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PDC2, a yeast gene essential for synthesis of pyruvate decarboxylase, encodes a novel transcription factor

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Abstract. A positive regulatory gene *PDC2* required for expression of the enzyme pyruvate decarboxylase (PDC) in the yeast *Saccharomyces cerevisiae* has been identified and cloned. The *pdc2* mutant lacks pyruvate decarboxylase activity and is unable to grow on a medium containing glucose. *PDC2* disruptants are viable on ethanol. The *PDC2* gene product is essential for transcription of *PDC1* and *PDC5*, the structural genes of pyruvate decarboxylase. The *PDC2* gene codes for a low-abundance mRNA of approximately 2·8 kb. Transformation of a wild-type strain with multiple copies of the promoter of *PDC1* leads to decreased pyruvate decarboxylase activity, presumably owing to titration of *trans*-acting factors. Normal activity is restored by multiple copies of *PDC2*, implicating involvement of *PDC2* in transcription of *PDC1*. The deduced *PDC2* protein (Pdc2p) sequence contains 925 amino acids, and is rich in asparagine and serine. We fused the DNA sequence encoding the N-terminal domain of Gal4p to the sequence encoding the C-terminal of Pdc2p; the hybrid protein (Gal4–Pdc2p) was able to activate transcription of the *GAL1–lacZ* fusion gene. The active domain consists of an unusual structure with a strikingly high asparagine content. We propose that this asparagine-rich domain represents a novel structural motif for transcriptional activation. *PDC2* maps on chromosome IV between *cdc34* and *aro1*; *PDC1* is on the left arm of chromosome XII, linked to *ppr1*.

Keywords. Pyruvate decarboxylase; gene expression; transcription factor; asparagine-rich box; *Saccharomyces cerevisiae*.

1. Introduction

Most of the glycolysis enzymes in *Saccharomyces cerevisiae* are coordinately regulated in response to glucose (Maitra and Lobo 1971b). The increased synthesis of these enzymes during growth on glucose is due to transcriptional activation of the corresponding genes (Moore *et al.* 1991). The mechanism by which glucose brings about high-level expression of genes encoding glycolysis enzymes remains unknown. Both positive and negative *cis*-acting regulatory sequences have been identified for many glycolysis genes (Uemura *et al.* 1986; Cohen *et al.* 1987; Stanway *et al.* 1987; Nishizawa *et al.* 1989; Bitter *et al.* 1991). The *trans*-acting factors Grf1p (also referred to as Tuf1p and Rap1p), Abf1p, Gcr1p and Gcr2p appear to be required for efficient transcriptional activation of the glycolysis genes (Holland *et al.* 1987; Buchman *et al.* 1988; Brindle *et al.* 1990; Santangelo and Tornow 1990). In addition, several of the glycolysis genes have binding sites for Reb1p/Grf2p in the vicinity of known upstream activation sequences (UAS) (Chasman *et al.* 1990). However, the roles played by many of these factors are not clear.

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Grf1p and Abf1p seem to play a pleiotropic role, and are essential for cell viability (Huet *et al.* 1985; Shore and Nasmyth 1987; Buchman *et al.* 1988b). The *GCR1* and *GCR2* genes play a central role in coordinate regulation of genes involved in glycolysis, since null mutations in these genes cause substantial reductions in the specific activities of many glycolysis enzymes (Clifton *et al.* 1978; Clifton and Fraenkel 1981; Baker 1986; Uemura and Fraenkel 1990). Gcr1p has been shown to bind to the CTTCC sequence motif found in the promoter of many glycolysis genes (Baker 1991; Huie *et al.* 1992). It has also been suggested that Gcr1p and Gcr2p might function as a complex, in which Gcr1p provides the DNA-binding function and Gcr2p the transcriptional activation function (Uemura and Jigami 1992). In the high-level expression of the triose phosphate isomerase gene *TPI1*, there seems to be a concerted action of various transcriptional activators such as Reb1p, Grf1p and Gcr1p (Scott and Baker 1993).

We investigated regulation of yeast pyruvate decarboxylase (PDC), an enzyme that catalyses conversion of pyruvate to acetaldehyde and carbon dioxide, as a model for regulation of glycolysis genes. In the presence of high glucose concentrations, yeast cells contain increased levels of PDC and produce ethanol at a high rate. At very low glucose concentrations, or on ethanol, levels of PDC are decreased by a factor of 10 to 20 and mainly oxidative metabolism takes place (Schmitt and Zimmermann 1982). The regulation of PDC activity is largely determined by changes in the level of *PDC1* mRNA (Schmitt *et al.* 1983).

Two structural genes for pyruvate decarboxylase have been identified in yeast: *PDC1* and *PDC5* (Schaaff *et al.* 1989; Seeboth *et al.* 1990). Recently, Hohmann has indicated the presence of a third structural gene, *PDC6*, which is silent (Hohmann 1991). However, in wild-type cells, only *PDC1* is expressed, while expression of *PDC5* is activated upon deletion of the *PDC1* coding region (Hohmann and Cederberg 1990). Two additional genes, *PDC2* and *PDC3*, required for expression of pyruvate decarboxylase have also been identified. *PDC2* was originally postulated to act post-transcriptionally (Schmitt *et al.* 1983), on the basis of evidence that *PDC1* mRNA levels were unaffected in a mutant whereas the enzyme activity was reduced to 10–20% of that in the wild-type. *PDC3* was proposed to act post-translationally (Wright *et al.* 1989), on the basis of altered affinity of the enzyme for thiamine pyrophosphate in a *pdc3* strain. In this paper, we show that *PDC2* is essential for transcription of *PDC1*. [During preparation of this manuscript, we received a communication from Dr S. Hohmann on cloning of *PDC2* by complementation of the mutant *pdc2-122* (reported in Schmitt *et al.* 1983). Comparison of the nucleotide sequence of this gene with that reported here showed the two to be identical. For this reason, the nomenclature remains the same (Hohmann 1993).] Null mutants for *PDC2* do not have any detectable *PDC1* mRNA. The protein encoded by *PDC2* is homologous to many transcription factors. We also show that Gal4–Pdc2p hybrid protein can activate transcription of *GAL1–lacZ*. The transcriptional activation domain of Pdc2p has a novel primary structure.

2. Materials and methods

2.1 *Strains, growth media, genetic methods and enzyme assays*

The yeast and *Escherichia coli* strains used in this study are shown in table 1.

Table 1. List of strains.

Strain	Genotype	Source or reference
E. coli		
DH5αF′	F′*lendA1 hsdR17* ($r_K^- m_K^+$) *supE44 thi-1 recA1 gyrA* (Nal^r) *relA1*Δ(*lacZYA-argF*) *U169* (ϕ*80dlac*Δ(*lacZ*)*M15*)	Hanahan 1983
DPWC	F^+ *supE42* Δ*recA*[*Sst*II–*Eco*RI] *srl*::Tn*10*-[Tet^s]	Strathmann *et al.* 1991
JGM	F^- *araD139* Δ(*ara-leu*)*7696* Δ(*lac*)*X74 galU galK hsdR2* ($r_K^- m_K^+$) *mcrB1 rpsL* (Str^+) Tn*5*seq1	Strathmann *et al.* 1991
S. cerevisiae		
MC3	a *ino1 ino2*	Henry *et al.* 1975
EG103	a *leu2 trp1 ura3*	B. Hall
Kn79	α *leu2 trp1*	B. Hall
EG103/EG103	Isogenic diploid of EG103	This work
pdc2-2T21B	α *pdc2-2 leu2 trp1 ura3*	This work
pdc2-2T17D	a *pdc2-2 leu2 trp1 ura3*	This work
pdc1-1T1D	a *pdc1-1 leu2 trp1 ura3*	This work
pdc1-1T2C	α *pdc1-1 leu2 trp1 ura3*	This work
Y526	a *gal4*Δ *gal80*Δ *ura3*::*GAL1–lacZ leu2 his3 trp1*	Legrain *et al.* 1993

The yeast strains used for mapping were obtained from the Yeast Genetic Stock Center at Berkeley, California. Standard yeast rich (YEP), minimal (SM) and sporulation media were prepared as described previously, and supplemented with appropriate amino acids and 1% (w/v) glucose or 1% (v/v) ethanol (Maitra and Lobo 1978; Sherman *et al.* 1986). Mutagenesis by ethyl methanesulphonate was done as described by Sherman *et al.* (1979), and the mutants were enriched either by inositol starvation (Henry *et al.* 1975) or by the heat-shock method (Walton *et al.* 1979). The mutations were isolated in either an MC3 or a Kn79 genetic background (see table 1), and the mutants were then crossed with either Kn79 or EG103 so that the mutations were in a *leu2 ura3 trp1* background, which facilitates isolation of transformants. Glycolysis enzymes were assayed as previously described (Maitra and Lobo 1971a).

2.2 *Plasmids and recombinant DNA*

Standard methods were used for recombinant-DNA manipulations (Sambrook *et al.* 1989). The YEp13 and YCp50 libraries were obtained through P. Sinha and U. Vijayraghavan respectively. Yeast transformations were performed either after making spheroplasts (Hinnen *et al.* 1978) or by the lithium acetate method (Ito *et al.* 1983). Transformants were selected on SM medium lacking appropriate amino acids.

For integration of the cloned DNA into the yeast chromosome plasmid YIpPDC2-1 (figure 1b) was constructed. This plasmid contains the 5·8-kb *Bam*HI–*Sal*I fragment of YEpPDC2-1 inserted into YIp5. After digestion with *Sac*I, YIpPDC2-1 was integrated into the chromosome by homologous recombination.

The construct used for disruption of the *PDC2* gene was as shown in figure 1b. The 1·5-kb and 0·7-kb *Eco*RI fragments were replaced by a 1·4-kb *Eco*RI fragment containing the *TRP1* gene. The resulting plasmid (YpDPDC2-1) was digested with *Sac*I and *Cla*I to expose the recombinogenic ends, and haploid and diploid *PDC2*

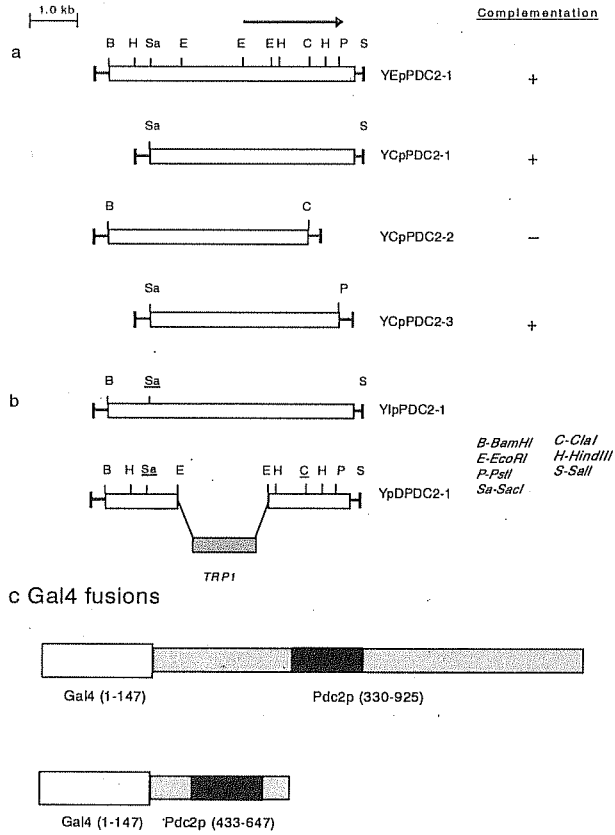


Figure 1. (a) Restriction map of YEpPDC2-1 and subcloning of *PDC2*. The arrow above the map denotes the open reading frame proposed to encode the *PDC2* gene product. (b) Integration and disruption of chromosomal *PDC2*. The restriction enzymes that were used to generate recombinogenic ends are underlined. (c) Gal4–Pdc2 hybrid proteins. Coding regions of Gal4p and Pdc2p are indicated in parenthesis. The darkly shaded area indicates the asparagine-rich region.

strains EG103 and EG103/EG103 (table 1) were transformed with it. Transformants were selected on complete synthetic medium lacking tryptophan and supplemented with 1% ethanol.

PCR amplification was done using the AmpliTaq kit according to the manufacturer's instructions. The primers used for amplification were 5′-ACGAATTCTTAGAGA-GCG-3′ and 5′-AGATGGATCCAAATCAGG-3′.

2.3 *OFAGE and chromosomal blotting*

Yeast cells were lysed in agarose blocks and the blocks containing the lysates were loaded for orthogonal-field-alternation gel electrophoresis (OFAGE) in a Pharmacia Pulsaphor unit. Electrophoresis in a 1% agarose gel was for 36 h, with a pulse time of 120 sec. The resolved chromosomes were blotted onto nylon

membranes (Hybond N, Amersham) and probed as described by Vollrath *et al.* (1988). Most radioactive probes were generated by the random priming reaction using the Boehringer-Mannheim kit.

2.4 *Northern analysis*

Fresh YEP medium supplemented with 1% ethanol was inoculated with cells from an overnight culture and the new cultures were grown to an OD_{650} of 1·0. Glucose was then added to some of the cultures to a final concentration of 1%. One hour after addition of glucose, total RNA was isolated, and resolved on 1% formaldehyde-agarose gels as described by Sherman *et al.* (1986). The resolved RNA was transferred to nylon membranes for the hybridization. Autoradiography was done for 6 h at −80°C on Fuji RX film.

2.5 *DNA sequencing*

We adopted the transposon-facilitated strategy for sequencing (Strathmann *et al.* 1991). The 4·5-kb *Sac*I–*Pst*I fragment (see figure 1) was cloned into pMOB and the transposon γδ was mobilized into the target DNA sequence by conjugation. The transposon insertion sites were mapped by digesting the plasmids with *Sac*I and *Sac*I/*Sal*I. Two oligonucleotides GD1 and GD2, complementary to the two ends of the transposon, were used to prime the dideoxy reaction. Both strands were completely sequenced. Regions that could not be sequenced by this method and DNA beyond the *Pst*I site were sequenced by subcloning.

2.6 *Gal4p fusion constructs and β-galactosidase assay*

The 2·1-kb *Eco*RI/*Sal*I fragment of YEpPDC2-1 was cloned into the plasmid pGBT9 (Chien *et al.* 1991) to generate pGPDC2-13. The plasmid pBDC5 was obtained by cloning 659-bp PCR-amplified fragment into pGBT9.

β-Galactosidase was assayed in liquid cultures. Exponentially growing cells were harvested (≈ 1 OD_{600} per ml) and β-galactosidase assays were performed as described by Miller (1972), except that the incubation temperature was 30°C. A β-galactosidase unit corresponds to $10^3 \times OD_{420}$ units per minute of reaction for 1 ml of a 1 OD_{600} culture. Under these conditions cells harbouring Gal4p provide approximately 1500 units.

2.7 *Nucleotide sequence accession number*

The Genbank accession number for the *PDC2* sequence is L19880.

3. Results

3.1 *Isolation of pdc2 mutant and cloning of PDC2*

We screened for mutants that did not grow on glucose, but grew on ethanol. The

mutants that accumulated pyruvate when incubated with glucose were further analysed. Colonies of glucose-negative, pyruvate-accumulating cells had a halo of turbidity due to pyruvic acid released during the first two to three days on glucose plates. The zone disappeared subsequently with growth of the colonies. Most of the mutants had reduced pyruvate decarboxylase activity. On the basis of complementation tests, the mutants were assigned to two classes, *pdc1* and *pdc2*. We isolated about 20 alleles each of *pdc1* and *pdc2*. The heterozygous diploid of *pdc1* (EG103/pdc1-1T2C) had only 50% of the PDC activity of the wild-type (EG103), whereas the heterozygous diploid of *pdc2* (EG103/pdc-2T21B) showed wild-type activity (table 2). The mutants pdc2-2T21B and pdc1-1T1D were transformed with yeast genomic libraries, and the complementing clones were analysed. Whereas the clones that complemented the *pdc1* mutant were all *PDC1* clones (Schmitt *et al.* 1983), those that complemented the *pdc2* mutant belonged to two groups, *PDC1* and *PDC2*, suggesting that multiple copies of *PDC1* gene can suppress the pdc2 phenotype. The plasmids containing *PDC2*, however, did not complement pdc1-1T1D. The *PDC2* clones did not exhibit any gene dosage effect, unlike *PDC1* clones (table 2).

3.2 *Integration and disruption of PDC2 gene*

The mutant pdc2-2T21B (*ura3*, see table 1), was transformed with linearized YIpPDC2-1 (*ura*$^+$), and transformants were selected on a synthetic complete medium lacking uracil. The transformants were crossed with EG103. Tetrad analysis showed that the integrated DNA was tightly linked to the *pdc2* locus. Therefore, the isolated sequence in YIpPDC2-1 should be derived from the *PDC2* gene. Southern analysis of the transformants also confirmed integration of the sequence (data not shown).

In the disruption experiment Trp$^+$ transformants were obtained from both haploid and diploid strains. The haploid disruptants were viable; however, they did not grow on glucose. The haploid transformants showed reduced pyruvate decarboxylase activity and failed to complement pdc2-2T21B, indicating disruption of *PDC2*. The

Table 2. Growth and PDC activity of the wild type, *pdc1* and *pdc2* mutants, and their transformants.

Strain	Ethanol		Glucose	
	Growth	PDC (Sp. act., mU/mg)	Growth	PDC (Sp. act., mU/mg)
EG103	+	103	+	564
pdc1-1T2C	+	13	–	15
pdc2-2T21B	+	15	–	25
pdc2Δ	+	11	–	20
EG103/pdc1-1T2C	+	64	+	234
EG103/pdc2-2T21B	+	97	+	514
pdc1-1T2C[YEpPDC1]	±	n.d.	+	2065
pdc2-2T21B[YEpPDC1]	±	n.d.	+	1250
pdc2Δ[YEpPDC1-1]	±	n.d.	+	865
pdc2-2T21B[YEpPDC2-1]	+	n.d.	+	614
pdc2-2T21B[YCpPDC2-1]	+	n.d.	+	650

n.d., Not determined; ±, doubling time ≈ 8–10 h

diploid transformants grew normally, and 19 tetrads examined showed 2 : 2 segregation of Trp^+Glu^- and Trp^-Glu^+. A Southern blot confirmed that the Glu^- strains contained the null mutation *pdc2*Δ :: *TRP1* (data not shown).

3.3 *Mapping of pdc1 and pdc2*

The *pdc1* and *pdc2* loci were localized on chromosomes XII and IV respectively by OFAGE (data not shown). The second division segregation frequencies with respect to *TRP1* as the centromere marker were, respectively: *pdc1*, 24 out of 55 tetrads; *pdc2*, 48 out of 60 tetrads. Meiotic analysis placed *pdc1* as a centromere marker on the left arm of chromosome XII linked to *ppr1* on the right arm of the chromosome, and *pdc2* 13 cM to the right of *cdc34* on the right arm of chromosome IV (table 3).

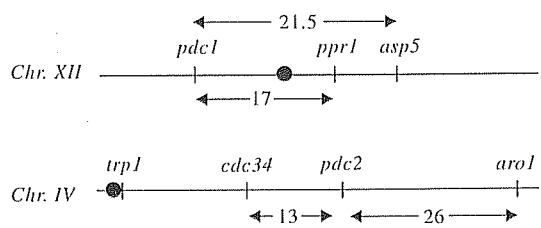
3.4 *The PDC2 gene is essential for transcription of PDC1*

We prepared and analysed total RNA from wild-type (EG103) and various mutant strains grown under a variety of physiological conditions (figure 2a). In the wild type (EG103), *PDC1* mRNA is induced 10–15-fold by glucose over the levels in exponential cultures growing on ethanol (lanes 1 and 2). There is a slight increase (2–3-fold) in the *PDC1* mRNA levels during stationary phase on ethanol (lane 3) over that in exponential phase. In the mutant pdc2-2T21B, and in the disruptant pdc2Δ, the 1·8-kb *PDC1* mRNA was not detectable from cultures grown either on glucose or on ethanol (lanes 4, 5, 6 and 7). The episomal and the centromeric plasmids carrying the *PDC2* gene restored *PDC1* mRNA to the wild-type level in the *pdc2* mutant (lanes 8 and 9). These data seem to suggest that the primary

Table 3. Mapping of *pdc1* and *pdc2*.

Gene pair	P:N:T	Distance (cM)
pdc1–ppr1	60:0:30	16·7
pdc1–asp5	108:1:72	21·5
pdc2–aro1	18:0:20	26
pdc2–cdc34	31:0:11	13

P, Parental ditype; N, non-parental ditype; T, tetratype; cM, centimorgan



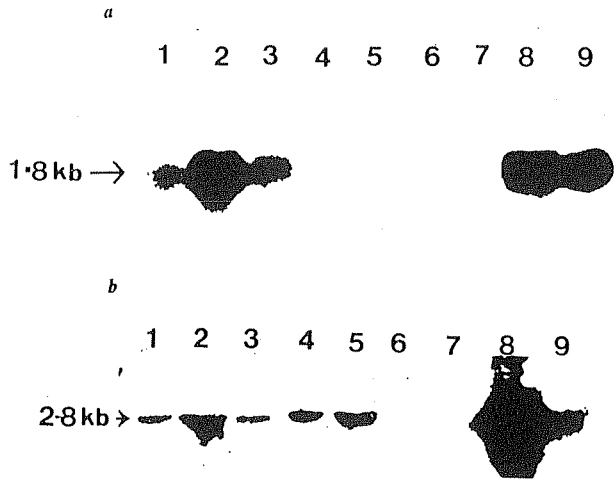


Figure 2. Northern analysis of (a) *PDC1* and (b) *PDC2* mRNA: wild type grown on ethanol (lane 1) and glucose (lane 2), and wild type in alcohol stationary culture (lane 3); pdc2-2T21B on ethanol (lane 4) and glucose (lane 5); pdc2Δ on ethanol (lane 6) and glucose (lane 7); pdc2-2[YEpPDC2] on glucose (lane 8) and pdc2-2[YCpPDC2] on glucose (lane 9).

defect in a *pdc2* mutant is in transcription of *PDC1*. The *PDC5* transcript, which is also recognized by the *PDC1* probe (Schaaff *et al.* 1989), was also absent in the *pdc2* mutants.

3.5 *Regulation of PDC2 gene expression*

To understand *PDC2* gene regulation, the same blot that was used to probe *PDC1* mRNA (described above) was probed with the 1·1-kb *Hin*dIII fragment of *PDC2*. Figure 2b shows that *PDC2* transcript is about 2·8 kb in size and is expressed very poorly compared to *PDC1* mRNA. Presence of glucose in the medium causes possibly a marginal induction of *PDC2* mRNA. The mutant pdc2-2T21B (EMS-induced mutation) shows presence of a 2·8-kb mRNA (lanes 4 and 5), whereas the pdc2Δ strain does not show a signal corresponding to the 2·8-kb mRNA (lanes 6 and 7), confirming disruption of *PDC2*. As expected, the transformant strain containing *PDC2* on an episomal plasmid shows a much stronger signal (lane 8) than the transformant carrying *PDC2* on a centromeric plasmid (lane 9).

3.6 *Titration of trans-acting factors by PDC1 promoter*

The 1·2-kb *Cla*I (– 1085)–*Bgl*II (+ 107) fragment containing the *PDC1* promoter (Butler and McConnell 1988) was cloned into the high-copy vector YRp7, and wild-type strain EG103 was transformed with the resulting construct YRpPRO1 (table 4). The transformant EG103[YRpPRO1] grew poorly on glucose as well as on ethanol. PDC activity on glucose was reduced to about 20% of normal level,

Table 4. Titration of *trans*-acting factors by *PDC1* promoter.

Strain	Ethanol			Glucose		
	Doubling time (h)	Sp. act. (mU/mg)		Doubling time (h)	Sp. act. (mU/mg)	
		PDC	ZWF		PDC	ZWF
EG103	3·2	108	86	1·8	565	128
EG103[YRpPRO1]	10·9	111	90	10·7	145	130
EG103[YRpPRO1, YEpPDC2]	10·8	170	78	2·8	430	146

PDC, Pyruvate decarboxylase, ZWF, glucose-6-P dehydrogenase; EG103, wild type; YRpPRO1, yeast replicating plasmid containing *PDC1* promoter; YEpPDC2, yeast episomal plasmid bearing *PDC2* gene.

while activity on ethanol was unaffected. Multiple copies of *PDC2* cloned into this strain partially rescued growth and PDC levels on glucose but did not rescue growth on ethanol.

The poor growth on ethanol of wild-type cells carrying multiple copies of the promoter might be because *PDC1* promoter interacts with factors that are essential for growth on gluconeogenic carbon sources, and sequestering of these factors by excess *PDC1* promoter would lead to decreased growth of the cells on ethanol.

3.7 *Nucleotide sequence of PDC2*

The nucleotide sequence of *PDC2* gene predicts a polypeptide of 925 amino acids (figure 3). The predicted protein (Pdc2p) is rich in asparagine (11·5%) and serine (12%). The codon adaptive index for the protein is 0·13 (Sharp and Cowe 1991), which suggests that Pdc2p is expressed at a low level and could be a regulatory protein.

Homology analysis done against the SWISSPROT protein database using the BLAST algorithm (Altshul *et al.* 1990) revealed regions of homology among various transcription factors. All the transcription factors showed homology with the stretch of amino acids from 505 to 575. The striking feature of this region is the presence of a cluster of asparagines (figure 4).

The high degree of homology observed among Pdc2p and yeast transcription factors such as Pho81p and Dal81p, and *Drosophila melanogaster* homeodomain proteins such as mastermind, caudal and cut (figure 4) indicates that the *PDC2* gene product might be involved in a similar function, *i.e.* transcriptional activation. The fact that the homology region, which we call the 'asparagine-rich box' (ARB), is conserved over two very different organisms suggests that it may be involved in an important function required for transcriptional activation. The fact that the ARB in Pdc2p is also present in Rpi1p, a negative regulator of Ras proteins (Kim and Powers 1991), supports a hypothesis involving protein–protein interaction rather than one involving protein–DNA interaction.

3.8 *Pdc2p is a transcriptional activator*

To test the hypothesis that Pdc2p is a transcriptional activator, we made hybrid proteins of Pdc2p with the N-terminal domain of Gal4p (residues 1–147; see figure 1c).

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-540 ACAACCGCCT ATCATAATAC TTTTCGCAAA TTTGCAAAGA AACTCTCATC CGGTACTTTA AATCTCCAAC AAATATTCGA ACGTCCTCTG
-450 TCCCACTATA ATAACTGTTG TATTTGAACA GCCTTGGAAT ATAATAACAA ACATCGCCTT CTTGATGAGC TATATGTCCG TACTTATCCA
-360 TGGCTCCAAC CCATTATTAA AAGCCAGCTT ATTGCTCTTT TTTCGCACAG AAATGGAATA TCAACAAGCA GGATTTCATA TATTCCAATG
-270 CTTACTTCTT TTTCCATGCC TTTACATACT TCTTTTTGGT GTGATTCGTG GTTTTGTTGT TAGAATAGCT GCTGCATATT TCCGTTCTGA
-180 GTGACTTTGC CCATCGAAGT CAATTACTTG AAAGTAAGAA ATTATCAAAA CCAATAGCTG CTTAAAAATC AGTTGCAAGA TTTGAATGAA
 -90 ATCAGCAAAG TTTGATCAAT CGTGACTAGT TCGCGTTTTG AATTTTGTGG TAACACCAAG CAGTAAAGAG ACAGCTTTAT TATAACCAGC
 001 ATG CTT TCC ATT CAG CAA AGA TAT AAT ATT TGT CTA ATG GCG GAG AGG CAC CCA AAG TGG ACG CAA CTT GAA TTG
       M   L   S   I   Q   Q   R   Y   N   I   C   L   M   A   E   R   H   P   K   W   T   Q   L   E   L  25
 076 GCA AAA TGG GCT TAT GAG ACG TTC CAG CTG CCA AAA ATT CCA TCC CAA GGC ACA ATA TCG CGT TTG TTG GCA AGG
       A   K   W   A   Y   E   T   F   Q   L   P   K   I   P   S   Q   G   T   I   S   R   L   L   A   R  50
 151 AAA TCA ACT TAT ATG AAT TGT AAA GAG CAT GAA AAA GAT GCG AAT AGA TTA AGG AAG CCA AAT AAT CTT TTG GTT
       K   S   T   Y   M   N   C   K   E   H   E   K   D   A   N   R   L   R   K   P   N   N   L   L   V  75
 226 CGG AAA ATT TTA CAA GAA TGG ATT TCT CAA AGT TTG TGG AAT GGA ATC CCT ATA ACG TCA CCT ATT ATT CAA GAC
       R   K   I   L   Q   E   W   I   S   Q   S   L   W   N   G   I   P   I   T   S   P   I   I   Q   D 100
 301 ACT GCA CAA GCT GTT TGG CAC AGA ATT CCT GCG GAG ACT CGC GAG GGA AAT GGT TCT TTT AGT TAT AAA TGG ATT
       T   A   Q   A   V   W   H   R   I   P   A   E   T   R   E   G   N   G   S   F   S   Y   K   W   I 125
 376 TCG AAT TTT TTA TCA AAA ATG GAT GTC AAT ATT TCT GTT TTA GAC GAA GAG TTA CCC AAA ACC CCA AAA GTC TGG
       S   N   F   L   S   K   M   D   V   N   I   S   V   L   D   E   E   L   P   K   T   P   K   V   W 150
 451 ACA TTT GAA GAG AGG GAT GTA TTG AAG GCT TAT TTC TCC AAA ATT CCT CCA AAG GAT TTA TTC ACT TTG ATA GAA
       T   F   E   E   R   D   V   L   K   A   Y   F   S   K   I   P   P   K   D   L   F   T   L   I   E 175
 526 GCG TTT CTC TCC TAC AAC CTA CCG TTG GAT TAT GCT CAA TAT GAA GCA AGT AGC ATT CAA AGG CGT ATA GAG GTG
       A   F   L   S   Y   N   L   P   L   D   Y   A   Q   Y   E   A   S   S   I   Q   R   R   I   E   V 200
 601 GCA ACT GTC ATG CTG TGC TCC AAT TTA GAT GGC TCT GAA AAG TTA AAA CCT GTT GTC GTG GGC AAA TAT GAT AGT
       A   T   V   M   L   C   S   N   L   D   G   S   E   K   L   K   P   V   V   V   G   K   Y   D   S 225
 676 TAC AAA TCA TTC AGG AAT TAT TTC CCC AAT GAA CCG AAT GAT CCT GTG TCA CAA TCA ATG TTG GGT ACT AAG ATG
       Y   K   S   F   R   N   Y   F   P   N   E   P   N   D   P   V   S   Q   S   M   L   G   T   K   M 250
 751 GCT AAG AAA TTT GAT ATC TCA TAC CAT AGT AAC AGG AAA GCA TGG CTA ACG AGC AAT CTT TTC CAC AAC TGG TTA
       A   K   K   F   D   I   S   Y   H   S   N   R   K   A   W   L   T   S   N   L   F   H   N   W   L 275
 826 GTC AGG TGG GAT AAG AGG TTG GTT GCT GTG AAT AGG AAG ATT TGG ATT GTT TTG GAT GAT TCT TGC TGT CAT CGA
       V   R   W   D   K   R   L   V   A   V   N   R   K   I   W   I   V   L   D   D   S   C   C   H   R 300
 901 ATA ATT AAT TTG CGC CTT CAA AAT ATA AAA CTT GTA TAC ACT TCC TCA AAT TCA AAG TTT TTG CCA TTT AAC TGG
       I   I   N   L   R   L   Q   N   I   K   L   V   Y   T   S   S   N   S   K   F   L   P   F   N   W 325
 976 GGT GTC TGG GAT GAA TTC AAA ACA CGA TAC AGA ATA CAA CAG TAT CAG GCG CTC ATT GAC TTG CAA AAT AGA ATT
       G   V   W   D   E   F   K   T   R   Y   R   I   Q   Q   Y   Q   A   L   I   D   L   Q   N   R   I 350
1051 TCG AAG AAT ATC CAA AAT AAA AAT AAA TCA GAA CGG AAC GAA TGC ATA CCC AAT GGT AAA AAA TGT TTG ATT AGC
       S   K   N   I   Q   N   K   N   K   S   E   R   N   E   C   I   P   N   G   K   K   C   L   I   S 375
1126 TTT GAG CAG AGT CAA CTC ACA ATG TCA AAT GCA TTC AAA TTT ATT AAA AAA GCT TGG GAT GAT ATA CCC GTT GAT
       F   E   Q   S   Q   L   T   M   S   N   A   F   K   F   I   K   K   A   W   D   D   I   P   V   D 400
1201 GCT ATC AAA GCA AAT TGG AAA AGT TCC GGT CTG CTT CCT CCT GAA ATG ATA CAT TTG AAT GAG AAT GTT AGT ATG
       A   I   K   A   N   W   K   S   S   G   L   L   P   P   E   M   I   H   L   N   E   N   V   S   M 425
1276 GCA TTT AAG AAA AAC GAA GTC TTA GAG AGC GTT TTG AAT AGA TTA TGT GAT GAA TAC TAC TGT GTT AAA AAA TGG
       A   F   K   K   N   E   V   L   E   S   V   L   N   R   L   C   D   E   Y   Y   C   V   K   K   W 450
1351 GAA TAT GAA ATG TTG TTA GAT TTA AAC ATT GAA AAC AAA AAC ACA AAC TTC TTG AGT ACA GAA GAA TTA GTG GAA
       E   Y   E   M   L   L   D   L   N   I   E   N   K   N   T   N   F   L   S   T   E   E   L   V   E 475
1426 AGT GCT ATT GTG GAG CCT TGT GAA CCT GAT TTT GAT ACT GCG CCA AAA GGT AAT GAG GTC CAT GAT GAT AAT TTT
       S   A   I   V   E   P   C   E   P   D   F   D   T   A   P   K   G   N   E   V   H   D   D   N   F 500
1501 GAT GTA TCA GTT TTT GCC AAT GAA GAT GAT AAT AAT CAA AAT CAT TTA AGC ATG TCA CAA GCT AGC CAC AAC CCC
       D   V   S   V   F   A   N   E   D   D   N   N   Q   N   H   L   S   M   S   Q   A   S   H   N   P 525
1576 GAT TAC AAC AGT AAT CAC AGC AAC AAT GCT ATT GAA AAT ACT AAT AAT AGA GGC AGT AAT AAT AAT AAC AAT AAT
       D   Y   N   S   N   H   S   N   N   A   I   E   N   T   N   N   R   G   S   N   N   N   N   N   N 550
1651 AAT GGT AGT AGT AAT AAT ATT AAT GAT AAT GAT AGT AGC GTA AAG TAT TTG CAA CAG AAT ACT GTT GAT AAT AGT
       N   G   S   S   N   N   I   N   D   N   D   S   S   V   K   Y   L   Q   Q   N   T   V   D   N   S 575
1726 ACC AAA ACA GGT AAC ATT TCT AGT ATG GAA TCG CAA AGG AAC TCT TCG ACT ACA GAT TTA CCT GGA CAA CCA AAT
       T   K   T   G   N   P   G   Q   P   N   I   S   S   M   E   S   Q   R   N   S   S   T   T   D   L 600
1801 GTT GTT GAC GGT AAT TAT GAC GTC AAT TTT AAC GGC CTT TTG AAT GAT CCA TAT AAT ACA ATG AAA CAG CCG GGC
       V   V   D   G   N   Y   D   V   N   F   N   G   L   L   N   D   P   Y   N   T   M   K   Q   P   G 625
1876 CCA TTA GAT TAT AAT GTC AGT ACA TTA ATC GAT AAA CCT AAT TTA TTC TTA AGT CCT GAT TTG GAT TTA TCT ACT
       P   L   D   Y   N   V   S   T   L   I   D   K   P   N   L   F   L   S   P   D   L   D   L   S   T 650
1951 GTT GGC GTT GAT ATG CAA CTA CCA TCA TCA GAA TAT TTT AGC GAA GTA TTT TCT TCA GCT ATC AGA AAC AAC GAA
       V   G   V   D   M   Q   L   P   S   S   E   Y   F   S   E   V   F   S   S   A   I   R   N   N   E 675
2026 AAA GCT GCC TCA GAT CAG AAC AAA TCA ACT GAT GAA CTT CCT TCA AGC ACG GCC ATG GCA AAT TCA AAC TCG ATA
       K   A   A   S   D   Q   N   K   S   T   D   E   L   P   S   S   T   A   M   A   N   S   N   S   I 700
2101 ACG ACT GCC CTT CTA GAG TCA AGA AAT CAA GCA CAG CCG TTT GAT GTC CCA CAT ATG AAT GGG TTG CTG AGC GAC
       T   T   A   L   L   E   S   R   N   Q   A   Q   P   F   D   V   P   H   M   N   G   L   L   S   D 725
2176 ACA TCA AAA AGC GGA CAT TCT GTT AAT TCC TCA AAT GCT ATA TCT CAA AAT TCT CTG AAT AAC TTT CAA CAT AAT
       T   S   K   S   G   H   S   V   N   S   S   N   A   I   S   Q   N   S   L   N   N   F   Q   H   N 750
2251 TCG GCG TCC GTC GCG GAA GCT TCG TCT CCT TCA ATT ACA CCA TCT CCT GTG GCA ATA AAC TCA ACA GGC GCT CCA
       S   A   S   V   A   E   A   S   S   P   S   I   T   P   S   P   V   A   I   N   S   T   G   A   P 775
2326 GCG AGA TCT ATT ATA TCT GCA CCC ATA GAC TCA AAT TCC TCT GCG TCA TCG CCA TCA GCT TTA GAA CAT CTT GAA
       A   R   S   I   I   S   A   P   I   D   S   N   S   S   A   S   S   P   S   A   L   E   H   L   E 800
2401 GGT GCT GTT TCC GGT ATG TCA CCC TCT TCC ACC ACA ATA TTA AGT AAC TTA CAA ACA AAT ATA AAT ATC GCC AAA
       G   A   V   S   G   M   S   P   S   S   T   T   I   L   S   N   L   Q   T   N   I   N   I   A   K 825
2476 TCA TTG AGT ACC ATT ATG AAA CAT GCA GAA TCA AAC GAA ATA TCA CTG ACG AAA GAA ACA ATA AAT GAA CTT AAT
       S   L   S   T   I   M   K   H   A   E   S   N   E   I   S   L   T   K   E   T   I   N   E   L   N 850
2551 TTC AAT TAT TTG ACA CTT TTA AAA AGG ATT AAA AAG ACT AGA AAA CAA TTA AAT AGC GAA AGC ATT AAA ATA AAC
       F   N   Y   L   T   L   L   K   R   I   K   K   T   R   K   Q   L   N   S   E   S   I   K   I   N 875
2626 AGT AAG AAT GCA CAA GAC CAT TTA GAA ACC CTT CTA TCT GGG GCT GCA GCT GCA GCT TCC GCC AAT AAC
       S   K   N   A   Q   D   H   L   E   T   L   L   S   G   A   A   A   A   A   T   S   A   N   N 900
2701 TTG GAC CTT CCG ACT GGT GGT TCA AAC CTC CCA GAC TCT AAT AAC TTA CAC TTA CCT GGT AAC ACA GGC TTT TTT
       L   D   L   P   T   G   G   S   N   L   P   D   S   N   N   L   H   L   P   G   N   T   G   F   F 925
2776 TAG ATATAAACTT AATAAGATCT CGATATTCAT TGCTCTTTTT TGTAGTTTTG CCTTTAACTC TCGGTTTTTT GAATTTATCA TTTTCCTGAT
       *
2869 GACCTTTGAC TGCTCTTTGA TCTATCCTGA ACACAAGAAA ACGAAAAGAA TAAATAAAAG TAGAGATATT TATTATTTAG CCTGTACCGA TC
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Figure 3. Nucleotide sequence of *PDC2*. The nucleotide sequence of 3500 base pairs of genomic DNA was determined as described in Materials and methods. The predicted amino acid sequence from a 925-codon open reading frame is indicated below the nucleotide sequence. The consensus polyadenylation site is underlined.

The N-terminal of Gal4p (1–147) can bind to UAS_G, but is unable to activate transcription as it lacks the activation domain. Plasmids carrying various gene fusions were introduced into strain Y526, which has deletions in both *GAL4* and

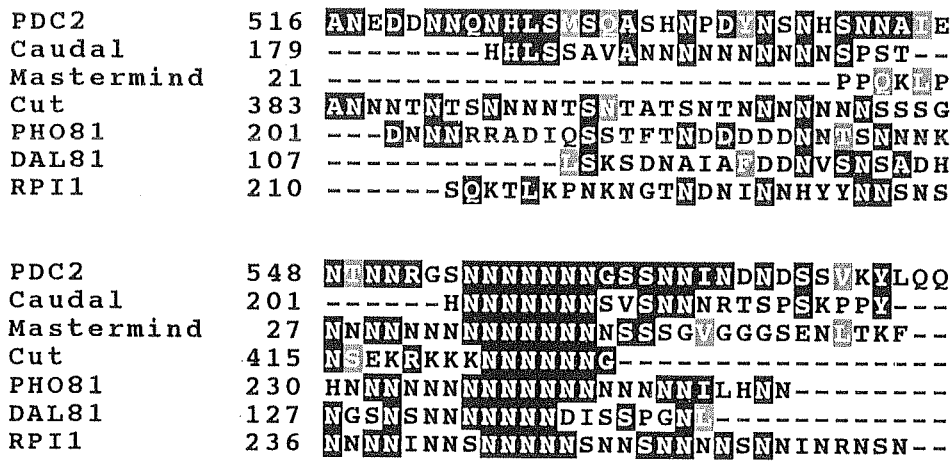

Figure 4. Protein sequence homology between Pdc2p and various regulatory proteins. Identical residues are in black boxes and similar residues in grey.

GAL80 and contains an integrated *GAL1–lacZ* fusion gene. Gal4p-mediated transcriptional activity can be assessed quantitatively by β-galactosidase activity. The data shown in table 5 seem to suggest that a region of Pdc2p can substitute for the Gal4p activation function that is absent in the N-terminal domain of Gal4p (residues 1–147). Pdc2p(330–925) is able to activate synthesis of β-galactosidase by about 30-fold, whereas the Gal4p(1–147) alone gave no induced activity. Further, the amino acid stretch from 433 to 647 of Pdc2p is sufficient for the fusion product to induce transcription of the *GAL1–lacZ* fusion gene to a level comparable with induction by Pdc2p(330–925).

These data indicate that the region 433–647 of Pdc2p contains a putative transcription activation domain, confirming our hypothesis based on the homology of Pdc2p with other transcription factors. As the amino acid sequence between 433 and 647 of Pdc2p does not have any similarity to previously identified transcription activation domains, we propose that this segment of the protein defines a novel class of activation domain: one containing the asparagine-rich box.

Table 5. Transcriptional activation by hybrid Gal4–Pdc2 proteins.

Plasmid	Description	β-Galactosidase activity (U)
None		34
pGBT9	Gal4(1–147)	38
pGPDC2-13	Gal4(1–147)–Pdc2(330–925)	1040
pBDC5	Gal4(1–147)–Pdc2(433–647)	946

Y526 cells were transformed with the indicated plasmids and assayed for β-galactosidase activity. All transformants were grown on minimal medium supplemented with 2% each of ethanol, glycerol and galactose. Values are means for three independent transformants in duplicates.

4. Discussion

This work has identified a regulatory gene, *PDC2*, which encodes a protein required for expression of pyruvate decarboxylase. Cells in which this gene was disrupted are viable but are unable to utilize glucose as carbon source. *PDC2* encodes a transcriptional activator essential for transcription of *PDC1*, the gene that codes for PDC. This conclusion is supported by the absence of *PDC1* mRNA in the deletion-disruption mutant pdc2Δ and in cells with point mutations in *PDC2*. Consistent with this suggestion is the presence in Pdc2p of a cluster of asparagines, which is also found in many transcription factors (figure 4). The final supportive evidence for Pdc2p having a potential transcription activation domain is the observation that the Gal4p–Pdc2p fusion lacking the activation domain of native Gal4p was able to activate transcription of *GAL1–lacZ* to a level comparable to activation by native Gal4p.

The absence of *PDC5* transcript in *pdc2* mutants seems to indicate that *PDC2* gene product is essential for transcription of *PDC5*. Hohmann *et al.* (Hohmann and Cederberg 1990) have suggested that autoregulation might control expression of *PDC1* and *PDC5* . The *PDC5* gene is expressed only when *PDC1* gene product is absent. Since *PDC1* and *PDC5* genes are highly homologous (Schaaff *et al.* 1989; Seeboth *et al.* 1990), the absence, in *pdc2* mutants, of an mRNA homologous to *PDC1* can be explained only if the *PDC2* gene product is essential for transcription of *PDC5* also. This result confirms the earlier observation (Hohmann and Cederberg 1990) that a *pdc1*Δ *pdc2* double mutant had no detectable enzyme activity, whereas a *pdc1*Δ mutant had normal enzyme activity.

The observation of Schmitt *et al.* (1983) that in a *pdc2* mutant the *PDC1* mRNA level was about the same as in wild type might be due to the fact that that mutant was considerably leaky compared to our mutants. The strongest reported mutant *pdc2* allele, *viz. pdc2–122*, allows 20–30% of wild-type PDC activity (Hohmann and Cederberg 1990), whereas the mutants described in our study, pdc2-2 and pdc2Δ, have less than 5% of normal PDC activity.

The absence of any appreciable difference in level of *PDC2* mRNA between cells grown on ethanol and cells grown on glucose suggests that regulation of pyruvate decarboxylase synthesis by Pdc2p is not due to a change in the level of Pdc2p. This inference is supported by the fact that cells carrying *PDC2* on an episomal plasmid do not synthesize increased amounts of PDC compared to cells carrying *PDC2* on a centromeric plasmid (see table 2). Translational control of *PDC2* mRNA cannot be ruled out at present. However, the fact that the pdc2 phenotype can be suppressed by mutations that are extragenic to *pdc2*, and that mutants carrying these 'suppressors' are able to synthesize pyruvate decarboxylase only when grown on glucose, argue in favour of factors in addition to Pdc2p that may be involved in the increased expression of PDC during growth on glucose (unpublished observations).

The pdc2 phenotype can also be suppressed by multiple copies of the *PDC1* gene. The amount of enzyme activity present in transformants with multiple copies of *PDC1* cannot be accounted for merely by 'leakiness' of the mutation as the similar phenotype of the *pdc2* disruptant can also be suppressed by multiple copies of *PDC1*. Kellermann and Hollenberg (1988) have identified putative *cis*-acting UAS and a negative element in the promoter of *PDC1*. They have shown that, in

the absence of the UAS, activity of the promoter decreases to less than 1% of that in the wild-type; when the negative element is also deleted, the TATA box of *PDC1* promoter alone is sufficient to give about 20% enzyme activity. The absence of UAS is analogous to a *pdc2* mutant, which lacks the upstream activating factor and consequently has reduced PDC activity. We believe that the suppression of *pdc2* by multiple copies of *PDC1* is due to the titration of a factor binding to the negative element in the promoter, and the TATA box of *PDC1* promoter alone is able to allow synthesis of enzyme. The same argument holds for the residual 20% activity of PDC found in wild-type cells containing multiple copies of *PDC1* promoter.

The above considerations suggest that *PDC2* may encode an activator protein required for expression of pyruvate decarboxylase. The activation of transcription of the *GAL1–lacZ* fusion gene by Gal4–Pdc2 hybrid protein strongly supports the hypothesis. We believe that Pdc2p might belong to a new class of transcription factors containing an asparagine-rich activation domain. At present we have no biochemical data demonstrating that the *PDC2* gene product binds to DNA.

There are at least two physiological states in which the *PDC2* gene product might perform its role in transcriptional activation: (i) when the cells are grown on non-glycolytic carbon sources (uninduced), and (ii) in 'glucose-induced' cells. The *PDC2* protein presumably activates transcription of *PDC1* by interacting with the UAS of *PDC1* or general transcription factors or both. When glucose is present, it induces *PDC1* transcription to the required level by interacting with 'glucose-specific' transcription factors or with general transcription factors.

Finally, the information presented in this work demonstrates the layered regulation to which the genes responsible for pyruvate decarboxylase activity are subject. Transcription of both *PDC1* and *PDC5* requires *PDC2* and *GRF1* gene products (Butler *et al.* 1990). The induced levels of PDC on glucose are probably due to a glucose-specific factor acting on the upstream elements of the structural genes in addition to the above two. The evidence for the existence of the negative factor indicates that the level of PDC enzyme in the cell is 'fine-tuned' by the interaction of more than one factor depending on the physiological conditions.

Our analysis of *PDC2* gene product has identified a distinct domain that may be important for transcriptional activation. This domain appears to map to an asparagine-rich segment. Unlike the activation domains that have been mapped in several transcription factors (Hope and Struhl 1986; Ma and Ptashne 1987), this domain is largely devoid of acidic residues in its primary sequence. Further, a large number of transcription factors contain asparagine-rich segments (figure 4), and some that have a pleiotropic role, such as the homeodomain proteins, have more than one such segment. Multiple domains may allow them to interact with a wide variety of factors at different positions. Further, these asparagine-rich segments are also rich in serine and threonine residues, whose phosphorylation might modulate the interaction mediated by the asparagine-rich box. It will be interesting to see if it is possible to generate a functional transcription factor by interchanging the asparagine-rich domains of these factors.

We believe that the mechanism of activation by the asparagine-rich segments will be similar to the mechanism of activation by glutamine-rich segments (Courey and Tjian 1988). There may be a specific class of molecules that would function as adaptors between an asparagine-rich activator and the general transcription

machinery. For example, the *ADA2* gene of yeast potentially encodes an adaptor protein for VP16p and Gcn4p but not for Hap4p (Berger *et al.* 1992). It seems likely that, when classified together, some of the activation domains that are now grouped superficially on the basis of primary sequence analysis might actually have diverse structural motifs that interact with distinct targets.

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References

Altshul S. F., Gish W., Miller W., Myers E. W. and Lipman D. J. 1990 Basic local alignment search tool. *J. Mol. Biol.* 215: 403–410

Baker H. V. 1986 Glycolytic gene expression in *Saccharomyces cerevisiae*: Nucleotide sequence of *GCR1* null mutants, and evidence for expression. *Mol. Cell. Biol.* 6: 3774–3784

Baker H. V. 1991 *GCR1* of *Saccharomyces cerevisiae* encodes a DNA binding protein whose binding is abolished by mutations in the CTTCC sequence motif. *Proc. Natl. Acad. Sci. USA* 88: 9443–9447

Berger S. L., Pina B., Silverman N., Marcus G. A., Agapite J., Reiger J. L., Triezenberg S. J. and Guarente L. 1992 Genetic isolation of ADA2: a potential transcriptional adaptor required for function of certain acidic activation domains. *Cell* 70: 251–265

Bitter G. A., Chang K. K. H. and Egan K. M. 1991 A multi-component upstream activation sequence of the *Saccharomyces cerevisiae* glyceraldehyde-3-phosphate dehydrogenase gene promoter. *Mol. Gen. Genet.* 231: 22–32

Brindle P. K., Holland J. P., Willet C. E., Innis M. A. and Holland M. J. 1990 Multiple factors bind the upstream activation sites of the yeast enolase genes *ENO1* and *ENO2*: ABF1 protein, like repressor activator protein RAP1, binds *cis*-acting sequences which modulate repression or activation of transcription. *Mol. Cell. Biol.* 10: 4872–4885

Buchman A., Kimberly W. J., Rhine J. and Kornberg R. D. 1988a Two DNA-binding factors recognize specific sequences at silencers, upstream activating sequences, autonomously replicating sequences and telomers in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* 8: 210–225

Buchman A. R., Lue N. F. and Kornberg R. D. 1988b Connections between transcriptional activators, silencers, and telomers as revealed by functional analysis of yeast DNA-binding protein. *Mol. Cell. Biol.* 8: 5086–5099

Butler G., Dawes I. W. and McConnell D. J. 1990 TUF factor binds to the upstream region of the pyruvate decarboxylase structural gene *PDC1* of *Saccharomyces cerevisiae*. *Mol. Gen. Genet.* 223: 449–456

Butler G. and McConnell D. J. 1988 Identification of an upstream activation site in the pyruvate decarboxylase structural gene *PDC1* of *Saccharomyces cerevisiae*. *Curr. Genet.* 14: 405–412

Chasman D. I., Lue N. F., Buchman A. R., LaPointe J. W., Lorch Y. and Kornberg R. D. 1990 A yeast protein that influences the chromatin structure of UAS_G and functions as a powerful auxiliary gene activator. *Genes Dev.* 4: 503–514

Chien C. T., Bartel P. L., Sternglanz R. and Fields S. 1991 The two-hybrid system: A method to identify and clone genes for proteins that interact with a protein of interest. *Proc. Natl. Acad. Sci. USA* 87: 9578–9582

Clifton D. and Fraenkel D. G. 1981 The *gcr* (glycolysis regulation) mutation of *Saccharomyces cerevisiae*. *J. Biol. Chem.* 256: 13074–13078

Clifton D., Weinstock S. B. and Fraenkel D. G. 1978 Glycolysis mutants in *Saccharomyces cerevisiae*. *Genetics* 88: 1–11

Cohen R., Holland J. P. and Holland A. E. P. M. J. 1987 Transcription of the constitutively expressed yeast enolase gene *ENO1* is mediated by positive and negative *cis*-acting regulatory sequences. *Mol. Cell. Biol.* 7: 2753–2761

Courey A. J. and Tjian R. 1988 Analysis of Sp1 *in vivo* reveals multiple transcriptional domains, including a novel glutamine-rich activation motif. *Cell* 55: 887–898

Hanahan D. 1983 Studies on transformation of *E. coli* with plasmids. *J. Mol. Biol.* 166: 557–580

Henry S. A., Donahue T. F. and Culbertson M. R. 1975 Selection of spontaneous mutants by inositol starvation in yeast. *Mol. Gen. Genet.* 143: 5–11

Hinnen A., Hicks J. B. and Fink G. R. 1978 Transformation of yeast. *Proc. Natl. Acad. Sci. USA* 75: 1929–1933

Hohmann S. 1991 Characterization of *PDC6*, a third structural gene for pyruvate decarboxylase in *Saccharomyces cerevisiae*. *J. Bacteriol.* 173: 7963–7969

Hohmann S. 1993 Characterization of *PDC2*, a gene necessary for the high level expression of pyruvate decarboxylase structural genes in *Saccharomyces cerevisiae*. *Mol. Gen. Genet.* 241: 657–666

Hohmann S. and Cederberg H. 1990 Autoregulation may control the expression of pyruvate decarboxylase structural genes *PDC1* and *PDC5*. *Eur. J. Biochem.* 188: 615–621

Holland M. J., Yokoi T., Holland J. P., Myambo K. and Innis M. A. 1987 The *GCR1* gene encodes a positive transcriptional regulator of the enolase and glyceraldehyde-3-phosphate dehydrogenase gene families in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* 7: 813–820

Hope I. A. and Struhl K. 1986 Functional dissection of a eukaryotic transcriptional activator protein, GCN4 of yeast. *Cell* 46: 885–894

Huet J., Cottrelle P., Cool M. L., Thiele D., Marck C., Buhler J., Sentenac A. and Fromageot P. 1985 A general upstream binding factor for genes of the yeast translational apparatus. *EMBO J.* 4: 3539–3547

Huie M. A., Scott E. W., Drazinic C. M., Lopez M. C., Hornstra I. K., Yang T. P. and Baker H. V. 1992 Characterization of the DNA-binding activity of GCR1: In vivo evidence for two GCR1-binding sites in the upstream activation sequence of *TPI* of *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* 12: 2690–2700

Ito H., Jukuda Y., Murata K. and Kimura A. 1983 Transformation of intact yeast cells treated with alkali cations. *J. Bacteriol.* 153: 163–168

Kellermann E. and Hollenberg C. P. 1988 The glucose and ethanol-dependent regulation of *PDC1* from *Saccharomyces cerevisiae* are controlled by two distinct promoter regions. *Curr. Genet.* 14: 337–344

Kim J.-H. and Powers S. 1991 Overexpression of RPI1, a novel inhibitor of the yeast Ras–cyclic AMP pathway, down-regulates normal but not mutationally activated Ras function. *Mol. Cell. Biol.* 11: 3894–3904

Legrain P., Chapon C. and Galisson F. 1993 Interactions between PRP9 and SPP91 splicing factors identify a protein complex required in prespliceosome assembly. *Genes Dev.* 7: 1390–1399

Ma J. and Ptashne M. 1987 Deletion analysis of GAL4 defines two transcriptional activating segments. *Cell* 48: 847–853

Maitra P. K. and Lobo Z. 1971a A kinetic study of glycolytic enzyme synthesis in yeast. *J. Biol. Chem.* 246: 475–488

Maitra P. K. and Lobo Z. 1971b Control of glycolytic enzyme synthesis in yeast by products of the hexokinase reaction. *J. Biol. Chem.* 246: 489–499

Maitra P. K. and Lobo Z. 1978 Reversal of glycolysis in yeast. *Arch. Biochem. Biophys.* 185: 535–543

Miller J. H. 1972 *Experiments in molecular genetics* (Cold Spring Harbor: Cold Spring Harbor Laboratory Press)

Moore P. A., Sagliocco F. A., Wood R. M. and Brown A. J. P. 1991 Yeast glycolytic mRNA are differentially regulated. *Mol. Cell. Biol.* 11: 5330–5337

Nishizawa M., Araki R. and Teranishi Y. 1989 Identification of an upstream activating sequence and an upstream repressible sequence of the pyruvate kinase gene of the yeast *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* 9: 442–451

Sambrook J., Fritsch E. F. and Maniatis T. 1989 *Molecular cloning: A laboratory manual*, second edition (Cold Spring Harbor: Cold Spring Harbor Laboratory Press)

Santangelo G. M. and Tornow J. 1990 Efficient transcription of the glycolytic gene *ADH1* and three translational component genes requires *GCR1* product, which can act through TUF/GRF/RAP binding sites. *Mol. Cell. Biol.* 10: 859–862

Schaaff I., Green J. B. A., Gozablo D. and Hohmann S. 1989 A deletion of the *PDC1* gene for pyruvate decarboxylase of yeast causes a different phenotype than previously isolated point mutations. *Curr. Genet.* 15: 75–91

Schmitt H. D., Ciriacy M. and Zimmermann F. K. 1983 The synthesis of yeast pyruvate decarboxylase is regulated by large variations in the messenger RNA level. *Mol. Gen. Genet.* 192: 247–252

Schmitt H. D. and Zimmermann F. K. 1982 Genetic analysis of the pyruvate decarboxylase reaction in yeast glycolysis. *J. Bacteriol.* 151: 1146–1152

Scott E. W. and Baker H. V. 1993 Concerted action of the transcriptional activators REB1, RAP1, GCR1 in the high-level expression of the glycolytic gene *TPI1*. *Mol. Cell. Biol.* 13: 534–550

Seeboth P. G., Bohnsack K. and Hollenberg C. P. 1990 *pdc1*^{s} mutants of *Saccharomyces cerevisiae* give evidence for an additional structural *PDC* gene: Cloning of *PDC5*, a gene homologous to *PDC1*. *J. Bacteriol.* 172: 678–685

Sharp P. M. and Cowe E. 1991 Synonymous codon usage in *Saccharomyces cerevisiae*. *Yeast* 7: 657–678

Sherman F., Fink G. R. and Hicks J. B. 1979 *Methods in yeast genetics* (Cold Spring Harbor: Cold Spring Harbor Laboratory Press)

Sherman F., Fink G. R. and Hicks J. B. 1986 *Methods in yeast genetics* (Cold Spring Harbor: Cold Spring Harbor Laboratory Press)

Shore D. and Nasmyth K. 1987 Purification and cloning of a DNA binding protein from yeast that binds to both silencer and activator elements. *Cell* 51: 721–732

Stanway C., Mellor J., Ogden J. E., Kingsman A. J. and Kingsman S. M. 1987 The UAS of the yeast *PGK* gene contains functionally distinct domains. *Nucleic Acids Res.* 15: 6855–6873

Strathmann M., Hamilton B. A., Mayeda C. A., Simon M. I., Meyerowitz E. M. and Palazollo M. J. 1991 Transposon-facilitated DNA sequencing. *Proc. Natl. Acad. Sci. USA* 88: 1247–1250

Uemura H. and Fraenkel D. G. 1990 *gcr2*, a new mutation affecting glycolytic gene expression in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* 10: 6389–6396

Uemura H. and Jigami Y. 1992 Role of GCR2 in transcriptional activation of yeast glycolytic genes. *Mol. Cell. Biol.* 12: 3834–3842

Uemura H., Shiba T., Paterson M., Jigami Y. and Tanaka H. 1986 Identification of a sequence containing the positive regulatory region of *Saccharomyces cerevisiae* gene *ENO1*. *Gene* 45: 67–75

Vollrath D., Davis R. W., Connelly C. and Heiter P. 1988 Physical mapping of large DNA by chromosome fragmentation. *Proc. Natl. Acad. Sci. USA* 85: 6027–6031

Walton E. F., Carter B. L. A. and Pringle J. R. 1979 An enrichment method for temperature-sensitive and auxotrophic mutants of yeast. *Mol. Gen. Genet.* 171: 111–114

Wright A. P. H., Png H.-L. and Hartley B. S. 1989 Identification of a new gene required for full pyruvate decarboxylase activity in *Saccharomyces cerevisiae*. *Curr. Genet.* 15: 171–175