The P64k Neisseria meningitidis *dihydrolipoamide dehydrogenase participates in catalysis of the pyruvate dehydrogenase multienzyme complex*

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ABSTRACT

The neisserial P64k antigen is a dihydrolipoamide dehydrogenase which is partially found on the cell envelope. Given its unusual localization pattern and taking into account that dihydrolipoamide dehydrogenases are generally associated with the bulky complexes of cytoplasmic α-ketoacid dehydrogenases, we have tried to determine if at least part of the P64k participates in either pyruvate dehydrogenase or a-oxoglutarate dehydrogenase catalysis. By using Northern blots of meningococcal knockout mutants for P64k and the E1 and E2 subunits of the putative pyruvate dehydrogenase of *Neisseria meningitidis*, it is shown that P64k is transcriptionally linked to the genes coding for the enzymes of this complex. Furthermore, by examining the growth patterns of these mutants on a defined media using acetate or succinate as the sole carbon source, as well as by measuring enzyme activity in cell extracts, it was proven that the P64k participates in the catalysis of this complex, and unlike many other Gram negative bacteria, it is not shared with the meningococcal a-oxoglutarate dehydrogenase complex.

Keywords: Neisseria meningitidis, pyruvate dehydrogenase, lipoamide dehydrogenase, complementation assay, P64k, mutant, metabolism, biochemistry, knock-out, Northern blot

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RESUMEN

La dihidrolipoamida deshidrogenasa P64k de *Neisseria meningitidis* participa en la catálisis del complejo multienzimático de la piruvato deshidrogenasa. El antígeno P64k de *Neisseria meningitidis* es una dihidrolipoamida deshidrogenasa localizada parcialmente en las membranas celulares del meningococo. Teniendo en cuenta que las dihidrolipoamida deshidrogenasas normalmente se encuentran asociadas a los grandes complejos multienzimáticos de las α-cetoacido deshidrogenasas en el citoplasma, este patrón de localización resulta inusual. En este trabajo se ha tratado de determinar si al menos parte de la P64k participa en la catálisis de la piruvato o la a-oxoglutarato deshidrogenasas, usando para ello cepas de *N. meningitidis* mutantes para la P64k y las subunidades E1 y E2 del complejo de la piruvato deshidrogenasa. Se demuestra que P64k está ligada transcripcionalmente a los genes codificantes para las enzimas de este complejo mediante el uso de Northern *blotting*; y mediante pruebas de crecimiento en medio definido usando acetato o succinato como fuente de carbono, así como mediante mediciones enzimáticas directas en dichos mutantes, se establece que P64k participa en la catálisis del complejo de la piruvato deshidrogenasa; y que en contraste con la mayoría de las bacterias Gram negativas, no es compartida con el complejo de la a-oxoglutarato deshidrogenasa.

Palabras Claves: Neisseria meningitidis, piruvato deshidrogenasa, lipoamida deshidrogenasa, ensayo de complementación, P64k, mutante, metabolismo, bioquímica

*I*ntroduction

Dihydrolipoamide dehydrogenase (LipDH) (EC 1.8.1.4) is a flavin-containing pyridine nucleotide disulfide oxidoreductase that catalyzes the NAD+- or NADP+-dependent oxidation of dihydrolipoamide [1]. In bacteria, it is usually found as part of the cytoplasmic a-oxoacid dehydrogenase multienzyme complexes, where it participates in the oxidative decarboxylation of ketoacids such as pyruvate and α-oxoglutarate as well as of branched ketoacids originating from the transamination of branched aliphatic aminoacids. LipDH is also a component of the glycine decarboxylase complex known as the glycine cleavage system [2].

Our group has cloned a dihydrolipoamide dehydrogenase, termed P64k, from the Gram negative bacterium *Neisseria meningitidis* by screening with polyclonal sera raised against meningococcal outer membrane proteins [3, 4]. In agreement with this, a significant portion of P64k has been found to be envelope-associated (manuscript submitted). However, in the neisserial chromosome the P64k gene clusters with the genes for the E1p and E2p components of the pyruvate dehydrogenase complex (PDC) [5], suggesting that it is the E3 component of this α-ketoacid dehydrogenase. Given the subcellular localization and sheer size of these multienzyme complexes, it is difficult to reconcile the partial cell envelope association of P64k with its putative participation in a-ketoacid oxidative decarboxylation, unless the envelope-associated and the cytoplasmic forms of P64k have different physiological roles.

Here, the possible involvement of at least part of the P64k LipDH in PDC catalysis is examined. Using knockout meningococcal strains for the E1p-, E2p- and P64k-coding genes, it is shown that P64k is transcriptionally linked to the other genes of the cluster, that it is indeed the E3 component of the PDC complex, and that unlike the situation in most other bacteria [6], it is not shared with the α-oxoglutarate dehydrogenase complex.

1. Williams CH Jr. Lipoamide dehydrogenase, glutathione reductase, thioredoxin reductase and mercuric reductase-family of flavoenzyme transhydrogenases. In: Müller F, editor. Boca Ratón, USA: CRC Press; 1992. p.121-211.

2. Berg A, de Kok A. 2-Oxo acid dehydrogenase multienzyme complexes. The central role of the lipoyl domain. Biol Chem 1997;378:617-34.

3. Alvarez A, Coizeau E, Fernández JR, González S, Morales J, Selman-Houssein M et al., inventors; Centro de Ingeniería Genética y Biotecnología, asignee. Nucleotide sequence coding for an outer membrane protein from *Neisseria meningitidis* and use of said protein in vaccine preparations. EP19910202291 19910906. 1992 Mar. 11.

Materials and Methods

Bacterial strains and growth conditions

Escherichia coli strain XL-1 Blue [7] was used for all cloning. It was grown in Luria Broth (LB) at 37 ºC, supplemented accordingly with 100 mg/mL ampicillin or 50 mg/mL kanamycin. *Neisseria meningitidis* strain H355 (B:15:P1.19,15) [8] and its mutant derivatives described in this work were grown at 37 ºC in brain heart infusion (BHI, Oxoid, UK) 1.5% (w/v) agar plates in a candle jar, or in BHI cultures inoculated to an initial OD_{620} of 0.1 and supplemented with 100 mg/mL kanamycin as necessary. Culture stocks were prepared in 10% (w/v) skim milk and stored at -70 ºC.

Plasmids and probes

The plasmids used in this study have inserts spanning different regions of the meningococcal PDC gene cluster where either the putative E1p (*aceE*), E2p (*aceF*), or E3 (P64k, *lpdA*) genes have been inactivated by insertion or replacement with the kanamycin resistance (Kan^r) cassette from pUC4K [9] (Figure 1). pM140 was constructed by the insertion of a blunted (*BamH* I-Klenow) KanR cassette between the *Sty* I sites of the meningococcal E1p (*aceE*) gene, previously amplified by PCR using the oligonucleotides 2195 (5' TTTCAAGTTTTCCCTTGTTT 3') and 2196 (5' TCGTCAACGGCGATGGTGTC 3') and cloned into pMOSBlue (Amersham Pharmacia Biotech UK Ltd.). pM117 was made by inserting the same cassette into the *EcoR* V site on the fragment of the meningococcal E2p gene (*aceF*) present in pM2 [3]. Plasmid pM110 was constructed by the digestion of pM3 [3] with *Xho* I and the subsequent Exonuclease III/S1 nuclease treatment [10], ligating the resulting DNA to the blunted Kan^r cassette. Only 249 and 105 bp of the P64k (*lpdA*) gene are retained in pM110.

DNA probes specific for the *aceE*, *aceF* and *lpdA* genes were prepared by purifying the *Kpn* I or *EcoR* I fragments from pM122 and pM2 respectively, or by amplifying *lpdA* entirely using PCR with oligonucleotides 1573 (5' TTCCATGGTAGATAAAAG 3') and 1206 (5' AAAAAAGAAAACGCCTCC 3').

Recombinant DNA techniques

Standard recombinant DNA techniques were carried out essentially as previously described [11]. DNA restriction and modification enzymes were used following the manufacturers' recommendations. For the transformation of meningococci, exponentially growing cells were resuspended in BHI supplemented with 10 mM $MgCl_2$ at 0.05 OD_{620} and incubated statically with plasmid DNA at 10 mg/mL for 1 h at 37 ºC. Afterwards they were plated onto BHI-kanamycin plates, grown 12 to 24 h, and resistant colonies were purified twice by streaking onto selective plates before preparing stocks for analyses. Purification of meningococcal chromosomal DNA and total RNA followed published procedures [12, 13]. Hybond-N+ nylon membranes and the ECL Direct Nucleic Acid Labelling and Detection System were used for Southern and Northern blots, following the instructions supplied by the manufacturer (Amersham Pharmacia Biotech UK Ltd.).

SDS-PAGE and Western blotting

Sodium-dodecyl sulphate polyacrylamide gel electrophoresis, protein transfer to nitrocellulose filters and immunodetection were performed as described [14, 15]. Protein concentration was determined with a modi-

4. Guillén G, Alvarez A, Silva R, Morera V, González S, Musacchio A et al. Expression in *Escherichia coli* of the *lpdA* gene, protein sequence analysis and immunological characterization of the P64k protein from *Neisseria meningitidis*. Biotechnol Appl Biochem 1998;27 (Pt 3):189-96.

5. Ala' Aldeen DA, Westphal AH, de Kok A, Weston V, Atta MS, Baldwin TJ et al. Cloning, sequencing, characterisation and implications for vaccine design of the novel dihydrolipoyl acetyltransferase of *Neisseria meningitidis*. J Med Microbiol 1996;45:419-32.

6. de Kok A, Hengeveld AF, Martin A, Westphal AH. The pyruvate dehydrogenase multi-enzyme complex from Gram-negative bacteria. Biochim Biophys Acta 1998;1385:353-66.

7. Bullock WO, Fernández JM, Short JM. XL-1Blue: A high efficiency plasmid transforming *recA Escherichia coli* K12 strain with beta-galactosidase selection. Biotechniques 1987;5:376-8.

8. Maiden MCJ, Suker J, McKenna AJ, Bygraves JA, Feavers IM. Comparison of the class 1 outer membrane proteins of eight serological reference strains of *Neisseria meningitidis*. Mol Microbiol 1991;5: 727-36.

9. Taylor LA, Rose RE. A correction in the nucleotide sequence of the *Tn903* kanamycin resistance determinant in pUC4K. Nucl Acids Res 1988;16:358.

10. Henikoff S. Unidirectional digestion with exonuclease III creates targeted breakpoints for DNA sequencing. Gene 1984;28:351-9.

11. Sambrook J, Fritsch EF, Maniatis T. Molecular cloning: A laboratory manual. New York, USA: Cold Spring Harbor Laboratory Press; 1989.

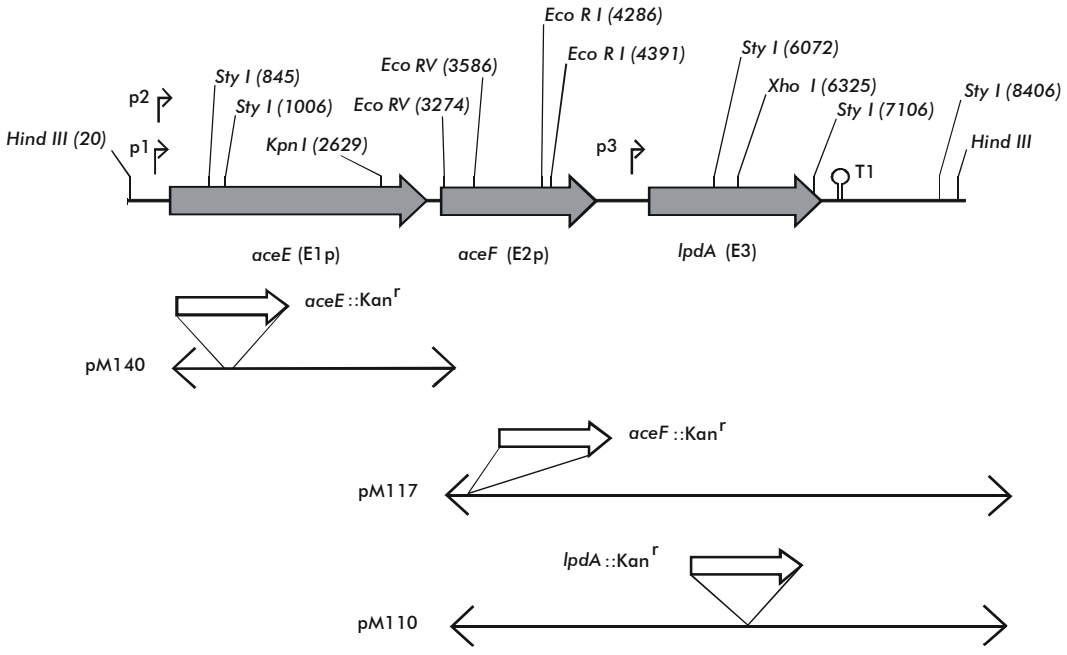

Figure 1. Regions of the *N. meningitidis* B385 PDC gene cluster spanned by the inserts of plasmids pM140, pM117 and pM110, and sites of insertion of the Km^r determinant. Only their relevant features and restriction sites are shown. p1, p2 and p3 are putative promoters found using a neural network-based prediction method [22]; T1 is the only canonical *rho*-independent transcriptional terminator found by manually scanning the sequence of the cluster.

fied micro Biuret method [16]. Monoclonal antibodies (mAb) 114 and 448 have also been described [17, 18].

Complementation assays

Complementation assays were carried out in an MCDA defined medium [19], using glucose as the carbon source. Briefly, fresh cultures of mutant meningococcal strains grown in 100 mg/mL kanamycin-BHI were washed and resuspended in MCDA, and plated to an approximate density of 300 colony-forming units (c.f.u.) per plate on MCDA-glucose supplemented with 2 mM acetate, 2 mM succinate, or both. Results are given after 72 h of growth at 37 ºC.

Enzyme assays

Fresh meningococcal cultures grown in BHI-kanamycin were washed twice and resuspended to an OD_{620} of 5 with 50 mM phosphate buffer pH 7 at 4 ºC, lysed by sonication, and spun at 20000 x g, 4 ºC for 1 h. The supernatant was immediately used for measuring dihydrolipoamide dehydrogenase (E3), pyruvate dehydrogenase (PDC) and a-oxoglutarate dehydrogenase (OGDC) activities as described [20], substituting 5 mM a-oxoglutarate for pyruvate in the later case.

Results

Construction of meningococcal mutant strains

Plasmids pM140 (E1p::Km^r), pM117 (E2p::Km^r) and pM110 (P64k::Km^r) were used for transforming *N. meningitidis* strain H355 and rescuing transformants in which the Kan^r cassette has been integrated into the genome via homologous recombination with its flanking homology arms; thus replacing the wild-type gene with an inactivated counterpart. Figure 2A shows the results of the analysis by Southern blotting of chromosomal DNA from potential H355 *aceE* (E1p$^-$) and H355 *aceF* (E2p$^-$) mutant strains. Only 1 hybridizing target is found in both cases, confirming the occurrence of a double recombination event. Since the size difference between the wild-type and mutated *Hind* III fragments is too small to be resolved by agarose gel electrophoresis, further proof for correct replacement of the wild type gene in the E1p$^-$ clones was obtained by PCR amplification of their *aceE* locus with primers 2195 and 2196 (Data not shown). Clones represented in lanes 1 and 3 were designated AM803 and AM802, and working stocks were prepared for further analysis.

Figure 2 B shows a Western blot using a mixture of MAbs specific for P64k against an H355 *lpdA* (P64k) transformant obtained with plasmid pM110 and previously checked by Southern blotting (data not shown). The absence of detectable P64k confirms the replacement of the wild type gene in all cases. This clone was designated AM801, and frozen stocks were prepared for later analysis.

Transcriptional organization of the PDC gene cluster

The transcriptional organization of the PDC gene cluster was analyzed by Northern blotting since the Km^r expression cassette alters the size and stability of the mRNA transcribed from the locus it is inserted on.

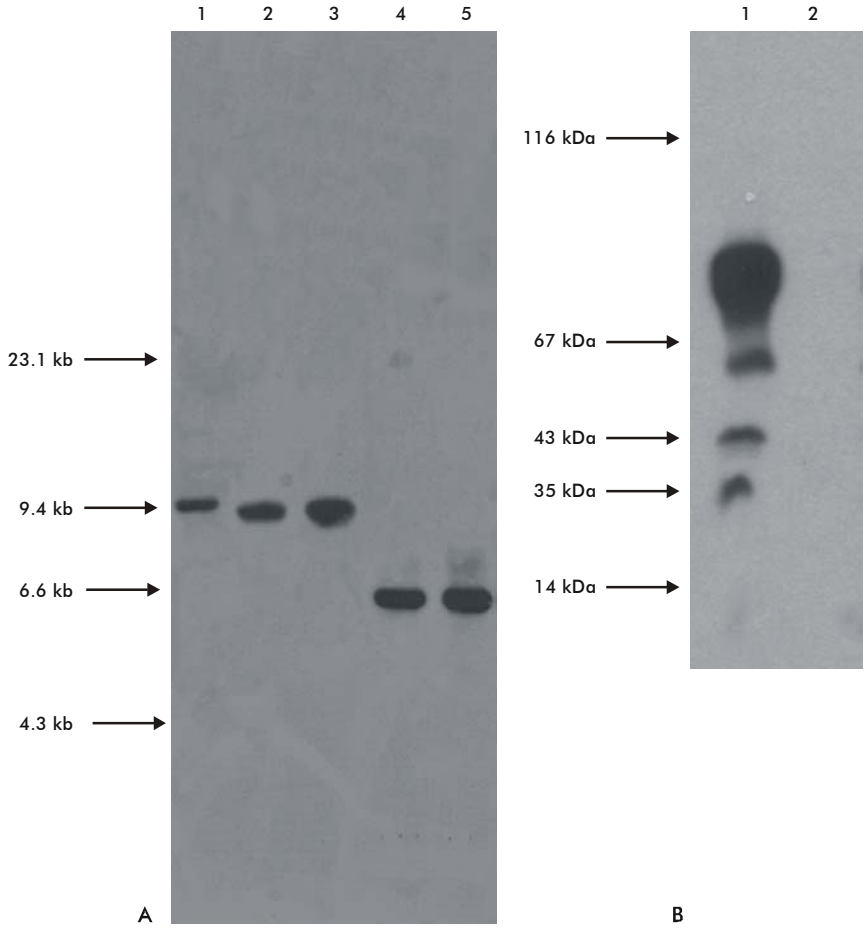

Figure 2. Analysis of meningococcal *aceE* (E1p), *aceF* (E2p) and *lpdA* (P64k) mutants. A) Southern blot of chromosomal DNA (1 μg/well) from wild type *N. meningitidis* H355 (lane 1) and either E1p$^-$ (lanes 2-3) or E2p$^-$ (lanes 4-5) mutants, digested with *Hind* III and probed with an *lpdA*-specific DNA fragment. See Figure 1 for a *Hind* III map of the analyzed region. B) Western blot of total cellular protein from wild type *N. meningitidis* H355 (lane 1) and P64k mutants (lanes 2-3) probed with a mixture of the P64k-specific MAbs 448 and 114.

Figure 3 shows Northern blots of total RNA from wild type, E1p$^-$, E2p$^-$, and P64k (E3$^-$)strains probed specifically for the *aceE, aceF* and *lpdA* genes.

In all three cases a mRNA of approximately 7.5 kb is detected in the wild type strain when using either *aceE*-, *aceF*- or *lpdA*-specific probes, suggesting that the three genes are transcribed into a single polycistronic mRNA. That this is indeed the case is confirmed by the disappearance or mobility shift of this RNA species upon insertion of the Km^r cassette into either *aceE, aceF or lpdA* in the three mutants analyzed, using any of the probes for detection.

A second RNA band is detectable in the wild type strain when using either *aceE* or *aceF*, but not *lpdA* probes; with a relative mobility of approximately 6 kb. This is most probably the result not of the endonucleolytic degradation of the 7.5 kb mRNA, but of an alternative termination of transcription at a site between the *aceF* and *lpdA* genes, since the amount of the 6 kb mRNA in the *lpdA* mutant compared to the wild type remains unchanged even though large changes in mobility and concentration can be detected for the larger messenger (see blots probed with the *aceE* and *aceF* genes).

12. Kath N. A rapid DNA isolation procedure from petri dish grown clinical bacterial isolates. Nucl Acids Res 1990;18:6462.

13. Shaw JH, Clewell DB. Complete nucleotide sequence of macrolide-lincosamide-streptogramin B-resistance transposon *Tn*917 in *Streptococcus faecalis*. J Bacteriol 1985;164:782-96.

14. Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 1970;227: 680-5.

15. Towbin H, Staehelin T, Gordon J. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Biotechnology 1979;24:145-9.

16. Goa J. A microbiuret method for protein determination: Determination of total protein in cerebrospinal fluid. Scand J Clin Lab Invest 1954;5:218-22.

17. Alvarez A, Guillén GE, Nazábal C, Quintana D, Carpio E, Duarte CA et al., inventors; System for the expression of heterologous antigens as fusion proteins. EP 0 816 506 B1. 1997 Jan. 17.

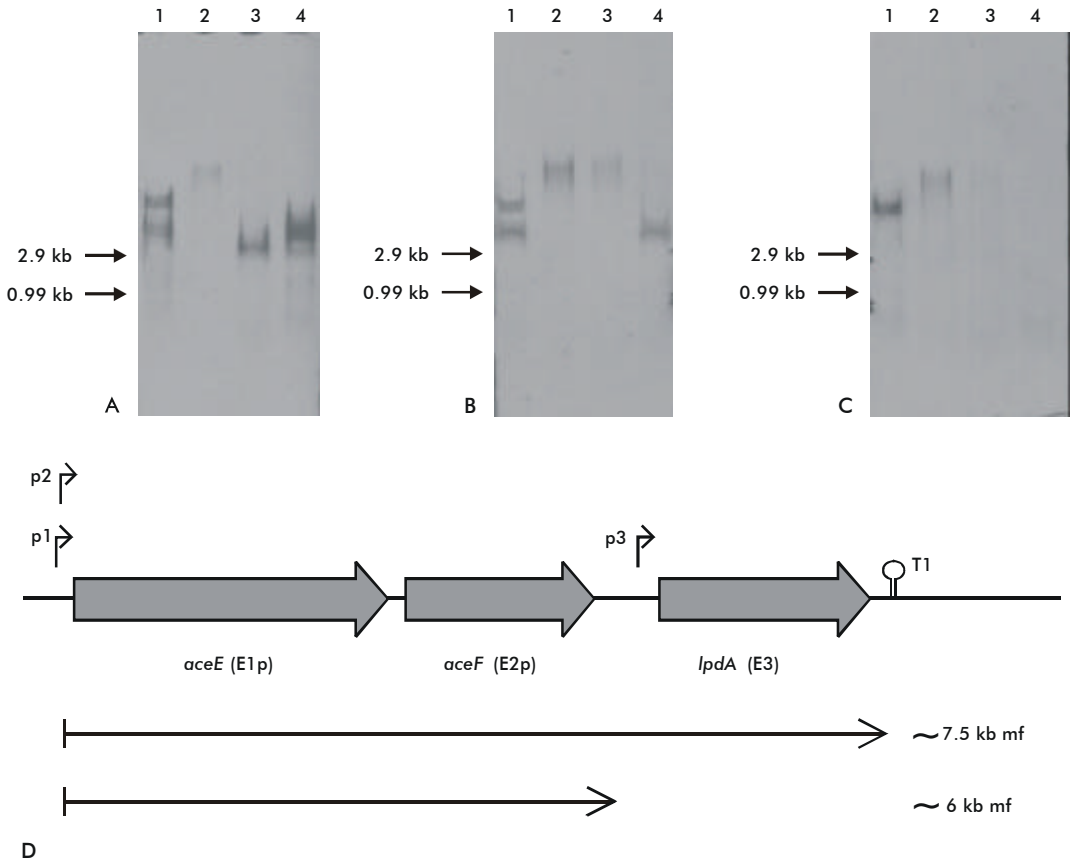

Figure 3. A-C) Analysis by Northern blot of total cellular RNA (5 mg) from wild type *N. meningitidis* H355 (lane1) and *aceE*::Km^r (E1p$^-$, lane 2), *aceF*::Km^r (E2p$^-$, lane 3), and *lpdA*::Km^r (P64k$^-$, lane 4) mutants; using *aceE*- (Panel A), *aceF*- (Panel B) or *lpdA*-specific (Panel C) probes. D) Proposed transcriptional organization of the meningococcal PDC gene cluster.

All these results are coherent with the model for the transcriptional organization of the meningococcal PDC gene cluster presented in Figure 3.

Complementation assays

Disruption of PDC or OGDC activity results in the disruption of the tricarboxylic acid cycle (TCA), which is lethal under aerobic growth using glucose as the carbon source unless acetyl-Coenzyme A (AcCoA) or succinyl CoA are fed to the cycle by adding acetate or succinate to the growth medium, and their conversion by the acetate kinase-phosphotransferase or succinyl-CoA synthetase pathways [21]. Thus, we have examined the growth of the *aceE* (E1p), *aceF* (E2p) and *lpdA* (E3, P64k) mutants in defined media with glucose as the carbon source, in the presence or absence of 2 mM acetate and succinate.

As shown (Table 1), the disruption of either the *aceE*, *aceF* or *lpdA* genes impairs growth in the absence of acetate, but not of succinate. This suggests that these genes are indeed the components of the neisserial PDC, and that P64k (the putative E3 component) is not shared with the α-oxoglutarate dehydrogenase complex (OGDC).

Assays for pyruvate-, α-oxoglutarate- and dihydrolipoamide dehydrogenase activity

In order to corroborate the findings of the previous experiments, PDC, OGDC and LipDH activities were measured in cleared lysates from wild type and P64k$^-$ (*lpdA*) neisserial strains as described in the experimental procedures (Figure 4). As implied previously by the capacity of exogenous acetate but not of succinate to restore growth to the neisserial mutants in MDCA-glucose, disruption of *lpdA* completely eliminates PDC activity without affecting OGDC, confirming that P64k is not involved in the catalysis of the later complex. Total LipDH activity levels dropped to approximately 50% of the wild-type levels, since other cellular dihydrolipoamide dehydrogenases are present to sustain OGDC function.

Discussion

We have prepared meningococcal mutants for the genes coding for the E1p, E2p and P64k polypeptides and used them to study the transcriptional or

18. Nazábal C, Carmenate T, Cruz S, González S, Silva R, Musacchio A et al. Mapping of monoclonal antibodies specific to P64k: A common antigen of several isolates of *Neisseria meningitidis*. Canadian Journal of Microbiology 2001;47:158-64.

19. Erwin AL, Stephens DS. Identification and characterization of auxotrophs of *Neisseria meningitidis* produced by Tn*916* mutagenesis. FEMS Microbiol Lett 1995; 127:223-8.

20. Bresters TW, de Abreu RA, de Kok A, Visser J, Veeger C. The pyruvate-dehydrogenase complex from *Azotobacter vinelandii* . Eur J Biochem 1975;59:335-45.

Table 1. Sensitivity of meningococcal E1p$^-$ (*aceE*), E2p$^-$ (*aceF*) or P64k$^-$ (*lpdA*) mutants to the absence of acetate or succinate during growth in MDCA-glucose plates. The figures shown are the average from 3 independent experiments, and represent colony-forming units. Ac: 2 mM acetate, Succ: 2 mM succinate.

Strains	Supplements			
	None	Ac	Succ	Ac + Succ
wild-type	153	97	125	102
E1p-	0	28	0	35
E2p-	0	30	0	19
P64k-	0	51	0	67

ganization and activity of the putative PDC gene cluster of *N. meningitidis*.

As expected, our results prove that *lpdA* (P64k) is transcriptionally linked to *aceE* (E1p) and *aceF* (E2p) as shown in Figure 3. This is a strong evidence for the involvement of P64k in PDC catalysis, and agrees in general with the pattern of the predicted transcriptional promoters and terminators of the *aceEF-lpdA* locus (see Figure 1). Additionally, a shorter mRNA comprising the *aceE* and *aceF* genes is synthesized, and no additional transcripts containing the *lpdA* gene are detected. This contrasts with most Gram negative bacteria studied so far [21], where a third mRNA is synthesized which codes for the E3 component alone, reflecting an increased need for E3 in species in which this protein is shared between the pyruvate, the α-oxoglutarate, and the branched-chain ketoacid dehydrogenase complexes [6]. However, our experiments do not exclude the possibility that a P64k-specific mRNA can be produced under growth conditions different from those tested here; and more sensitive techniques such as ribonuclease protection assays could be used to detect such RNA species. In fact, a strong s^{70} promoter (p3, Figure 1) is predicted to be located upstream of the *lpdA* gene using the neural network-based prediction algorithm of Reese *et al.* [22], a method with low sensitivity but also a very low false-positive rate.

The assays for growth in a defined medium of *aceE, aceF* and *lpdA* mutants and the measurements of PDC, OGDC and LipDH activity confirm the involvement of at least part of the cellular P64k in PDC catalysis, and reveal that this LipDH is not shared with other cellular α-ketoacid dehydrogenases. It will be interesting to find out what function, if any, is performed by the envelope-associated fraction of the cellular P64k. Other LipDHs have been found in archaebacteria and in the mammalian bloodstream form of *Trypanosoma brucei*, where neither α-oxoacid complexes nor glycine cleavage systems

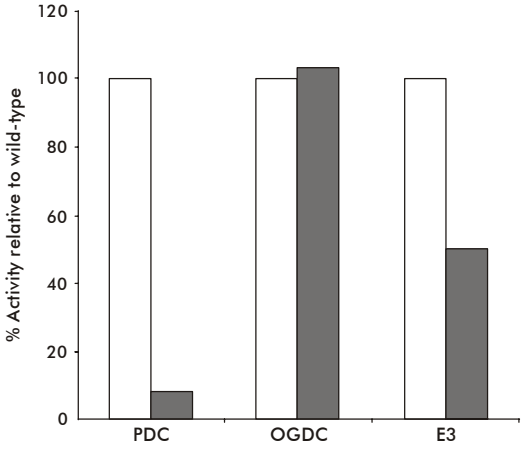

Figure 4. Measurement of PDC, OGDC and E3 (LipDH) activity in a *N. meningitidis* P64k$^-$ mutant (AM801) as compared to the wild type. Average results from 2 independent experiments are shown.

are found [23, 24], evidencing that LipDH may fulfill additional, as yet undiscovered cellular roles. Also, LipDH has been suggested or proven to be envelope-associated in a variety of eubacteria [25-30]. In this context it is worth noting that the dithiol-disulphide exchanges that are typical of LipDH catalysis might be ideally suited for oxidation-reduction reactions involved in membrane transport and signal transduction, and in fact the presence in *E. coli* of lipoic acid-dependent transport systems which are inhibitable by dithiol-specific arsenicals have been reported [31, 32]. However, given the absence of NAD^+ and $NADP^+$ outside the cytosol, questions on the role of a LipDH like P64k besides its involvement in PDC catalysis are hard to answer. Our work provides a starting point to clarify these issues.

21. Guest JR, Angier J, Russell GC. Structure, expression and protein engineering of the pyruvate dehydrogenase complex of *Escherichia coli*. Ann NY Acad Sci 1989;573:76-99.

22. Reese MG, Harris NL, Eeckman FH. Large scale sequencing specific neural networks for promoter and splice site recognition. Biocomputing: Proceedings of the 1996 Pacific Symposium; Hawaii, USA. Singapore: World Scientific Publishing Co.; 1996.

23. Danson MJ, Conroy K, McQuattie A, Stevenson KJ. Dihydrolipoamide dehydrogenase from *Trypanosoma brucei*. Characterization and cellular location. Biochem J 1987; 243:661-5.

24. Kerscher L, Oesterhelt D. Pyruvate: ferredoxin oxidoreductase - new findings on an ancient enzyme. Trends Biochem Sci 1982;7:371-4.

25. Owen P, Kaback HR, Graeme-Cooke KA. Identification of antigen 19/27 as dihydrolipoyl dehydrogenase and its probable involvement in ubiquinone-mediated NADH-dependent transport phenomena in membrane vesicles of *Escherichia coli*. FEMS Microbiol Lett 1980;7:345-8.

26. Smith LD, Bungard SJ, Danson MJ, Hough DW. Dihydrolipoamide dehydrogenase from the thermoacidophilic archaebacterium *Thermoplasma acidophilum*. Biochem Soc Trans 1987;15:1097.

27. Freudenberg W, Mayer F, Andreesen JR. Immunocytochemical localization of proteins P1, P2, P3 of glycine decarboxylase and of the selenoprotein P_A of glycine reductase, all involved in anaerobic glycine metabolism of *Eubacterium acidaminophilum*. Arch Microbiol 1989;152:182-8.

28. Dietrichs D, Bahnweg M, Mayer F, Andreesen JR. Peripheral localization of the dihydrolipoamide dehydrogenase in the purinolytic anaerobe *Clostridium cylindrosporum*. Arch Microbiol 1991;155:412-4.

29. Hemilä H. Lipoamide dehydrogenase of *Staphylococcus aureus*: nucleotide sequence and sequence analysis. Biochim Biophys Acta 1991;1129:119-23.

30. Berks BC, McEwan AG, Ferguson SJ. Membrane-associated NADH dehydrogenase activities in *Rhodobacter capsulatus*: purification of a dihydrolipoyl dehydrogenase. J Gen Microbiol 1993;139 (Pt 8):1841-51.

31. Richarme G. Possible involvement of lipoic acid in binding protein-dependent transport systems in *Escherichia coli*. J Bacteriol 1985;162:286-93.

32. Richarme G, Heine HG. Galactose- and maltose-stimulated lipoamide dehydrogenase activities related to the binding-protein-dependent transport of galactose and maltose in toluenized cells of *Escherichia coli*. Eur J Biochem 1986;156:399-405.

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