The Nucleotide Sequence of the *LPD1* Gene Encoding Lipoamide Dehydrogenase in *Saccharomyces cerevisiae*: Comparison between Eukaryotic and Prokaryotic Sequences for Related Enzymes and Identification of Potential Upstream Control Sites

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The complete nucleotide sequence of the *LPD1* gene, which encodes the lipoamide dehydrogenase component (E3) of the pyruvate dehydrogenase and 2-oxoglutarate dehydrogenase multienzyme complexes of *Saccharomyces cerevisiae*, has been established. The flanking region 5' to the *LPD1* gene contains DNA sequences which show homology to known control sites found upstream of other yeast genes. The primary structure of the protein, determined from the DNA sequence, shows strong homology to a group of flavoproteins including *Escherichia coli* lipoamide dehydrogenase and pig heart lipoamide dehydrogenase. The amino acid sequence also reveals the presence of a potential targeting sequence at its N-terminus which may facilitate transport to and entry into mitochondria.

INTRODUCTION

Lipoamide dehydrogenase (EC 1.8.1.4) is a component of the multienzyme complexes pyruvate dehydrogenase and 2-oxoglutarate dehydrogenase, which catalyse the oxidative decarboxylation of pyruvate and 2-oxoglutarate to acetyl-CoA and succinyl-CoA respectively (Reed, 1974). Both complexes contain multiple copies of three component enzymes: pyruvate dehydrogenase (EC 1.2.4.1) or 2-oxoglutarate dehydrogenase (EC 1.2.4.2) (E1); dihydrolipoamide acetyltransferase (EC 2.3.1.12) or dihydrolipoamide succinyltransferase (EC 2.3.1.61) (E2) and lipoamide dehydrogenase (E3). The E1 and E2 components are specific to their respective complexes whereas lipoamide dehydrogenase has been shown to be functionally interchangeable between both (Mukherjee *et al.*, 1965), and in *Escherichia coli* and *Saccharomyces cerevisiae* is encoded by a single gene (Guest & Creaghan, 1973; Dickinson *et al.*, 1986).

In higher eukaryotes lipoamide dehydrogenase has an additional role in the multienzyme complexes which specifically catalyse the oxidative decarboxylation of branched-chain 2-oxoacids derived from leucine, valine and isoleucine by transamination (Lawson *et al.*, 1983). The reversible oxidative decarboxylation of glycine has also been shown to involve lipoamide dehydrogenase in the aerobic bacterium *Arthrobacter globiformis* (Kochi & Kikuchi, 1976), in the anaerobe *Peptococcus glycinophilus* (Robinson *et al.*, 1973) and in rat liver mitochondria (Kochi & Kikuchi, 1976).

Regulation of the synthesis of the E1, E2 and E3 components of pyruvate dehydrogenase and 2-oxoglutarate dehydrogenase in *E. coli* has been studied extensively (Guest & Rice, 1984; Spencer & Guest, 1985), and it has been shown that the *E* and *F* genes of the *ace* operon and the *A* and *B* genes of the *suc* operon encode the E1 and E2 subunits of the pyruvate dehydrogenase and 2-oxoglutarate dehydrogenase complexes respectively. Lipoamide dehydrogenase is encoded by a single gene, *lpd*, which is linked to the *ace* operon but can operate under the control of its own promoter. Transcription from *sucAB*, *aceEF* and *lpd* genes shows differential

regulation in response to the nature of the carbon source, thereby ensuring the necessary ratio of subunit components when pyruvate and/or 2-oxoglutarate dehydrogenase enzyme complexes are required. The absence of operons in eukaryotic organisms dictates that the regulation of synthesis of the E1, E2 and E3 components in yeast must differ from the system found in *E. coli*.

The role of lipoamide dehydrogenase in at least two multienzyme complexes raises several interesting questions concerning the regulation of synthesis and control of distribution of an enzyme which functions within two different complexes. The enzyme is encoded in the nucleus but must be transported to its site of action in the mitochondrion. Biochemical studies of the initiation of sporulation in *S. cerevisiae* have shown that the regulation of 2-oxoglutarate dehydrogenase activity may be of crucial importance in the switch of cells from vegetative growth via mitosis to the developmental processes of meiosis and sporulation (Dickinson *et al.*, 1986). In *S. cerevisiae* the enzyme has also been shown to be subject to catabolite repression (Roy & Dawes, 1987).

Determination of the DNA sequence for the genes which encode the E1, E2 and E3 components of both pyruvate and 2-oxoglutarate dehydrogenase is necessary in order to study how their syntheses are regulated at the molecular level.

METHODS

Sources of DNA. The 3.7 kb *XhoI* fragment which contains the *LPD1* gene was obtained from pGP-R1 (Roy & Dawes, 1987) for 'shotgun' cloning into M13 vectors. Large-scale plasmid and phage RF2 DNA isolation was performed by alkaline-SDS lysis; the DNA was purified by centrifugation in CsCl/ethidium bromide gradients (Maniatis *et al.*, 1982). Restriction fragments were separated by electrophoresis in agarose gels and extracted by electro-elution onto dialysis membrane (Smith, 1980).

Cloning in the M13-based vector. The 3.7 kb *XhoI* fragment was digested with three restriction enzymes for shotgun cloning into M13mp18 (Messing, 1983). *TaqI* and *MspI* fragments were cloned into the *AccI* site of M13mp18, and *Sau3AI* fragments were cloned into the *BamHI* site of the same vector. In addition, the 1.45 kb *XbaI* fragment was cloned into the *XbaI* site of M13mp18. Transfection of *E. coli* JM101 [$\Delta(lac-pro) supE thi/F'$ *traD36 proAB lacI^a Z Δ M15*] was performed according to published procedures (Winter & Fields, 1980).

Nucleotide sequence analysis. Single-stranded M13 DNA templates were prepared and sequenced by the dideoxy chain-termination method using a 17-nucleotide synthetic primer (Sanger *et al.*, 1977). Two 15-nucleotide synthetic primers, based on information obtained from the *LPD1* sequence, were also used. The nucleotide sequence was compiled and analysed using the University of Wisconsin Genetics Computer Group programs (Devereux *et al.*, 1984).

Materials. Restriction enzymes were purchased from Bethesda Research Laboratories, Boehringer, and Northumbria Biologicals. Phage T4 DNA ligase was from either Boehringer or Northumbria Biologicals. The 17-nucleotide synthetic primer was obtained from Pharmacia; the 15-nucleotide synthetic primers were purchased from OSWEL DNA Synthesis Service, Chemistry Department, Edinburgh University. DNA polymerase (Klenow fragment), dideoxynucleotides and deoxynucleotides were obtained from Boehringer, [α - 32 P]dATP from Amersham, and reverse transcriptase from Northumbria Biologicals.

RESULTS AND DISCUSSION

The nucleotide sequence

Based on deletion analysis of the plasmid pGP1 (Roy & Dawes, 1987), a YEpl3-based vector containing a 5.5 kb yeast DNA insert carrying the *LPD1* gene, a 3.7 kb *XhoI* fragment was chosen for 'shotgun' cloning analysis. A region of 2701 bp has been sequenced. All of the sequence was obtained from at least two independent clones, it was fully overlapping and was, with the exception of a 0.1 kb region 0.8 kb upstream of the coding region, derived from both DNA strands. The program MAP was used to identify the coding region representing the *LPD1* gene. One large open reading frame of 1.5 kb was found, which is consistent with the known size of the yeast lipoamide dehydrogenase subunit polypeptide (Wieland, 1983) and which exhibits a consistently high score with respect to preferred codon usage in *S. cerevisiae* determined using the programs CODONFREQUENCY and CODONPREFERENCE (data not shown). The complete and unambiguous sequence of the *LPD1* gene and the primary structure of lipoamide dehydrogenase, translated from the DNA sequence, are presented in Fig. 1.

In the region 5'-distal to the *LPD1* sequence are two potential open reading frames: one (at -571), which would be read in the opposite sense, is capable of encoding a polypeptide of 77 residues; the other (sequence not complete, terminating at -858) could encode a polypeptide of at least 76 amino acids. A search of the NBRF protein data base showed no strong homology between the two predicted amino acid sequences and any known protein.

Primary structure of yeast lipoamide dehydrogenase

The primary structure, translated from the nucleotide sequence of the *LPD1* gene, contains 499 amino acid residues that correspond to a protein of M_r 54010 (54730 including the FAD cofactor). These M_r values are in good agreement with previous estimates (Wieland, 1983).

Lipoamide dehydrogenase, encoded in the nucleus and synthesized on cytoplasmic ribosomes, is a component of two multienzyme complexes which function in the mitochondrial matrix-inner membrane compartment. The presence of an N-terminal targeting sequence, which directs the protein to its correct subcellular location and facilitates its entry into the mitochondrion, has been shown in mammalian cells. Antibodies raised against the E1, E2 and E3 components of mitochondrial 2-oxoglutarate dehydrogenase from ox heart have been used to show the presence, in cultured pig kidney cells, of initial cytoplasmic translation products that are larger than the mature proteins (Hunter & Lindsay, 1986). The E3 component is synthesized as a polypeptide 10–20 amino acids larger than the mature protein. The primary structure, derived from the *LPD1* nucleotide sequence of *S. cerevisiae*, supports the conclusion that, in this organism, the lipoamide dehydrogenase N-terminal region represents a mitochondrial targeting sequence. The first 20 amino acids are rich in seryl, threonyl and basic residues, and show a complete absence of acidic residues. All of these features are typical of mitochondrial targeting sequences in yeast (von Heijne, 1986). In *E. coli* such a targeting sequence would be unnecessary; and when the primary structures of *E. coli* and *S. cerevisiae* lipoamide dehydrogenase (Stephens *et al.*, 1983) are aligned (see Fig. 2) the yeast sequence is seen to contain an additional 20 amino acids at its N-terminus which are absent from the inferred *E. coli* protein.

The primary structures of several flavoproteins have been determined either partially or completely. Pig heart lipoamide dehydrogenase, *E. coli* lipoamide dehydrogenase, human erythrocyte glutathione reductase and *Pseudomonas aeruginosa* transposon mercuric reductase amino acid sequences have previously been compared (Williams *et al.*, 1984) and four regions were identified which show strong homology between the four proteins. Two of these (FAD-1 and FAD-2) are involved in the binding of FAD, and the others constitute a pyridine nucleotide binding domain and an 'interface' domain involved in the interaction between subunits of the enzyme. Fig. 2 shows the primary structure of these four proteins and of *S. cerevisiae* lipoamide dehydrogenase aligned for maximum homology using the computer programs GAP and PRETTY. The primary structure of *S. cerevisiae* lipoamide dehydrogenase is in full agreement with the established 'domain homology' found between the other proteins. Comparison of the three lipoamide dehydrogenase primary structures reveals that the strongest homology lies between those of pig heart and yeast. The higher level of homology is particularly noticeable in the dimer interface region concerned with the interaction of the two polypeptides in formation of the active dimeric form (see Fig. 2). This may indicate that there are differences between prokaryotes and eukaryotes in the recognition of the subunits during assembly, which is reflected in the different organization of the multienzyme complexes in terms of subunit composition, especially for pyruvate dehydrogenase (Reed, 1974). Despite these differences the overall degree of homology that exists between all three polypeptides indicates the heavy evolutionary constraints placed upon an enzyme which functions within two different multienzyme complexes. This is also reflected in the low immunogenicity of the yeast E3 subunit relative to the other enzymes of the pyruvate and 2-oxoglutarate dehydrogenase complexes (De Marcucci *et al.*, 1985).

General features of the noncoding regions

The sequences upstream and downstream which flank the coding region of the *LPD1* gene share several characteristics common to the 5'- and 3'-noncoding regions of many sequenced yeast genes.

TCGAAAGAAAGATCATTTCATCAACTGGGGCTATGTGGCCACATCGAGACATTGCAGTAAACTCCAAGTATCAGGGCCAAAGGTTTGG	
-1087	-1001
GCAAGCTCTTGATTGATCAATTGGTAACATATCGGCTTGACTACGGTTGTITATAAGATTATTTAGATTGGCATGAGAAAAATGTCAAATTCATGAAAA	-901
ATGTGGGTTTAGCAACGCGCGGTGAAATGCAAAATAGAAATAGTATTGCTGTATAGTACATAGAGGTGACATATATATATATATATAATTTTAT	-801
ATGGCATCTAGAGCCTCTAGGGATATTATGGAGTTGCCCTAATGACGGTCTGCAAGCCTAGCTGGTGGTTATGGCAGGGTCTTCACCATTTCCTCCA	-701
TTGCATCATCAAGAAGCAGCTTTAAGAAAAATTCGTAGCCTCGCAAAATTCCTGCCACTTTTCCTAGATCCATTATATTCAGCAATATCTTCTGTGCCAT	-601
ATATTTCTTCAGTTGAGGGGTAGAAACCATTCGTATCTGTGGGTGTGTTGGGTAACTGCTGCTATGCCGGTACTCTATATCGGTGTACTTTCTTATA	-501
CATTTCATGGCACCCTTAGAGATTAAATTTTCTTACCACATTGTTCTATCAGCACAACGTAAGAGCGTAGGCGAGGTATAGGAAAAAGGCGAAAT	-401
GAAAGACAGAGAAATATCGAACAATGCCACTGACAAACGTATGGGTGACTACGACGAATAGTCATCATGCAATGTAGCCTCATATATGCCACTAAAAATA	-301
TATACTTACTAATGGTTTATGCCAGTGAATATCACTGAATCGTTTTAACTGATGACTCGTTTTAGAATACTTTATCATCTCGAAGCGGTGTTCTCTCA	-201
TTGGCGAGAAGTCTCGCGGAGGAACTTAATGAAACTTAGTGTATTATAATGCTAATAAACAATTTGATGATAATTCGTACCGTTTGACTCACTCGAA	-101
TATATATAGATATATATATACATATAACGTATATTATATATATACGGTTTTGTTGCGATTGCTCTGTCGTACCATCAAGAACATAGTAAACAGTTCACA	-1
Met Leu Arg Ile Arg Ser Leu Leu Asn Asn Lys Arg Ala Phe Ser Ser Thr Val Arg Thr Leu Thr Ile Asn Lys	
ATG TTA AGA ATC AGA TCA CTC CTA AAT AAT AAG CGT GCC TTT TCG TCC ACA GTC AGG ACA TTG ACC ATT AAC AAG	74
Ser His Asp Val Val Ile Ile Gly Gly Gly Pro Ala Gly Tyr Val Ala Ala Ile Lys Ala Ala Gln Leu Gly Phe	
TCA CAT GAT GTA GTC ATC ATC GGT GGT GGC CCT GCT GGT TAC GTG GCT GCT ATC AAA GCT GCT CAA TTG GGA TTT	149
Asn Thr Ala Cys Val Glu Lys Arg Gly Lys Leu Gly Gly Thr Cys Leu Asn Val Gly Cys Ile Pro Ser Lys Ala	
AAC ACT GCA TGT GTA GAA AAA AGA GGC AAA TTA GGC GGT ACC TGT CTT AAC GTT GGA TGT ATC CCC TCC AAA GCA	224
Leu Leu Asn Asn Ser His Leu Phe His Gln Met His Thr Glu Ala Gln Lys Arg Gly Ile Asp Val Asn Gly Asp	
CTT CTA AAT AAT TCT CAT TTA TTC CAC CAA ATG CAT ACG GAA GCG CAA AAG ACA GGT ATT GAC GTC AAC GGT GAT	299
Ile Lys Ile Asn Val Ala Asn Phe Gln Lys Ala Lys Asp Asp Ala Val Lys Gln Leu Thr Gly Gly Ile Glu Leu	
ATC AAA ATT AAC GTA GCA AAC TTC CAA AAG GCT AAG GAT GAC GCT GTT AAG CAA TTA ACT GGA GGT ATT GAG CTT	374
Leu Phe Lys Lys Asn Lys Val Thr Tyr Tyr Lys Gly Asn Gly Ser Phe Glu Asp Glu Thr Lys Ile Arg Val Thr	
CTG TTC AAG AAA AAT AAG GTC ACC TAT TAT AAA GGT AAT GGT TCA TTC GAA GAC GAA ACG AAG ATC AGA GTA ACT	449
Pro Val Asp Gly Leu Glu Gly Thr Val Lys Glu Asp His Ile Leu Asp Val Lys Asn Ile Ile Val Ala Thr Gly	
CCG GTT GAT GGG TTG GAA GGC ACT GTC AAG GAA GAC CAC ATA CTA GAT GTT AAG AAC ATC ATA GTC GCC ACG GGC	524
Ser Glu Val Thr Pro Phe Pro Gly Ile Glu Ile Asp Glu Glu Lys Ile Val Ser Ser Thr Gly Ala Leu Ser Leu	
TCT GAA GTT ACA CCC TTC CCG GGT ATT GAA ATA GAT GAG GAA AAA ATT GTC TCT TCA ACA GGT GCT CTT TCG TTA	599
Lys Glu Ile Pro Lys Arg Leu Thr Ile Ile Gly Gly Gly Ile Ile Gly Leu Glu Met Gly Ser Val Tyr Ser Arg	
AAG GAA ATT CCC AAA AGA TTA ACC ATC ATT GGT GGA GGA ATC ATC GGA TTG GAA ATG GGT TCA GTT TAC TCT AGA	674
Leu Gly Ser Lys Val Thr Val Val Glu Phe Gln Pro Gln Ile Gly Ala Ser Met Asp Gly Glu Val Ala Lys Ala	
TTA GGC TCC AAG GTT ACT GTA GTA GAA TTT CAA CCT CAA ATT GGT GCA TCT ATG GAC GGC GAG GTT GCC AAA GCC	749
Thr Gln Lys Phe Leu Lys Lys Gln Gly Leu Asp Phe Lys Leu Ser Thr Lys Val Ile Ser Ala Lys Arg Asn Asp	
ACC CAA AAG TTC TTG AAA AAG CAA GGT TTG GAC TTC AAA TTA AGC ACC AAA GTT ATT TCT GCA AAG AGA AAC GAC	824
Asp Lys Asn Val Val Glu Ile Val Val Glu Asp Thr Lys Thr Asn Lys Gln Glu Asn Leu Glu Ala Glu Val Leu	
GAC AAG AAC GTC GTC GAA ATT GTT GTA GAA GAT ACT AAA ACG AAT AAG CAA GAA AAT TTG GAA GCT GAA GTT TTG	899
Leu Val Ala Val Gly Arg Arg Pro Tyr Ile Ala Gly Leu Gly Ala Glu Lys Ile Gly Leu Glu Val Asp Lys Arg	
CTG GTT GCT GTT GGT AGA AGA CCT TAC ATT GCT GGC TTA GCG GCT GAA AAG ATT GGA TTA GAA GTA CAC AAA ACG	974
Gly Arg Leu Val Ile Asp Asp Gln Phe Asn Ser Lys Phe Pro His Ile Lys Val Val Gly Asp Val Thr Phe Gly	
GGA CGC CTA GTC ATT GAT GAC CAA TTT AAT TCC AAG TTC CCA CAC ATT AAA GTG GTA GGA GAT GTT ACA TTT GGT	1049

(continued on facing page)

Fig. 1. Nucleotide sequence of the *LPDI* gene and deduced primary structure of lipamide dehydrogenase. The nucleotide sequence of a 2.7 kb region containing the sense strand of the *LPDI* gene as well as over 1 kb of flanking DNA is shown in the 5' to 3' direction. The specific features of the 5' flanking region are noted as follows: The 'TATA' consensus sequences are overlined; the sequences which show homology to known regulatory sequences are boxed. Specific features of the 3' flanking sequence are noted as follows: the proposed consensus polyadenylation signal, AATAAA, is denoted by overdots; the proposed polyadenylation/termination signal TAG...TATGT...TTT is denoted by underdots.

Downstream of the TGA translation termination codon an additional 114 bp has been sequenced. The motif AATAAA postulated to specify a site involved in polyadenylation in higher eukaryotes (Proudfoot & Brownlee, 1976) and seen in many yeast genes is found at position +12. The sequence TAG...TA(T)GT...(AT rich)...TTT has been postulated to be a signal for transcription termination or polyadenylation (Zaret & Sherman, 1982), and a version of this is found at position +18: CAGTATAGTATATATATTT.

These motifs are illustrated in Fig. 3. At position -247 there is a TGACTC sequence with an adjacent run of T residues that conforms very closely to the consensus sequence obtained from the GCN4 protein binding sites studied to date (Hill *et al.*, 1986). This protein mediates the general control of amino acid biosynthesis response that is involved in modulating during amino acid starvation the expression of some genes for amino acid synthesis (Struhl, 1982). It is interesting that a second, almost perfect direct repeat sequence (TGAATCGTTTTT), is also present 17 bp away at position -265. At -114 there is another TGACTC motif, although this conforms less well to the GCN4 consensus. All other yeast genes found so far to be subject to this GCN4-mediated control are concerned directly with synthesis of amino acids or charged tRNA species. Regulation of lipoamide dehydrogenase by general amino acid control may be less

1 70
 YLPDH mlrirsllnn krafssstvrt ltinkshDVV IIGGGPaGYv AAiKAAqLGF nTacVEkrgk LGGTCLNVGC
 PLPDH adqpidaDVt VIGSGPGGYv AAiKAAqLGF kTVcIEkneT LGGTCLNVGC
 ECLPDHsteiktqVV VIGaGPaGYs AAfrcAdLGL eTViVErynt LGGvCLNVGC
 HGR acrq epqqpgdpda agavasyDyl VIGGGsGGla sArrAAeLga raavVE.shk LGGTCvNVGC
 PAMR adnrvglldk vrgwmaaaek hsgneppvqV aVIGsgGaam AAalkAveqg aqVtlierT iGGTCvNVGC
 -----DVV VIGGGPGGY- AA--AA-LGF -TV-VE---T LGGTCLNVGC

139
 IPSKALLNns HlfHqMhtea qkrgi.dvng DIKiNvanfq kakDdaVkl TGGielLFKk nkVtyykGng
 IPSKALLNns HyyH.Mahgk dfasrGiems EVrLNlekm* *****aL TGGiahLFK* *****
 IPSKALL... HvakvieEak alaehGivfg EpKtdidkir twkEkvInqL TGGlagMaKg rkVkvVnGlg
 VPkKvMwNta vhsfMhDha ...dyGfpse EgKfNwrvik ekrDayVsrL naiyqnnLtk shLeIIrGha
 VPSKiMiraa HiaHlrrEsp fdgglaatvp tIdrskllaq qqarvdelrh akyegilGgn paItVVhGea
 IPSKALLN-- H--H-M-E-- -----G---- EIK-N----- ---D--V--L TGG---LFK- ---VV-G--

208
 sFedetkirV tpvdGlegatV kedhildvkn IiVATG.Sev TPFFgIeIDE ekivsStGAL sLkEIPkRLt
 vngygyk* **ad gsteVintkN IliATG.Sev TPFFgItIDE *****kMv
 kFtgantiev egenG..... .ktVinfdN aiIAaG.Srp iqLPfIphED priwdStDAL eLkEVPeRLl
 aF..... tsdpkptieV sgkkytaph. IliATGmpS TPhesqipga slgitSdgff qLeElPgRsv
 rFkddqsltV rlineGgerVv mfdrcL.... .VATG.asp avpPipglkE spywtSteAL asdtIPeRLa
 -F-----V ----G-----V ----V-----N I-IATG-S- TPFP-I--DE -----ST-AL -L-EIP-RL-

278
 IIGgGIIGLE MgsVYsrLGS KVtVvefqpq IgasmDgeVa katqkflkkq gldFklstkv isakrnddkn
 VIGaGVIGvE ..***** ***** *****
 VmGgGIIGLE MgtVYhaLGS qIdVvemfdq VipaaDkdIv kvftkriskk fnlMletkvt aveakedgiy
 IVGaGylavE MagILsaLGS Ktslmirhdk VlrsfDsmIs tncteeleena gvevlkfsqv kevkktslgl
 VIGssVvaLE LaqaFarLGS KVtVlarntL ffredpaige avtaafraeg ievLehtqas qva.....
 VIG-G-IGLE M--VY--LGS KV-V----- V-----D--I-----

346
 vveiv..ved tktnkqenle aEvLLVAVGR rPyiagLgaE KiGleVdDkrG rIVIDdqFns kfPhIkVGD
 ***** LVcIGR ePftQnLgLE elGIElr*** ***** *IPnIaAIGD
 v.....tme gkkapaepqr yDavLVAIGR vPngknLdag KaGVEVDdrG firVDkqLrT nVPhIFAIGD
 evsmvtavpg rlpvmtmipd vDcLLWAIGR vPNTkdLsLn KlGIqtDdkG hIIVDefqnt nVkgIYAVGD
 ...hmdgef vltttthgelr aDkLLVatGR tPNTTrsLaLD aaGvtVnaqG aIVIDqgMrT snPnIYAaGD
 -----D-LLVAIGR -PNT--L-LE K-G-EVD--G -IV-D---T -VP-IYA-GD

412
 VtfGpMLAHK AeeEG..iAa VemLktGhgh vnYnnIPSVm YsHPEVAWVG kT..EeqLKE aGIDYKigkF
 VvagPMLAHK AedEG..iic VegMagGavh iDYNcVPSVI YTHPEVAWVG k..SEeqLKE eGIEYKvgkF
 IvGqPMLAHK gvhEGhvaAe Viagk..khy FDPkvIPSIa YTePEVAWVG lT..EkeaKE kGIsYetatF
 VcgkaLLtpv AiaaGrklah rlfeykedsk LDYNnIPtVV FshPpIgtVG lTedEaihKy gienvKtyst
 ctdqPqFvyv AaaaG..tra ainMtgGdaa LDltamPaVV FTdPqVatVG ..ysEaeahh dGIEtdsrtL
 V--PMLAHK A--EG---A- V--M--G--- LDYN-IPSVV YTHPEVAWVG -T--E---KE -GIEYK---F

482
 PFAansRAKT NqDTEGFVKi liDsktERIi GaHIIGPnAG EMiAeAgLaI EyGAsaEDVA rvcHAHPTLS
 PFA***RAKT NaDTDGmVKI lgqkstDRVl GaHIIGPgAG EMInEAALaI EyGascEDIA rvcHAHPTLS
 PWAasgRAia sdcadGmtKL IfDkeshRVI GgaIVGtnGg ELlgEigLaI EmGcdaEDIA ltiHAHPTLS
 sFtpmyhAvT krtTkcvmkM VcankeEkVV GiHmqGlged EmlqgfAvAV kmGatkaDfd ntvaiHPTLS
 tLdnvpRala NfDTRGFIKL ViEegshRII GvqaVaPeAG ELIqtAALAI rnrmvtqEla dqlfpylTMv
 PFA---RA-T N-DTGDG-VKL V-D---ERVI G-HI-GP-AG EMI-EAALAI E-GA--EDIA ---HAHPTLS

499

EaFkeAnmaa ydkaihc... ..
 EaFreAnlaa sfgkainf.. ..
 EsvglAaevf egsitdlpnp kakkk
 EeLvtlr... ..
 EgLklAaqtf nkdvkqlscc ag...
 E---A---

(a)			
GCN4 binding site consensus sequence		rrTGACTCattt---	
<i>LPD1</i>	-264	CGTGAATCGTTTTTAG	-253
<i>LPD1</i>	-247	GATGACTCGTTTTTAA	-236
<i>LPD1</i>	-114	TTTGACTCACCTCGAA	-108
(b)			
<i>CYC1</i> UAS1		CTCTTTGGCCGGGGTTT	
<i>CYC1</i> UAS2		CTCTTTGGCGAGCGTTT	
<i>GAL1-10</i> control region sequence		CTCTTTGGAACTTTCAG	
<i>LPD1</i>	-204	CTCATTGGCGAGAAGTC	-189
(c)			
Heat-shock consensus sequence		C--GAA--TTC--G	
<i>LPD1</i>	-348	CACGAATAGTCATG	-335

Fig. 3. Alignment of potential regulatory sequences with known motifs. In each case the established regulatory sequence is displayed, with the upstream regions of the *LPD1* gene which show homology shown directly below. (a) Consensus sequence for the general control regulatory site. Highly conserved nucleotides are shown as capitals, conserved residues are shown in lower case, and nonconserved residues are indicated by dashes. (b) Upstream activation sites (UAS1 and UAS2) of *CYC1* and a sequence located in the *GAL1-10* control region. (c) Heat-shock promoter consensus sequence.

surprising than it seems at first sight, since some of the tricarboxylic acid (TCA) cycle intermediates are substrates for the synthesis of such amino acids as glutamate, arginine, lysine and proline. There is one report that fumarase activity is derepressed in arginine bradytrophs; it has been suggested that this TCA cycle enzyme may also be under general amino acid control (Delforge *et al.*, 1975).

A second putative control region, at position -204, together with its homology with the upstream activation sites of *CYC1*, is shown in Fig. 3. The function of this region is not known but both genes are involved in aerobic metabolism and are subject to catabolite repression (Guarente *et al.*, 1984; Roy & Dawes, 1987); a related sequence with homology over 8 bases can be found in the *GAL1-10* control region (Johnston & Davis, 1984).

A third potential control site shown in Fig. 3 is the sequence at -352 which shows a 7 out of 8 match with the general heat-shock consensus sequence (Pelham, 1985).

Several further sequences are also of interest. One is an inverted repeat CTCCGCGGAG at position -188 which is similar to a general repressor protein binding sequence proposed by

Fig. 2. Protein sequence comparison. The primary structures of *S. cerevisiae* lipoamide dehydrogenase (YLPDH), pig heart lipoamide dehydrogenase (PLPDH), *E. coli* lipoamide dehydrogenase (ECLPDH), human red blood cell glutathione reductase (HGR) and *Pseudomonas aeruginosa* mercuric reductase (PAMR) are aligned for maximum homology. The residue numbering is based on *S. cerevisiae* lipoamide dehydrogenase. A consensus line below the five sequences shows residues common to three or more of the amino acid sequences. The borders of the domains representing the four regions of strong homology are based on glutathione reductase and are marked by arrowheads below the consensus sequence. The 80 residues at the N-terminus of mercuric reductase have been omitted. The individual sequences show residues in agreement with the consensus sequence in upper case and all others in lower case. The alignment was performed using the program PRETTY with the default protein comparison file (Gribskov & Burgess, 1986), which regards certain amino acids as similar for the purposes of the consensus line. The asterisks in the pig heart lipoamide dehydrogenase sequence indicate regions of unknown sequence. Residues common to pig heart and yeast lipoamide dehydrogenase but not to the *E. coli* lipoamide dehydrogenase are shown by circles above the yeast sequence.

T. Cooper (personal communication); a second is the sequence CACCTCGA at position -109 which is homologous to one found upstream of the *ARG3* and *CAR1* genes involved in arginine biosynthesis and degradation respectively (Crabeel *et al.*, 1985). In addition to the above, the upstream region contains a number of direct repeats and inverted repeats which may also have a role in the regulation of *LPD1* expression. One of these at -283 is very interesting since it is a 23 bp inverted repeat, containing direct repeats of 9 bp each within it. It is represented in a symmetrically truncated form at -361. We are currently investigating the functional significance of these upstream regions.

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