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Induction of pyruvate decarboxylase in glycolysis mutants of *Saccharomyces cerevisiae* correlates with the concentrations of three-carbon glycolytic metabolites

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Abstract. Pyruvate decarboxylase, PDCase, activity in wild-type yeast cells growing on ethanol is quite low but increases up to tenfold upon addition of glucose, less with galactose and only slightly with glycerol. PDCase levels in glycolysis mutant strains growing on ethanol or acetate were higher than in the wild-type strain. These levels correlated with the sum of the concentrations of three-carbon glycolytic metabolites. The highest accumulation was observed in a fructose bisphosphate aldolase deletion mutant concomitant with the highest PDCase activity ever observed under gluconeogenic conditions. Glucose addition induced an increase in PDCase activity in all mutants. However, the enzyme activities never reached wild-type level. On the other hand, the PDCase levels in the different mutants again correlated with the sum of the concentrations of the three-carbon glycolytic metabolites. This was interpreted to mean that full induction of PDCase activity requires the accumulation of hexose- and triosephosphates.

Key words: *Saccharomyces cerevisiae* – Pyruvate decarboxylase – Pyruvate kinase – Signalling – Glycolysis mutants

Glycolysis in *Saccharomyces cerevisiae* is an inducible pathway which is activated by fermentable sugars (reviewed by Entian and Barnett 1992). Variations in the activities of glycolytic enzymes in response to the availability of glucose were first reported by Maitra and Lobo (1971a). This was subsequently shown to be due to regulation at the transcriptional level for pyruvate kinase (Burke et al. 1983), pyruvate decarboxylase (Schmitt et al. 1983; Schaaff et al. 1989; Hohmann and Cederberg 1990) and several other glycolytic enzymes (Moore et al. 1991, and references therein).

Abbreviations: PDCase, pyruvate decarboxylase; dw, dry weight; PEP, phosphoenolpyruvate; WT, wild-type

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Pyruvate decarboxylase is the key enzyme of alcoholic fermentation in yeast. It converts pyruvate to acetaldehyde which is the substrate for alcohol dehydrogenase. Three structural genes coding for pyruvate decarboxylase have been identified in *Saccharomyces cerevisiae* and sequenced: *PDC1* (Kellermann et al. 1986; Hohmann and Cederberg 1990), *PDC5* (Hohmann and Cedermann 1990; Seeboth et al. 1990) and *PDC6* (Hohmann 1991a, b). The three structural genes are differentially expressed at the transcriptional level (Schaaff et al. 1989; Hohmann 1991b). However, the *PDC1* encoded enzyme accounts for most of the pyruvate decarboxylase activity in wild-type yeast cells growing on glycolytic and gluconeogenic substrates (Hohmann and Cederberg 1990). Cis-acting elements and trans-acting factors are responsible for the regulation of *PDC1* expression (Butler et al. 1990; Butler and McConnell 1988; Kellermann and Hollenberg 1988; Hohmann 1992).

Induction of glycolytic enzyme activities by fermentable sugars must be triggered by specific glycolytic metabolites. Maitra and Lobo (1971b) were the first to use glycolytic mutants to identify the inducing metabolites. Recently, Boles et al. (1993) could show that different metabolic signals control the activation of glycolysis in *S. cerevisiae*. While metabolization of sugars beyond fructose-6-phosphate is dispensable for the complete induction of pyruvate kinase, full induction of pyruvate decarboxylase requires the formation of one or several metabolites beyond the phosphofructokinase reaction. Here we report the use of disruption/deletion mutants in the middle and lower part of glycolysis to identify additional metabolites required for full induction of pyruvate decarboxylase.

Materials and methods

Yeast strains and culture conditions

The isogenic wild-type strains ENY.WA-1A (*MATα, ura3–52, leu2–3, 112, trp1–289, his3-delta1, MAL2-8^c, MAL3, SUC3*) and ENY.WA-14A (*MATα, leu2–3, 112, trp1–289, his3-delta1, MAL2–8^c, MAL3, SUC3*) and the isogenic *hxk1/hxk2* deletion strain WAY.10-1C (*MATa, ura3–52, his3-delta1, hxk1Δ::HIS3,*

hxk2Δ::LEU2, MAL2–8°, MAL3, SUC3) were kindly provided by K. D. Entian and M. Rose (Universität Frankfurt/Main, Germany). Strain EBY8 is a *fba1* disruption mutant of ENY.WA-1A (Boles and Zimmermann 1993) obtained by transformation with the gene disruption plasmid of Schwelberger et al. (1989). All mutants constructed in this work were derived from strain ENY.-WA-1A. The yeast cells were grown at 28 °C in a YEP medium (1% yeast extract, 2% bactopeptone) or in a synthetic medium containing 0.17% Difco yeast nitrogen base, 0.5% $(NH_4)_2SO_4$ and amino acids. These two basic media were supplemented with different carbon sources.

Molecular biology techniques

DNA was prepared and manipulated according to the procedures described in Sambrook et al. (1989). pUC18, pUC19 and plasmids from the series of Gietz and Sugino (1988) served as vectors. *E. coli* strains JM101 and SURE (Stratagene GmbH, Heidelberg, Germany) were used for the propagation of plasmids. Yeast specific techniques were described by Guthrie and Fink (1991). Plasmids were transformed into yeast according to Schiestl and Gietz (1989).

Construction of deletion plasmids

a) pEB77 *(pgk1Δ::LEU2):* A 3.0-kb *Hin*dIII fragment carrying *PGK1* from plasmid pPGK1-1 (Seehaus et al. 1985) was subcloned into pUC19 resulting in pEB7. The 1.8-kb *Dra*I/*Bg*/II fragment of pEB7 containing most parts of the *PGK1* gene from −659 bp to +1157 bp according to the published sequences (Hitzemann et al. 1982; Ogden et al. 1986) was replaced by a 2.5-kb *Bgl*II/*Sca*I fragment carrying *LEU2* from plasmid YEp13.

b) pEB66 *(gpm1Δ::LEU2):* A 1.3-kb *Hin*dIII/*Sal*I fragment carrying *GPM1* from plasmid pPGM-7e (Seehaus et al 1985) was subcloned into pUC18 resulting in pEB6. The 0.8-kb *Msc*I/*Sfu*I fragment of pEB6 containing most parts of the *GPM1* gene from −161 bp to +641 bp according to the published sequences (White and Fothergill-Gilmore 1988; Heinisch et al. 1991) was replaced by a 2.5-kb *Sma*I/*Acc*I fragment with the *LEU2* gene originally cloned into pUC19.

c) pEB55 *(pyk1Δ::LEU2)* A 6.9-kb *Hin*dIII/*Xba*I fragment carrying *PYK1* from plasmid pPYK1–6 (J. Heinisch, unpublished; Seehaus et al. 1985) was subcloned into pUC18 resulting in pEB5. The 1.7-kb *Xba*I/*Hpa*I fragment of pEB5 containing the *PYK1* gene from +4 bp to 168 bp behind the stop codon according to the published sequence (Burke et al. 1983) was replaced by a 3.1-kb *Xba*I/*Sma*I fragment with the *LEU2* gene originally cloned into pUC19.

Construction of mutant strains

The deletion mutants were constructed following the one-step gene replacement procedure of Rothstein (1983). pEB77 was digested with *Hin*dIII, pEB66 with *Hin*dIII and *Sal*I, and pEB55 with *Bam*HI and *Hin*dIII. The fragments were transformed separately into the haploid strain ENY.WA-1A selecting for leucin prototrophy on a medium supplemented with 3% ethanol and 3% glycerol. The resulting mutant strains EBY71 *(pgk1Δ::LEU2)*, EBY66 *(gpm1Δ::LEU2)* and EBY55 *(pyk1Δ::LEU2)* did not grow on a 2% glucose medium. The *TPI1* gene in plasmid pΔTPI of Özcan et al. (unpublished data) had been completely replaced by the *HIS3* gene. pΔTPI was digested with *Eco*RI/*Sna*BI and transformed into strain ENY.WA-1A selecting for histidin prototrophy on a medium supplemented with 3% ethanol and 0.1% galactose, resulting in strain EBY88 *(tpi1Δ::HIS3)*. The attempted deletion mutants were characterized by Southern-blot analysis, their lack of enzyme activity or their growth properties (data not shown). Strain WAY10-1C *(hxk1Δ::HIS3, hxk2Δ::LEU2)* was crossed with strain ENY.-WA-14A and tetrad analysis was performed, resulting in strains W1-1 and W1-3 *(hxk2Δ::LEU2)* and W1-2 and W1-4 *(hxk1Δ::HIS3)*.

Enzyme activities and metabolite concentrations

Determination of enzyme activities and metabolite concentrations were performed essentially as described by Boles and Zimmermann (1993) and Boles et al. (1993).

Results

Recently we could demonstrate that full induction of pyruvate decarboxylase depends on the formation of metabolites in the lower part of glycolysis (Boles et al. 1993). In order to characterize the inducing metabolite(s) in more detail we constructed mutants with disruptions or deletions of the genes coding for fructose bisphosphate aldolase *(FBA1)*, triose phosphate isomerase *(TPI1)*, phosphoglycerate kinase *(PGK1)*, phosphoglycerate mutase *(GPM1)* and pyruvate kinase *(PYK1)*.

During gluconeogenic growth on ethanol (*tpi1Δ*, *pgk1Δ*, *gpm1Δ* and *pyk1Δ* mutants) or acetate (*fba1Δ* mutant) levels of pyruvate decarboxylase were higher than in wild-type by a factor 3–4 in the *fba1*, *gpm1* and the *pgk1* mutants, but only about twofold in the *tpi1* and *pyk1* mutants (Fig. 1). Induction of pyruvate decarboxylase by glucose added to these cultures was less than in the wild-type strain (Fig. 1). Interestingly, a distinct hierarchy between the different mutant strains could be observed. Glucose addition to the *fba1Δ* mutant caused nearly no increase in pyruvate decarboxylase activity. An increasingly enhanced induction could be observed in the

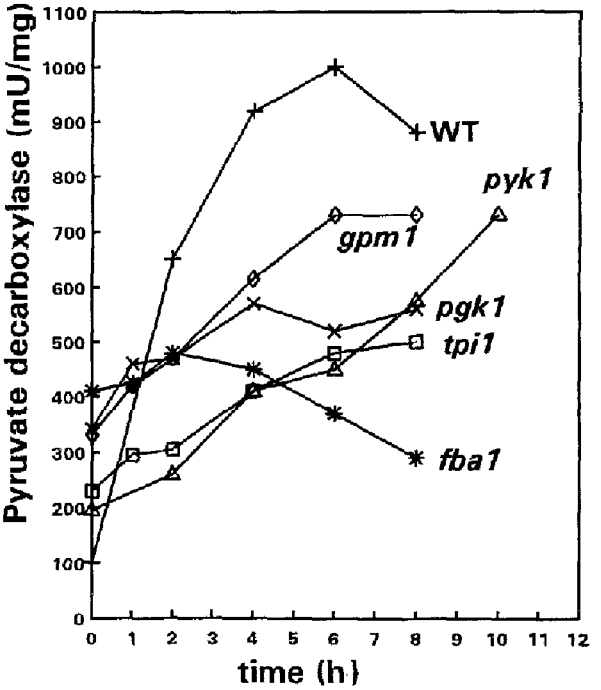

Fig. 1. Pyruvate decarboxylase activity in cells of the wild-type strain ENY.WA-1A (+), strains EBY8 *(fba1Δ)* (*), EBY88 *(tpi1Δ)* (□), EBY71 *(pgk1Δ)* (×), EBY66 *(gpm1Δ)* (◇) and EBY55 *(pyk1Δ)* (△) after the addition of 1% glucose to ethanol-grown cells (to acetate-grown cells in the case of EBY8)

Table 1. Concentrations of glycolytic metabolites. Extracts were prepared after growth on rich media with 1% ethanol (EtOH) and 2 h after the addition of 2% glucose (Glc) to the cultures. Additionally, extracts of wild-type cells were prepared after growth on rich media with 3% glycerol (Glyc) or 2% galactose (Gal). Concentrations of glycerate-1,3-bisphosphate were not detectable. Wild-type = ENY.WA-1A; *tpi1Δ* = EBY88; *pgk1Δ* = EBY71; *gpm1Δ* = EBY66; *pyk1Δ* = EBY55; PEP = phosphoenolpyruvate; dw = dry weight

Metabolites (nmol/mg dw)	*tpi1Δ*		*pgk1Δ*		*gpm1Δ*		*pyk1Δ*		Wild-type			
	EtOH	Glc	EtOH	Glc	EtOH	Glc	EtOH	Glc	Glyc	Gal	EtOH	Glc
Glc-6-P	0.4	2.6	2.4	1.1	1.0	0.8	0.9	0.6	2.5	1.2	1.1	3.3
Fru-6-P	0.2	0.5	0.5	0.3	0.2	0.3	0.2	0.1	0.3	0.1	0.2	0.3
Fru-1,6-P_2	0.5	6.2	1.0	16.7	1.8	9.3	0.2	3.4	0.2	1.4	0.2	8.5
Triose-P	4.3	9.5	0.3	9.0	0.1	1.9	<0.1	1.0	0.3	0.4	0.3	1.0
3-P-glycerate	5.1	0.1	19.5	0.2	0.3	22.4	0.2	7.0	2.6	0.8	0.4	1.3
2-P-glycerate	0.9	0.1	1.0	0.2	2.4	0.5	0.1	2.9	0.5	0.3	0.2	0.2
PEP	2.3	<0.1	8.8	0.1	13.3	0.2	0.2	11.2	1.7	0.2	0.3	0.1
Pyruvate	0.9	0.4	2.0	0.4	4.5	0.2	0.2	0.2	0.4	0.4	0.3	2.4
ATP	3.3	2.1	0.7	0.6	1.8	1.2	1.4	1.1	3.0	4.5	3.5	3.1

mutants with blocks further downstream in the glycolytic pathway.

The different mutant strains accumulated unusually high levels of glycolytic intermediates below the block under gluconeogenic growth conditions or above the block two hours after glucose addition (Table 1; Boles and Zimmermann 1993). The molar concentrations under gluconeogenic and glycolytic growth conditions of all the three-carbon metabolites from the triose phosphates to pyruvate were added. The sums of these metabolites correlated well with the pyruvate decarboxylase activities under gluconeogenic as well as glycolytic growth conditions (Fig. 2). Such a correlation did not apply to wild-type cells where the levels of three-carbon metabolites increased only threefold from gluconeogenic to glycolytic growth conditions. This indicated that full induction depends on a certain ratio between hexose phosphates and three-carbon metabolites, a notion supported by the fact that the *gpm1* mutant under glycolytic growth conditions accumulated the highest concentrations of three-carbon metabolites and concomitantly showed the highest glucose-induced pyruvate decarboxylase activities.

Galactose is slowly metabolized by *S. cerevisiae*. It induced pyruvate decarboxylase to only 500 mU/mg protein. Schmitt et al. (1983) observed that pyruvate decarboxylase activity was still lower in cells growing on glycerol but nevertheless higher than on ethanol. These

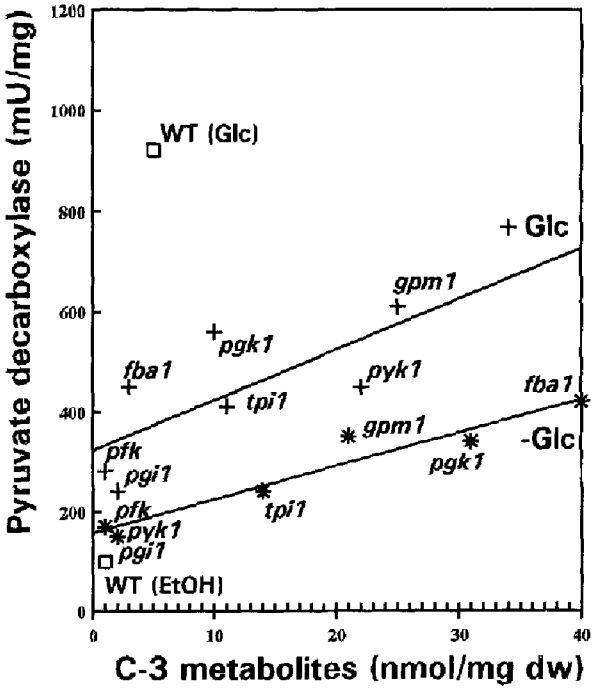

Fig. 2. Pyruvate decarboxylase activity in different strains as a function of the sum of three-carbon glycolytic metabolites after growth on ethanol (or acetate in the case of *fba1Δ*) (*) and 4 h after the addition of glucose to the cultures (–) (metabolite concentrations from 2 hours after glucose-addition). (□) = wild-type values. Glc = glucose; EtOH = ethanol; dw = dry weight; *pfk* = *pfk1*/*pfk2* double deletion mutant

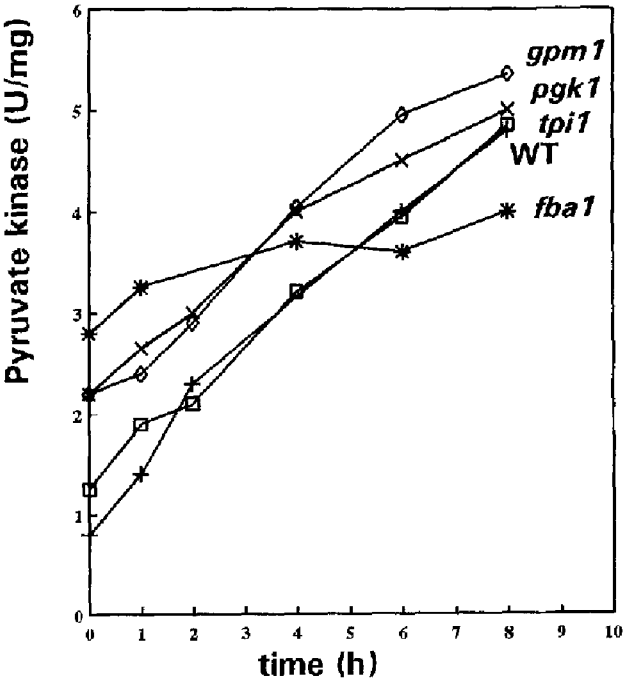

Fig. 3. Pyruvate kinase activity in cells of the wild-type strain ENY.WA-1A (+), strains EBY8 *(fba1Δ)* (*), EBY88 *(tpi1Δ)* (□), EBY71 *(pgk1Δ)* (×) and EBY66 *(gpm1Δ)* (◇) after the addition of 1% glucose to ethanol-grown cells (to acetate-grown cells in the case of EBY8)

low activities corresponded in both cases with slightly elevated levels of hexose phosphates and three-carbon glycolytic metabolites (Table 1; Boles and Zimmermann 1993).

We investigated whether phosphorylated hexoses or the hexokinases themselves are involved in pyruvate decarboxylase induction and used mutants with deletions of one or both of the hexokinase genes. Pyruvate decarboxylase was normally induced by glucose in *hxk1* or *hxk2* single deletion mutants (1513 mU/mg and 1279 mU/mg protein, respectively, 6 h after addition of 2% glucose to ethanol-grown cells) as previously shown for *hxk2* mutants by Entian and Zimmermann (1980). Fructose cannot be phosphorylated in *hxk1/hxk2* double mutants and did not induce pyruvate decarboxylase at all (37 mU/mg protein 6 h after addition of 2% fructose to ethanol-grown cells) whereas glucose did (1162 mU/mg protein 6 h after addition of 2% glucose to ethanol-grown cells) because it is phosphorylated by an additional glucokinase (Maitra and Lobo 1983).

Pyruvate kinase and decarboxylase are induced by different signals (Boles et al. 1993). As shown in Fig. 3, the pyruvate kinase activities after gluconeogenic growth were three times higher than in the wild-type in the *fba1* and two times higher in the *gpm1* and the *pgk1* mutants. In contrast to this, pyruvate kinase levels in the wild-type range were observed in the *tpi1* mutant (Fig. 3), the *pgi1* single and the *pfk1/pfk2* double mutants (Boles et al. 1993). Glucose induced wild-type level pyruvate kinase activities in *tpi1*, *pgk1* and *gpm1* mutants. In contrast to this, induction was reduced in the *fba1* mutant.

Discussion

Induction of glycolysis must be triggered by either free hexoses or their metabolites. This can be tested for fructose in hexokinase mutants. There are two hexokinases in yeast coded by *HXK1* and *HXK2* (Lobo and Maitra 1977) which phosphorylate fructose and glucose whereas an additional glucokinase coded by *GLK1* (Maitra and Lobo 1983) phosphorylates only glucose. Glucose, but not fructose, can induce pyruvate decarboxylase in a *hxk1/hxk2* double mutant. It was originally proposed that hexose uptake in yeast requires an immediate phosphorylation (Bisson and Fraenkel 1984). However, subsequent experiments by Fuhrmann et al. (1989) using yeast vesicles prepared from different mutant strains showed that hexose uptake did not depend on phosphorylation. This means that fructose is taken up in the *hxk1/hxk2* double mutant but intracellular fructose does not induce pyruvate decarboxylase.

Glucose-6-phosphate accumulates to high levels in phosphoglucose isomerase mutants as already shown by Maitra (1971). This demonstrates also that glucose is taken up. However, there is hardly any induction of pyruvate decarboxylase by glucose in *pgi1* deletion mutants (Boles and Zimmermann 1993). Consequently, neither glucose nor glucose-6-phosphate alone induce this enzyme. On the other hand, fructose leads to a partial induction in this mutant. However, this does not necessarily mean that fructose-6-phosphate causes this partial induction because fructose-6-phosphate can be degraded in a *pgi1* deletion mutant (Boles et al. 1993). Nevertheless, fructose metabolism in a *pgi1* mutant is partially blocked at the level of glyceraldehyde-3-phosphate dehydrogenase (Boles et al. 1993) probably due to a depletion of free inorganic phosphate (Hohmann et al. 1993). Therefore, the concentrations of glycolytic metabolites below the glyceraldehyde dehydrogenase reaction are low while those above increase. Interestingly enough, pyruvate decarboxylase induction under such conditions is only about 50% of the corresponding wild-type values (Boles and Zimmermann 1993).

Pyruvate kinase cannot be induced with fructose in a *pgi1* mutant whereas glucose is sufficient for a partial induction. This indicates that formation of glucose-6-phosphate promotes at least a partial induction of pyruvate kinase. Full induction of pyruvate kinase by glucose is observed in a *pfk1/pfk2* double mutant (Boles et al. 1993). This indicates that neither fructose-1,6-bisphosphate nor any of the three-carbon glycolytic metabolites are required for full induction.

Consequently, formation of glucose-6-phosphate contributes to the induction of these two enzymes. However, a full induction of pyruvate kinase requires in addition to the synthesis of glucose-6-phosphate fructose-6-phosphate or another nonglycolytic metabolite derived from it. Pyruvate decarboxylase cannot be fully induced neither in a *pfk1/pfk2* double (Boles et al. 1993) nor in a *fba1* single mutant (Fig. 1). This indicates that full induction requires in addition to the formation of glucose-6-phosphate one or several three-carbon glycolytic metabolites.

Metabolites of the lower glycolytic pathway can partially induce pyruvate kinase and decarboxylase. However, they are not required for full induction of pyruvate kinase, they represent a group of less efficient alternative inducers. In contrast to this, they are indispensable for full induction of pyruvate decarboxylase in combination with glucose-6-phosphate. Consequently, induction of pyruvate decarboxylase is under a more complex control than induction of pyruvate kinase.

Induction of pyruvate kinase by metabolites of the lower glycolytic pathway appears to be a cumulative effect of all three-carbon metabolites (Fig.3). There is no obvious inducing key metabolite. This applies also to the limited induction of pyruvate decarboxylase under gluconeogenic conditions and to the co-induction under glycolytic conditons (Fig. 1 and 2).

Pyruvate decarboxylase and pyruvate kinase synthesis are under transcriptional control (Schmitt et al. 1983; Burke et al. 1983). This means that the inducing signal(s) must affect a transcription initiation complex in the nucleus. At present, there is no obvious candidate or candidates for the sensing of so many different metabolites generating a signal which finally activates transcription of the *PDC1* and *PYK1* structural genes. A direct interaction of a metabolite with a transcription factor has been reported by Sze et al. (1992) who showed that the yeast Leu3 protein binds as a transcriptional activator of the upstream activation sites of leucine biosynthetic

genes *LEU1*, *LEU2*, and *LEU4* but only in the presence of an intermediate of leucine biosynthesis, α-isopropylmalate. Consequently, it is conceivable that one or several components of a transcription initiation complex in the upstream sequences of the *PDC1* and *PYK1* genes are only fully activated in the presence of a gene-specific complement of glycolytic intermediates.

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