 1989) were used for plasmid propagation. *E. coli* strains containing plasmids were grown in LB medium with antibiotics and the plasmids were obtained using the protocol described elsewhere (BIRNBOIM and DOLY 1979).

Isolation of revertants: All the suppressed strains were isolated by plating the *pdc2* mutant on YEP plates containing 1% glucose or 1% glucose + 0.3 mM azide. Plates were incubated at 30° for a few days to a few weeks.

Enzyme assay: All the glycolytic enzymes were assayed using the fluorimetric method described earlier (MAITRA and LOBO 1971a). β-galactosidase activity was assayed as described (GUARENTE 1983). All of the substrates and enzymes were purchased from either Sigma Chemical Co., USA, or Boehringer Mannheim, Germany.

Construction of diploids: The diploids used in this study were constructed by mating the respective strains in opposite mating type and incubating them at 30° for 6–10 hr. Zygotes were isolated using a micromanipulator and confirmed by checking either for mating or for sporulation or for complementing auxotrophic markers.

Northern blot analysis: Yeast RNA was prepared from the respective strains and characterized by Northern analysis as described (SHERMAN *et al.* 1986). DNA fragments labeled by random priming with a α-[^{32}P]dATP labeling kit from Boehringer Mannheim, Germany, were used as probes. *ENO1*- and *ENO2*-specific oligonucleotide probes were made by end-labeling with γ-[^{32}P]dATP using T4 polynucleotide kinase from New England Biolabs, England. The sequences of the oligonucleotide probes used were (NISHI *et al.* 1995) as follows: *ENO1*-3′, CTTTTGGATCGATAAAAAGTATTTTTTGGTTCGTTGACGAATAGTTGTG 5′ (49 mer); *ENO2*-3′, GGTCGTTGATTATGATATTGTATGTTATTAT 5′ (31 mer).

Hybridization was performed at 60° and radioactive bands were detected by autoradiography.

Plasmid construction and gene replacement: To make a *pdc2* deletion by gene replacement, we used the following construct. YEp24 plasmid was digested with *Hin*dIII and a 1.2-kb fragment containing *URA3* was isolated and ligated to a partially digested genomic DNA clone of *PDC2* obtained from a CV13 genomic DNA library. The resulting construct was selected by restriction enzyme analysis. This construct was cut with *Bam*HI and *Cla*I. The resulting fragments were transformed by the lithium acetate method (GIETZ *et al.* 1992) into the appropriate strains to make the *pdc2* deletion mutant.

TABLE 2

Phenotypes of the suppressors

Strains	YGlu		YAlc		YGlu + azide	
	30°	36°	30°	36°	30°	36°
Kn79 (WT)	+	+	+	+	+	+
pdc2 T17B	−	−	+	+	−	−
pdc2 pfk1 T4A	+	+	+	+	−	−
pdc2 XSP18 T2D	+	−	+	+	+	−
pdc2 xsp37 T5B	+	+	−	−	+	+
pdc2 pfk1 XSP18 T7B	+	−	+	+	−	−
PDC2 XSP18 T2B	+	+	+	+	+	+
PDC2 xsp37 T4C	+	+	−	−	+	+
pdc2Δ pfk1Δ	+	+	+	+	−	−
pdc2Δ pfk2Δ	+	+	+	+	+	+
pdc2 pfk1 pfk2	−	−	+	+	−	−
pdc2 pgi[a]	−	−	+	+	−	−

Growth on YEP agar plates containing carbon supplements (glucose 1% ± azide 0.3 mM; alcohol 1%) was scored in 3 days at two different temperatures.

[a] Glucose-free fructose was used in place of glucose.

Only those transformants that showed a gene disruption at the *PDC2* locus by integration of the construct bearing *URA3* were used as *pdc2* null mutants. These null alleles were confirmed by Northern analysis (Figure 2).

RESULTS

Isolation of suppressed strains: With the aim of identifying new genes involved in the regulation of pyruvate decarboxylase, we looked for spontaneous revertants from a *pdc2* segregant, *pdc2-2*T21B. Revertants were isolated on glucose media with or without 0.3 mM sodium azide to allow isolation of fermentation-competent strains. The reversion frequency was $\sim 10^{-6}$ on glucose plates and $\sim 5 \times 10^{-8}$ in plates containing glucose-azide. A large number (>1000) of such revertants were purified, retested on plates, and assayed for pyruvate decarboxylase activity following growth on glucose and on alcohol. The revertants showed varying levels of PDC activity on glucose while on alcohol the majority were comparable to *pdc2-2*T21B. Based on their ability to grow under different growth conditions, we classified these revertants into three groups (Table 2). The predominant class of revertants obtained on glucose plates in the absence of azide were able to grow on glucose only under aerobic conditions. These were associated with the loss of soluble phosphofructokinase activity due to a mutation in the structural gene *pfk1* (LOBO and MAITRA 1982) that renders yeast incompetent in fermentative growth; about two-thirds of the revertants belong to this group (*pfk1*). The second major class of revertants were able to grow on glucose as well as on glucose-azide (*XSP18*). A third group of revertants surprisingly were unable to grow on alcohol (*xsp37*). Three such revertants were isolated and all of them were able to grow on glucose and glucose-azide. At least five revertants each from the first two groups and two from the third group were out-crossed. Representative segregants from each were used for further analysis (Table 2). Other than two from the second group, all revertants were found to be extragenic to *pdc2*.

TABLE 3

Dominance of the suppressors

Diploid genotype	YGlu		YAlc		YGlu − azide	
	30°	36°	30°	36°	30°	36°
Kn79/EG103 (WT)	+	+	+	+	+	+
pdc2/pdc2	−	−	+	+	−	−
pdc2 pfk1/pdc2	−	−	+	+	−	−
pdc2 XSP18/pdc2	+	−	+	+	+	−
pdc2 xsp37/pdc2	+	+	+	+	+	+

The diploids were grown on YEP agar plates containing the respective carbon sources (glucose 1% ± azide 0.3 mM; alcohol 1%) at two different temperatures and scored in 3 days.

Dominance: To test for dominance, at least two diploids that were homozygous for *pdc2* and heterozygous for each suppressor mutation were isolated and tested for their ability to grow on glucose (Table 3). This analysis indicated that suppressors from group II (*XSP18*) and group III (*xsp37*) were dominant while the suppressor mutation in group I was recessive. This classification is only based on their ability to suppress the mutant phenotype. Regarding temperature sensitivity, *XSP18* is dominant; *xsp37* is recessive with respect to alcohol negativity (Table 3). Based on these observations we call *XSP18* a dominant gain of function mutation and *xsp37* a recessive gain of function mutation.

PDC enzyme activity in the suppressors: The enzyme activity of various strains was measured as described in MATERIALS AND METHODS. The specific activity of glucose-inducible pyruvate decarboxylase increases gradually after the addition of glucose (data not shown). PDC attains its maximum specific activity 7 hr after the addition of glucose. All the suppressed strains were able to synthesize PDC enzyme to various levels on glucose media (Table 4). PDC activity in the suppressed strains *pdc2 XSP18* and *pdc2 xsp37* on glucose was 60% of the wild type; the *pdc2 pfk1* strain had up to 50% of the wild-type activity. However, the level of PDC activity on alcohol was low and comparable to that of the starting mutant strain *pdc2-2* T21B. It is noteworthy that the suppression occurs only in the presence of glucose.

Suppression works by stimulating transcription: Total RNA was isolated from various strains and electrophoresed under denaturing conditions (SHERMAN *et al.* 1986). Northern analysis using the 0.5-kb *Eco*RI-*Sal*I fragment of *PDC1* as probe revealed that all the suppressors were able to synthesize *PDC1* mRNA (Figure 1).

TABLE 4

Suppressors restore pyruvate decarboxylate activity

Strains	Enzyme activity, mU/mg of protein			
	PDC		ZWF	
	YAlc	YGlu	YAlc	YGlu
Kn79 (WT)	210	2236	174	191
pdc2 T17B	28	124	177	166
pdc2 pfk1 T4A	41	1192	202	192
pdc2 XSP18 T2D	71	1365	168	165
pdc2 xsp37 T5B	76	1476	191	176
pdc2 pfk1 pfk2 T14D	82	937	204	212
PDC2 pfk1 pfk2 T4C	46	1214	179	196
pdc2 pfk1 XSP18 T7B	171	2176	175	176

Yeast cultures were grown in YEP medium containing 1% alcohol except *pdc2 xsp37*, which was grown in YEP glucose 1% until it reached late log phase. This culture was then harvested, washed and reinoculated into respective media to continue the exponential growth for 8 hr. The *pdc2 xsp37* strain was, for the purpose of comparison, transferred to an alcohol medium and incubated for 8 hr. Cells were harvested and disrupted by a French pressure cell. The enzyme activity was assayed in the cell-free extracts. PDC, pyruvate decarboxylase; ZWF, glucose 6-P dehydrogenase.

However, the synthesis of *PDC1* mRNA was observed only in glucose-grown cultures and not in alcohol-grown cultures. This is consistent with the enzyme activity data (Table 4), that is, the suppression requires the presence of glucose.

Suppressor acts on *PDC1* gene: To determine which of the two structural genes coding for PDC, *viz. PDC1*

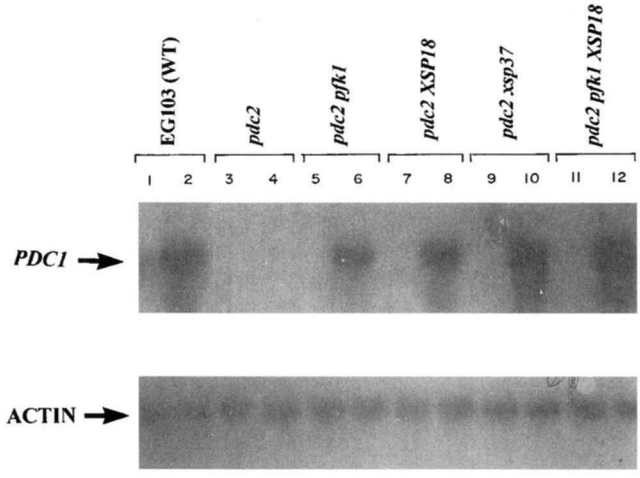

FIGURE 1.—Analysis of the *PDC1* transcript. Total cellular RNA was isolated from various strains grown on glucose or on alcohol. Approximately 50 μg of RNA was electropheresed in 1.5% formaldehyde-agarose gel and transferred to Amersham Hybond-N membrane and probed using *PDC1* and actin probes prepared by random priming. Odd numbers represent the alcohol-grown cultures whereas even numbers represent glucose-grown ones. Actin mRNA, which is not regulated by glucose, serves as an internal control. The top panel refers to the strains used.

TABLE 5

Suppressors act on *PDC1* independently of Pdc2p

Strains	YGlucose		YAlcohol	
	30°	36°	30°	36°
Kn79 (WT)	+	+	+	+
*pdc2*Δ	−	−	+	+
*pdc2*Δ *pfk1*	+	+	+	+
*pdc2*Δ *XSP18*	+	+	+	+
*pdc2*Δ *xsp37*	+	+	−	−
pdc1 pdc2 pfk1	−	−	+	+
pdc1 pdc2 XSP18	−	−	+	+

Growth was observed on YEP agar plates with carbon supplements (1% glucose; 1% alcohol) till the third day at two different temperatures.

and *PDC5*, gets activated, the suppressed strains were crossed to a *pdc1* mutant strain. Strains carrying mutations in *pdc1* were not suppressed by *pfk1* or *XSP18* (Table 5). The above result suggests that in these suppressed strains *PDC1* is the only gene that is transcribed. *PDC5* appears to stay unexpressed as in the wild type.

Suppression by *pfk* mutants: We mentioned earlier that the great majority of spontaneously isolated suppressors of *pdc2* have lost their soluble phosphofructokinase activity due to lesion in *pfk1*. Among >50 such suppressor strains not one was defective in the locus *pfk2*. However, deletion constructs in either *PFK1* or *PFK2* conferred suppressibility of *pdc2* (Table 2, lines 9 and 10). Earlier studies from this laboratory had suggested (GAYATRI and MAITRA 1991) that the polypeptide Pfk2p confers a scaffolding function to the catalytic subunit of Pfk1p, thus providing structural integrity to the soluble phosphofructokinase. The absence of such a function in the *pfk2*Δ mutant is likely to lead to a very considerable attenuation of the soluble phosphofructokinase activity and consequently suppression of the *pdc2* mutation. In fact results in Tables 2 and 5 suggest that *pdc2* suppression does not require any of the gene products of Pdc2, pfk1 or *pfk2* locus.

Mutation in *PGI1* fails to suppress *pdc2*: To rule out the possibility of the role of other upstream enzymes in *pdc2* suppression, we crossed *pdc2* T21B with a *pgi1* mutant and obtained the *pdc2 pgi1* segregant, which is fructose-negative (Table 2, last line). Thus *pgi1* fails to suppress the *pdc2* mutation.

***XSP18* interacts with *pdc2*:** We have constructed various *trans*-heterozygotes to check the interaction of missense mutant alleles with the suppressors (data not shown). Surprisingly one of the *trans*-heterozygotes *pdc2 XSP18/pdc2*Δ *xsp18* showed rescue of growth at 36° and thus revealed that *pdc2* and *XSP18* interact.

Suppressors act independently of Pdc2p: To check the role of Pdc2p in suppression, we have deleted *pdc2* by homologous recombination using a deletion-disruption construct. All the suppressors suppress a *pdc2* dele-

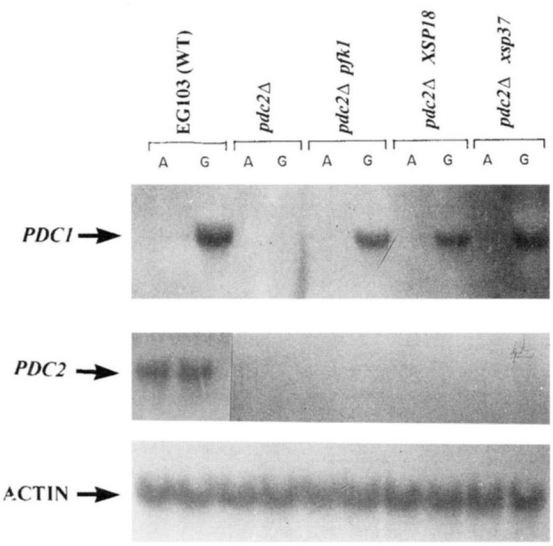

FIGURE 2.—Northern blot analysis of *PDC1, PDC2* and actin mRNAs in strains carrying the suppressors with *pdc2*Δ mutation. Cellular RNA was isolated, electrophoresed and transferred as mentioned in Figure 1. A represents alcohol-grown culture; G represent glucose-grown culture. The blot was hybridized using *PDC1, PDC2,* and actin probes prepared using random priming. Yeast actin mRNA serves as an internal control. The top panel represents the strains. The middle panel with *PDC2* RNA was exposed for longer time compared to the other two panels with *PDC1* and actin transcripts.

tion allele (Table 5). The levels of *PDC1* and *PDC2* transcripts were checked by Northern blot analysis (Figure 2). However, the reversal of the temperature-sensitive phenotype in the *pdc2*Δ *XSP18* (Table 5, line 4) in contrast to *pdc2 XSP18* (Table 2) shows the interaction of *pdc2* with *XSP18*. This correlates with our earlier observation in using *trans*-heterozygotes. The nature of interaction is under investigation.

***XSP18* affects induction of enolase:** When we measured the levels of various glycolytic enzymes in the *pdc2 XSP18* strain, the induction of the enolase enzyme by glucose was found to be affected by the suppressor mutation (Table 6). Enolase is induced at least two- to fivefold by glucose in wild-type strains, which is consistent with earlier observations (COHEN *et al.* 1986). In the *pdc2 XSP18* strain, the enolase levels fail to increase appreciably when cells were grown on glucose as compared to cells grown on alcohol (Table 6, line 4). Surprisingly the *pfk1* mutation was able to rescue the defect in the induction of enolase in the background of *XSP18* (Table 6, line 6) even though it cannot rescue the temperature-sensitive phenotype (Table 2, line 6). This also shows that the effect of *XSP18* on the temperature-sensitive phenotype is mainly due to the absence of suppression, not because of its additional effect on enolase. Since there are two enolases in yeast (HOLLAND *et al.* 1981), we confirmed that the effect of *pdc2 XSP18* was on the induction of *ENO2,* and not on *ENO1.* This was demonstrated using the *ENO1-lacZ* (data not shown) construct as well as by Northern blot analysis (Figure 3), using *ENO1*- and *ENO2*-specific probes (NISHI *et al.* 1995). The glucose-induced *ENO2* transcript is completely absent in *pdc2 XSP18* (Figure 3, lane 6) in contrast to the wild-type and the parental *pdc2* mutant strains grown on glucose (Figure 3, lanes 2 and 4). In *PDC2 XSP18* the enolase activity is similar to that in *pdc2 XSP18* (Table 6, last line). This traces the origin of the effect to the suppressor locus *XSP18.*

The temperature-sensitive phenotype in the *pdc2 XSP18* strain is due to lack of suppression: Since the *pdc2 XSP18* strain showed an additional effect on enolase 2 induction, we checked the reason for the temperature sensitivity by transforming *pdc2 XSP18* with either *PDC1* or *PDC2* multicopy clones, which is known to suppress or rescue the *pdc2* defect, respectively. (RAGHURAM *et al.* 1994). The strain *pdc2 XSP18* bearing either of the multicopy clones grew at high temperature.

***XSP18* is temperature-sensitive and interacts additively with *pfk1*:** Suppressors belonging to the *XSP18* class did not grow on glucose medium at 36°; however, growth on alcohol was not affected at this temperature. In the background of wild-type *PDC2,* the suppressor mutations did not result in any visible growth defect on

TABLE 6

***XSP18* affects enolase induction**

Strains	ZWF		ENO		PK	
	YAlc	YGlu	YAlc	YGlu	YAlc	YGlu
Kn79 (WT)	174	191	815	3883	181	2076
pdc2 T17B	177	166	630	1840	240	2360
pdc2 pfk1 T4A	202	192	1009	4073	243	2130
pdc2 XSP18 T2D	168	165	480	560	153	1726
pdc2 xsp37 T5B	191	176	745	2836	238	2741
pdc2 pfk1 XSP18 T7B	175	176	752	3478	268	3188
PDC2 XSP18 T2B	143	171	373	421	270	2199

Cultures were grown in liquid enriched medium supplemented with the indicated carbon source (glucose or alcohol 1% each) and harvested in lograthmic growth, 8 hr after inoculation to break using French pressure cell. Specific enzyme activity was assayed in the cell free-extracts. ZWF, glucose 6-P dehydrogenase; ENO, enolase; PK, pyruvate kinase.

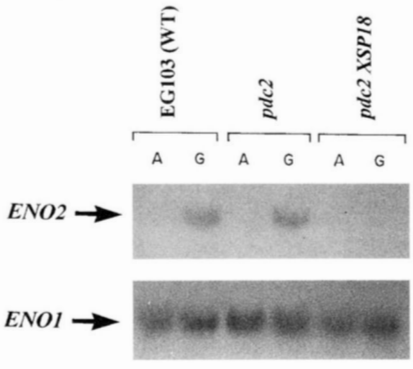

FIGURE 3.—Visualization of transcripts of constitutive (*ENO1*) and glucose-inducible (*ENO2*) enolases using specific molecular probes. Total RNA was isolated from EG103 (WT), *pdc2* and *pdc2 XSP18* strains and electrophoresed in 1.5% formaldehyde-agarose gel. The blotted RNA was hybridized using *ENO1*- and *ENO2*-specific oligonucleotide probes. Constitutive synthesis of *ENO1* serves as an internal control for an enzyme not responsive to glucose induction. A, alcohol-grown culture, G, glucose-grown culture. Other details are the same as in Figure 1.

either glucose or alcohol at the temperatures we tested (Table 2, line 7).

To determine if there is any interaction between these two suppressors, we generated recombinants carrying both *pfk1* and *XSP18*. The level of PDC in this strain was more than that in either of the individual suppressor-bearing strains (Table 4, last line). The enzyme activity was as much as that in the wild type and was nearly equivalent to the sum of the activities of the individual suppressed strains. However, this strain did not grow on glucose at elevated temperatures, indicating that the phenotype of *XSP18* is epistatic to *pfk1* (Table 2, line 6) at high temperature.

DISCUSSION

Our selection scheme for suppressors of *pdc2* was designed to reveal loci that permit *PDC1* transcription in the absence of its cognate regulatory activator protein Pdc2p. This might come about by mutational alteration of an existing transcriptional activator protein perhaps at the cost of growth in an otherwise permissive condition. We have described mutations in three different genetic loci that can suppress the defect caused either by point mutation or deletion-disruption in *PDC2*. The suppressors affect the expression of *PDC1* transcription and are dependent on the presence of glucose for the synthesis of PDC activity. The expression of the *PDC1* transcript is seen only in glucose-grown cultures (Figures 1 and 2), supporting that the suppression is a consequence of restored pyruvate decarboxylase activity.

Mutations in the gene encoding the catalytic subunit of phosphofructokinase (*PFK1*) (LOBO and MAITRA 1982) were identified as suppressors of *pdc2*. Interestingly, the identification of *pfk1*Δ and *pfk2*Δ as suppressors of *pdc2* seems to implicate the role of accumulated glycolytic intermediates as an inducer in relieving the transcriptional block, although we have no clue as to how it works. Mutation in *PGI1* fails to rescue the *pdc2* growth defect on the fermentable carbon source fructose. The triple mutant *pdc2 pfk1 pfk2* is also glucose-negative (Table 2, line 11) due to a blocked metabolism. However, it is still capable of inducing pyruvate decarboxylase to nearly half of the wild-type level when induced in a mixture of alcohol and glucose. It accumulates nearly 3 to 14 nmol of F6P/mg dry yeast (ARVANITIDIS and HEINISCH 1994; P. K. MAITRA and Z. LOBO, unpublished results) that could be responsible for triggering the synthesis of pyruvate decarboxylase. Complete induction of PDC perhaps requires additional signals (BOLES and ZIMMERMANN 1993; MULLER *et al.* 1995).

Mutations at the genetic locus designated *XSP18* are also able to suppress the *pdc2* phenotype. These mutations are dominant "gain in function" of a gene product. The observation that this suppressor extinguishes the glucose-mediated induction of enolase 2 (Figure 3) shows its multiple functions. The likely explanation for such a mutation could be an alteration of a transcription factor in such a way as to activate the transcription of *PDC1* in the absence of Pdc2p. This could be a factor that mediates glucose-specific induction of enolase 2.

The fact that *XSP18* is epistatic over *pfk1* at high temperature suggests that *XSP18* is also involved in the same mechanism by which *pfk1* suppresses *pdc2*. The additive PDC level in the double suppressor *pdc2 pfk1 XSP18* could be because of absence of cooperativity between these two suppressors. We believe that in glucose-containing medium the signal mediated by fructose 6-phosphate is transmitted via *XSP18* to a complex of transcription factors that brings about *PDC2*-independent transcription of *PDC1*. The absence of any effect of this suppressor in alcohol-grown culture supports a specific role of glucose on *XSP18*.

The *trans*-heterozygote *pdc2 XSP18/pdc2*Δ could rescue the temperature-sensitive property associated with this suppressor. This shows the interaction of *pdc2* with *XSP18*. Similar results have been obtained in *pdc2*Δ *XSP18* (Table 5, line 4), which is not temperature sensitive. Moreover, the strain *pdc2 XSP18* bearing a *PDC1* multicopy genomic DNA clone is also 36° positive. The fact that multicopy *PDC1* suppresses both *pdc2* and *pdc2*Δ mutant strains (RAGHURAM *et al.* 1994) indicates that the temperature sensitivity of the *XSP18*-bearing strain is due to lack of suppression in restoring PDC activity and not due to the other pleiotropic effects like enolase induction or interaction with *pdc2*. This suggests that the thermolabile nature of Xsp18p is due to its inability to compete with Pdc2p for the *PDC1* promoter site. Further investigation is in progress to understand the role of *XSP18* in suppression of the *pdc2* mutation.

To account for the suppression of the *pdc2* phenotype

by *xsp37* we suggest that there is a transcription factor essential for growth on alcohol and that it interacts with the *PDC1* promoter for the regulation of *PDC1* synthesis with respect to gluconeogenesis. Such an interpretation is supported by our earlier observation that multiple copies of the *PDC1* promoter lead to decreased growth of the cells in alcohol (RAGHURAM *et al.* 1994). A rare mutation could change the affinity of this factor to bind to the *PDC1* promoter or to interact with other unknown factor(s) acting at the level of the *PDC1* promoter and result in activation of transcription in the absence of *PDC2*. Mutations of *XSP37* that result in the suppression of *pdc2* might also affect the ability of Xsp37p to do its normal function, that is, to control other genes required for growth on gluconeogenic carbon sources. We have cloned this locus by complementing the alcohol-negative phenotype. It is located on chromosome *III*. Further characterization of this clone may throw light on the mechanism of suppression.

In conclusion we have identified three different classes of extragenic suppressors of *pdc2*, namely *pfk1*, *XSP18* and *xsp37*, that are able to restore the ability of the *pdc2* mutant to grow on glucose. The rescue of growth on glucose is due to restoration of PDC activity by increasing transcription of *PDC1*. All the suppressors not only suppress missense but also the *pdc2*Δ mutations, suggesting a bypass mechanism independent of Pdc2p. *XSP18* is also involved in the glucose-mediated induction of enolase 2 (Figure 3). Suppressor mutation in *XSP37* leads to the loss of growth in alcohol. Further molecular characterization of these suppressor mutations may lead to better understanding of glucose-mediated induction of glycolytic enzymes as well as the coordinated regulation of gluconeogenesis and glycolysis at the level of the *PDC1* promoter.

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