Organization and Expression of the Pyruvate Dehydrogenase Complex Genes of *Escherichia coli* K12

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(Received 6 May 1981)

The organization and expression of the pyruvate dehydrogenase complex genes, *aceE* (E1), *aceF* (E2) and *lpd* (E3), were investigated using a series of λ transducing phages carrying different segments of the *nadC*–*aroP*–*aceE*–*aceF*–*lpd* region of the chromosome of *Escherichia coli*. The polypeptides synthesized following the infection of u.v.-irradiated lysogenic and non-lysogenic hosts were analysed by sodium dodecyl sulphate–polyacrylamide gel electrophoresis and autoradiography.

The *aceE* and *lpd* gene products were readily identified by their sizes, M_r 100 000 (pyruvate dehydrogenase, E1 component) and 56 500 (lipoamide dehydrogenase, E3 component), but considerable heterogeneity was detected for the *aceF* gene product (acetyltransferase, E2 component). The main E2 polypeptides, M_r 80 000 and 83 000, were accompanied by a family of minor polypeptides, M_r 86 000, 89 000 and 91 000, but no precursor–product relationships were apparent. A very large polypeptide, M_r 190 000, was also detected and found to contain the E1 component but in uncertain combination. In addition, the product of a gene in the *nadC*–*aroP* region was detected as a polypeptide with M_r 36 500.

The existence of a single promoter for the *aceE* and *aceF* genes and a separate promoter for the *lpd* gene was confirmed. The *lpd* gene was shown to be transcribed with the same polarity as the *ace* genes (clockwise with respect to the *E. coli* linkage map). The relative rates of expression of the three genes from the bacterial promoters were estimated as 0.94:1.0:1.4–2.3 (E1:E2:E3) on a molar basis. The excess production of lipoamide dehydrogenase components (E3) indicates that the *lpd* promoter functions independently, at least in supplying components for the 2-oxoglutarate dehydrogenase complex. When expressed from P_L , the powerful phage promoter, the *ace* and *lpd* gene products accounted for the greater part of the newly synthesized protein.

INTRODUCTION

The pyruvate dehydrogenase complex of *Escherichia coli* is a multienzyme complex catalysing the overall reaction:

It contains multiple copies of three enzymic components: E1, pyruvate dehydrogenase (EC 1.2.4.1); E2, dihydrolipoamide acetyltransferase (EC 2.3.1.12); and E3, lipoamide dehydrogenase (EC 1.6.4.3). Recent estimates of the molecular weights (M_r) of the corresponding polypeptides are 100 000 for E1, 83 000 and 89 000 for E2, and 56 500 for E3 (Vogel, 1977; Gebhardt *et al.*, 1978), although there is evidence that the E2 polypeptide may be much smaller (M_r 64 500; Bleile *et al.*, 1979). The complex has a structural core comprised

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of E2 components surrounded by independently bound E1 and E3 components. However, the size and polypeptide chain stoichiometry of the native complex, if constant, are still uncertain. A recent study has indicated that the major fraction of the isolated complex has a molecular weight of 6×10^6 , corresponding to a core of 24 E2 chains, and a disproportionate chain ratio (E1:E2:E3) of 1.6:1.0:0.8 (Danson *et al.*, 1979). There is also evidence that the structure of the complex is not unique and that it exhibits a limited degree of heterogeneity (Schmitt & Cohen, 1980).

The dehydrogenase (E1) and acetyltransferase (E2) structural genes (*aceE* and *aceF*) constitute an operon with *aceEF* polarity, which is very close to the *nadC* (quinolinate phosphoribosyltransferase) and *aroP* (general aromatic amino acid permease) genes at 2.6 min on the *E. coli* linkage map (Guest, 1978). The lipoamide dehydrogenase (E3) structural gene (*lpd*) is linked to the *aceF* gene thus: *nadC-aroP-aceE-aceF-lpd*. The specific components of the analogous 2-oxoglutarate dehydrogenase complex are encoded by the *sucA* and *sucB* genes of the *suc* operon, which is situated at 16.4 min and has the same functional polarity, *sucAB* (Guest, 1978). The E3 components of the 2-oxoglutarate dehydrogenase complex are encoded by the identical *aceF*-linked *lpd* gene. This poses the interesting problem of how the expression of the single *lpd* gene is geared to the expression of the separately regulated *ace* and *suc* operons. Indirect evidence from biochemical studies with polar and deletion mutants supports the view that the *lpd* gene can be expressed independently of the *ace* and *suc* genes (Creaghan & Guest, 1972; Langley & Guest, 1978).

Recently, recombinant λ phages containing different segments of the *nadC* to *lpd* region have been constructed (Guest & Stephens, 1980) and a physical map of the corresponding chromosomal region has been deduced by restriction analysis. Furthermore, studies with dilysogens have proved that the cloned *lpd* gene can be transcribed from its own promoter in the absence of a functional *ace* operon and when the major λ promoters are repressed. The polarity of *lpd* expression relative to the polarity of the *ace* operon was not established. In the present work the technique of analysing phage-specified proteins by labelling u.v.-irradiated cells has been used with a variety of artificially constructed transducing phages, (a) to identify the products expressed in vivo from genes in the *nadC* to *lpd* region, (b) to establish the corresponding polarities of gene expression relative to the phage and bacterial genomes, particularly for the *lpd* gene relative to the *ace* operon, and (c) for further correlation of segments of the physical map with the corresponding genetic and biochemical functions.

METHODS

Phages and bacteria. The phages were almost all derived from one *EcoRI* vector, NM781 (*srI* λ 1-3, *cI*857 *nin*5, containing a replaceable *E. coli supE* fragment between *srI* λ 1 and *srI* λ 3; Murray *et al.*, 1977). They were originally isolated from artificially constructed hybrid phage pools of Dr N. E. Murray and characterized by Guest & Stephens (1980). Some properties of these phages and their derivatives are shown in Fig. 1. Phages G78N (λ *nadC*), G82L (λ *lpd*) and G83L (λ *lpd*) were selected for their ability to transduce the *nadC* and *lpd* genes. They contain *EcoRI* fragments of 10.1 and 8.2 kilobase pairs (kb) that are adjacent in the bacterial chromosome. The 8.2 kb fragment is inserted with opposite polarity in the two λ *lpd* phages, G82L and G83L. Phage G78N contains a functional *aroP* gene and the *EcoRI* target shared by the λ *nadC* and λ *lpd* phages lies within the *aceE* gene. Phages with extended transducing activities were G90 (λ *nadC-lpd*; from G78N) and G93 (λ *lpd-aceE*; from G83L). Both contain functional *ace* operons, which are oppositely polarized relative to the phage genome, but only G90 contains the *aroP* gene. Another phage, G105 (λ *lpd*), was kindly provided by P. E. Stephens. It was constructed by inserting the 5.4 kb *HindIII* plus *EcoRI* double-digestion product between *srI* λ 1/2 and *shn* λ 3 of the vector phage NM540 (*srI* λ 1-2, *shn* λ 3⁺ *att*⁺ *imm*²¹ *nin*5 *shn* λ 6^o). The bacterial fragment was inserted with the same polarity as it occurs in G83L and G93.

Escherichia coli strain S159 (*sup*^o *gal* *utrA* *rpsL*) and its lysogenic derivative, S159 (λ *imm*^h *ind*⁻), were used as u.v.-irradiated hosts for infection and pulse-labelling. Strain C600 (*thr* *leu* *thi* *supE* *tonA*) was used for routine phage assays. The methods used for preparation and assay of high titre phage lysates have been described previously (Guest & Stephens, 1980).

Fig. 1. The genomes of the λ *nadC* and λ *lpd* transducing phages and the phages with extended transducing activities (λ *nadC-lpd*, λ *lpd-aceE*) of Guest & Stephens (1980) are represented diagrammatically together with the physical and genetic maps of the corresponding segments of bacterial DNA. Phage G90 (λ *nadC-lpd*) was derived from G78N (λ *nadC*) and the cloned DNA is inserted with the same polarity as in G82L (λ *lpd*). Phage G93 (λ *lpd-aceE*) was derived from G83L (λ *lpd*) and in these phages, and G105 (λ *lpd*), the bacterial DNA is inserted with the alternate polarity relative to the phage genome. The positions of the bacterial genes cloned in transducing phages are aligned with the bacterial linkage map (2.5 to 2.8 min) and the physical map (where necessary, the vector arms have been reversed). The polarity of *ace* gene expression is denoted by the arrow above the genetic map. The dashed arrows indicate the polarities of expression of genes defined during the course of this work. Vector DNA is indicated by thick single lines and bacterial DNA by double lines. The broken lines denote regions where transition from bacterial to phage DNA is uncertain. The phage promoters, P_L (early) and P'_R (late), are indicated by the $\blacktriangle$ symbols on the phage DNA with arrows showing the direction of transcription. The restriction targets are denoted by the symbols $\blacktriangle$ for *EcoRI* and ∇ for *HindIII*. The sizes of the corresponding restriction fragments are indicated in kilobase pairs (kb).

Analysis of polypeptides formed after u.v.-irradiation and phage infection. Cultures of *E. coli* S159 (or its λ *imm*⁺ lysogen) were grown to a density of 10^9 bacteria ml^{-1} in minimal medium (M9 salts; Miller, 1972) with glucose (1%, w/v) at 37 °C. They were resuspended at 4×10^9 bacteria ml^{-1} in fresh medium containing MgSO_4 (20 mM) and irradiated with u.v. light (900 J m^{-2}). Samples (100 μl) plus fresh medium (400 μl) were infected at a multiplicity of 5 (5 μl of 4×10^{11} plaque-forming units ml^{-1}) and pulse-labelled with L-[³⁵S]methionine [final concentration 80–125 $\mu\text{Ci ml}^{-1}$ ($2.96\text{--}4.63 \text{ MBq ml}^{-1}$); Amersham] for periods of 3–35 min at 37 °C. After incorporation, L-methionine was added to a concentration of 120 $\mu\text{g ml}^{-1}$ and the pelleted cells were washed in cold saline. The samples were prepared for sodium dodecyl sulphate (SDS)–polyacrylamide gel electrophoresis by heating for 5 min at 100 °C in dissociation buffer (100 μl) containing SDS (2%, w/v), glycerol (10%, v/v), 2-mercaptoethanol (5%, v/v) and Tris/HCl (125 mM; pH 6.8). The radioactive proteins were analysed by SDS–polyacrylamide gel electrophoresis using acrylamide concentrations of 7.5, 10 or 15% (w/v). A selection of unlabelled proteins were used as standards for M_r determination: glutamate dehydrogenase (cross-linked monomer, 56 000–280 000), RNA polymerase (165 000, 155 000, 39 000), β -galactosidase (116 000), phosphorylase b (94 000), conalbumin (type I, 78 000), bovine serum albumin (68 000), ovalbumin (43 000), lactate dehydrogenase (35 000), carbonic anhydrase (29 000), trypsin inhibitor protein (21 500) and cytochrome c (12 500). The gels were stained and then dried for autoradiography.

Peptide mapping. Peptide mapping by limited proteolysis of [³⁵S]methionine-labelled polypeptides in SDS gels was done according to Cleveland *et al.* (1977). Bands from stained and autoradiographed gels were treated with

2 ng *Staphylococcus aureus* V8 protease (Miles Laboratories) for 15 min in the stacking gel (5%) above a second separating gel (15% acrylamide).

Densitometry. Strips of autoradiogram that had been exposed for different periods were scanned at 600 nm, using a Unicam SP1809 scanning densitometer fitted in the sample compartment of a Unicam SP1800 spectrophotometer. The molar ratios of the *aceE*, *aceF* and *lpd* gene products were estimated by dividing the corresponding peak areas by the methionine contents of the respective components. The latter were assumed to be 20 mol per 100 000 g for E1, 16 mol per 80 000 g for E2 and 10 mol per 56 000 g for E3, as reported by Vogel & Henning (1971, 1973) and Vogel *et al.* (1971).

Materials. Acrylamide, *N,N'*-methylenebisacrylamide and *N,N,N',N'*-tetramethylethylenediamine were obtained from Eastman Chemicals. The sources of other chemicals, enzymes and proteins were: BDH – sodium dodecyl sulphate, Triton X-100 and glutamate dehydrogenase; Boehringer – β -galactosidase, phosphorylase b, lactate dehydrogenase and trypsin inhibitor (soybean); Koch-Light – catalase; Sigma – conalbumin, ovalbumin and carbonic anhydrase; Armour Pharmaceuticals – bovine serum albumin. X-ray film (NS-2T) was from Kodak.

RESULTS

The structures of the phages used in this study are summarized in Fig. 1. They contain different segments of an 18.3 kb region of the *E. coli* chromosome encoding the *nadC*, *aroP*, *aceE*, *aceF* and *lpd* structural genes. The primary phages, G78N (λ *nadC*–*aroP*), G82L (λ *lpd*) and G83L (λ *lpd*), were originally constructed by cloning *Eco*RI fragments of bacterial DNA and selected by their ability to transduce and complement specific mutants. Derivative phages, G90 (λ *nadC*–*lpd*, from G78N) and G93 (λ *lpd*–*aceE*, from G83L), carrying functional *aceE*, *aceF* and *lpd* genes were subsequently isolated by prophage integration in the *nadC* or *lpd* regions and aberrant excision. It should be noted that the bacterial genes of two pairs of phages, G82L and G83L plus G90 and G93, are inserted with opposite polarities. Furthermore, because the orientation of the *ace* genes relative to the genetic and physical maps is known, it can be deduced that the transcription of the *ace* operon (*aceE* $\overrightarrow{F}$) is polarized *rightward* in G90 (λ *nadC*–*aceE* $\overrightarrow{F}$ –*lpd*) and *leftward* in G93 (λ *lpd*–*aceE* $\overleftarrow{F}$).

Polypeptides synthesized in a λ -immune host

Cultures of the homoimmune host S159 (*imm⁺ ind⁻*) were irradiated and labelled after infecting with several transducing phages to analyse phage-directed protein synthesis (Fig. 2). Under these conditions the only bacterial genes that can be expressed are those accompanied by their own promoters. This is because the phage promoters, which could affect the cloned genes, are silent in the immune host.

With G78N (λ *nadC*) only one specifically-labelled polypeptide, M_r 36 500, was detected. The same labelled product was also formed with G90 (λ *nadC*–*lpd*) but not with G83L (λ *lpd*) or G93 (λ *lpd*–*aceE*). This polypeptide, M_r 36 500, could be the *nadC* or *aroP* gene product.

The *lpd* transducing phage, G83L, directed the synthesis of only one specific product and this was readily identifiable, by its size (M_r 56 500), as lipoamide dehydrogenase (Fig. 2). This confirms that the *lpd* gene has its own promoter and can be expressed independently of the *ace* operon. With the phages containing functional *ace* and *lpd* genes, G90 and G93, the labelled polypeptides corresponded to the three components of the pyruvate dehydrogenase complex: E1, 100 000 (*aceE*); E2, 80 000–83 000 (*aceF*); and E3, 56 500 (*lpd*). The E2 region was noticeably diffuse, occupying an M_r range of 80 000 to 83 000. This is probably due to heterogeneity of the E2 component. The presence of at least two polypeptides migrating at different rates was an invariant feature of this investigation. Quantitative densitometry of the autoradiograms (Fig. 3) gave reproducible values for the relative incorporation of methionine into the pyruvate dehydrogenase complex components during the 20 min period of synthesis. The ratios (E1:E2:E3) were 1.13:1.00:0.87 (G93) and 1.18:1.00:1.44 (G90). By making assumptions about the size and composition of the components (see Methods) these correspond to the molar ratios (E1:E2:E3) or 0.94:1.00:1.40 (G93) and 0.94:1.00:2.30

Fig. 2. Autoradiogram of ^{35}S -labelled polypeptides synthesized in a u.v.-irradiated λ -immune host, S159 ($\lambda\text{imm}^+ \text{ind}^-$), after infection with several transducing phages and fractionation in SDS-polyacrylamide gel (10%). Labelling was for 20 min. The sizes of the products specifically directed by cloned genes were estimated by comparison with unlabelled standards. The tracks correspond to the following phages: 1, no phage (uninfected bacteria); 2, G78N (λnadC); 3, G83L (λlpd); 4, G93 ($\lambda\text{lpd-aceE}$); 5, G90 ($\lambda\text{nadC-lpd}$).

(G90). The same ratios were observed with other transducing phages of similar types. The reason for the different level of incorporation into the E3 component is unknown, but it should be remembered that the bacterial genes are cloned with opposite polarities in these phages.

Fig. 3. Densitometer traces of autoradiograms of ^{35}S -labelled polypeptides expressed from pyruvate dehydrogenase complex transducing phages. The polypeptides were labelled for 20 min following infection of the λ -immune host described in Fig. 2. The traces correspond to tracks 4 (a) and 5 (b) in Fig. 2: only the regions containing components of the multienzyme complex are shown.

Polypeptides synthesized in a λ -sensitive host

Expression of the cloned genes was also examined by infecting and pulse-labelling a u.v.-irradiated λ -sensitive host (S159). Under these conditions the products of the cloned genes are seen against a background of λ -specified proteins. Also, depending on the orientation of the cloned genes, expression may be enhanced or retarded by the powerful leftward phage promoter P_L (during the early phase of infection) or it may be affected by the late rightward promoter P_R (during the late phase of infection, when transcription from P_L is repressed). Thus, genes with a *leftward* (*l*) orientation benefit from the early effects of P_L and genes with the opposite, *rightward* (*r*) orientation are inhibited in the early phase but can later be expressed at their inherent rate or at a rate enhanced by rightward transcription originating at P_R . In addition, genes without promoters can be expressed from the phage promoters, and hybrid genes, generated by fusion of phage and bacterial DNA at restriction targets, can be expressed from phage or bacterial promoters, and these are subject to the same temporal constraints imposed by their orientations. The labelling periods used were 3–13 min (early) and 25–35 min (late) after infection.

Orientation of *lpd* and *ace* gene expression. Two λ *lpd* phages were examined (G82L and G83L) with λ *nadC* (G78N) as a control (Fig. 4). During the early period, incorporation into the lipoamide dehydrogenase polypeptide (M_r 56 500) was much higher with G83L than G82L, but during the late period there was very little difference. The E3 component is better resolved from a phage protein of about the same size in the 7.5% gel (Fig. 4b). Because expression of the *lpd* gene is enhanced in G83L, this phage can be designated λ *lpd*^l to denote the *leftward* orientation of the *lpd* gene. By contrast, the *lpd* gene of G82L must have the *rightward* orientation, because its expression appears to be inhibited until transcription from P_L is shut off by the λ *cro* function. Phage G82L may thus be designated λ *lpd*^r. Another phage, G105 (λ *lpd*), was also designated λ *lpd*^l because, as with G83L, labelling of the *lpd* product was high in both the early and late periods. This is consistent with the physical structures of G105 and G83L, which show the bacterial DNA cloned with the same polarity (Fig. 1).

Polypeptides corresponding to the dihydrolipoamide acetyltransferase component (E2, *aceF*) were also labelled with phages G82L (late) and G83L (early and late). Their molecular weights, in decreasing order of intensity, were 80 000 (major), 83 000 and 86 000 (minor). The M_r 80 000 and 83 000 components sometimes appeared in equivalent amounts (Fig. 4a); the

minor component (M_r 86 000) was better resolved in 7.5 % gels (Fig. 4*b*). The reason for this heterogeneity is not known. Some lessening of the relative amounts of the larger components (M_r 83 000 and 86 000) was observed following storage of the samples for 2 weeks at -20°C before analysis.

Phage G83L specifically directed the labelling of a prominent polypeptide of M_r 90 000 (Fig. 4). This was shown to contain part of the *aceE* gene product (E1) by peptide mapping studies with the isolated band. Parallel digestion of comparable samples of this polypeptide and the E1 component (M_r 100 000) with *S. aureus* V8 protease produced 19 and 23 labelled fragments, respectively. Of these, 12 were common to both, the others being specific to the M_r 90 000 polypeptide (7) and E1 (11). Thus it would appear that the M_r 90 000 polypeptide is the product of fusion between a phage gene and the *aceE* gene. The failure to detect this product in the late period of labelling with G82L, in which the *EcoRI* fragment is inverted, is consistent with this conclusion. The results indicate that the *EcoRI* fragment (8.2 kb) cloned in G82L not only contains a functional *lpd* gene but also an intact *aceF* gene and a significant part of the *aceE* gene. However, these *ace* sequences must be separated from the *ace* promoter because their products were not observed in an immune host, where the λ promoters are inactive, but they were expressed when linked to active λ promoters. These results are consistent with the known polarity of the *ace* operon and the conclusions of Guest & Stephens (1980) that the critical *EcoRI* target is in the *aceE* gene and that the corresponding *lpd* phages contain all of the *aceF* gene but only part of the *aceE* gene. No comparable fusion products were detected with the *lpd* phage, G105. This contains the 5.4 kb *HindIII* plus *EcoRI* fragment but should encode part of the *aceF* gene as well as the *lpd* gene.

Other polypeptides observed when the E2 component was strongly labelled had molecular weights of 72 000, 69 000, 43 000 and 42 000 (Fig. 4*b*). These could be E2 degradation products because this component is known to be unstable. It generates a series of comparably sized intermediates and eventually active sub-components of about 39 000 to 35 000 (Hale & Perham, 1979; Gebhardt *et al.*, 1978). If formed, the ultimate product may be obscured by the prominent λE gene product at 37 000 (Fig. 4).

In addition to several identifiable phage proteins, two other peptides, M_r 78 000 (G82L) and 120 000 (G83L), were synthesized as early- and late-labelled products (Fig. 4). These were probably generated by fusion between phage and bacterial genes and their orientation-dependent formation supports this conclusion.

Comparable early- and late-labelling experiments with phages carrying functional *ace-lpd* regions, G93 (λ *lpd-aceE*) and G90 (λ *nadC-lpd*), are shown in Fig. 5. These confirmed that the *ace* and *lpd* genes are both transcribed with the same polarity, their expression being *leftward* in G93 (λ *lpd^l-ace^l*) and *rightward* in G90 (λ *nadC-ace^r-lpd^r*). The *aceE* and *lpd* products were identifiable as polypeptides of M_r 100 000 and 56 500, respectively. However, with these phages the heterogeneity in the acetyltransferase (E2) region was even greater. There appeared to be a family of polypeptides of increasing size (M_r 80 000, 83 000, 86 000, 89 000 and 91 000), but decreasing intensity. These were quite specific and distinct from a phage protein that migrates to this region, the late λH gene product (M_r 88 000). Also with G93 (λ *lpd^l-ace^l*), a very large component (M_r 190 000) was invariably present but its exact nature was difficult to define (see below). The postulated E2 degradation products were also visible.

Polypeptide products of the nadC-aroP region. Studies with the λ -immune host identified a polypeptide, M_r 36 500, which was specifically expressed from phages carrying the *nadC-aroP* region (G78N and its derivative, G90). A product of the same size, formed at an enhanced rate in the late phase of λ *nadC* infection, was detected with the λ -sensitive host. This suggests that the corresponding gene is transcribed with *rightward* orientation. However, this could not be correlated with *nadC* or *aroP*, the only genes known to be located in the 10.1 kb *EcoRI* fragment. Other specific polypeptides, probably derived by *rightward* (29 500 and

Fig. 4. Autoradiograms of the ^{35}S -labelled polypeptides formed after infection of a u.v.-irradiated λ -sensitive host (S159) with $\lambda nadC$ and λlpd phages. Labelling was for 10 min in either the early (3–13 min. tracks 1–4) or late (25–35 min. tracks 5–8) periods after infection with the following phages: 1 and 5, no phage; 2 and 6, G78N ($\lambda nadC$); 3 and 7, G82L ($\lambda lpd'$); 4 and 8, G83L ($\lambda lpd'$). Samples of the labelled cultures were analysed by electrophoresis in SDS gels containing 10% (a) and 7.5% (b) acrylamide. A sample of the material shown in Fig. 3(a) provided radioactive markers for the E1, E2 and E3 components (track 9).

Fig. 5. Autoradiogram of ³⁵S-labelled polypeptides produced after infection of a u.v.-irradiated λ-sensitive host (S159) with transducing phages containing the three pyruvate dehydrogenase complex genes (*λnadC-lpd* and *λlpd-aceE*). The early- and late-labelling procedures were as described in Fig. 4 and the SDS gel contained 7.5% acrylamide. The tracks correspond to the following phages: 1 and 5, no phage (uninfected bacteria); 2 and 6, G78N (*λnadC*) control for phage gene expression; 3 and 7, G90 (*λnadC-aceEF-lpd*); 4 and 8, G93 (*λlpd-aceFE*).

Fig. 6. Autoradiographic analysis of ^{35}S -labelled polypeptides produced in a u.v.-irradiated λ -sensitive host (S159) after infection with G93 ($\lambda\text{lpd}^l\text{-ace}^l$) and exposure to [^{35}S]methionine for 3 or 6 min periods before electrophoresis through a 7.5% polyacrylamide gel containing SDS. The numbers on each track indicate the time (min) of termination of each pulse (3 min for the 3 to 18 min samples and 6 min for the 24 to 36 min samples). The track marked U represents uninfected bacteria labelled in the 30 to 36 min period.

28 500) and *leftward* (47 000 and 39 000) transcription, were also detected with the λ -sensitive host but their origin is unknown.

Progress of *ace*-*lpd* gene expression. Temporal changes in expression of the *aceE*, *aceF* and *lpd* genes were investigated by infecting the λ -sensitive host with G93 ($\lambda\text{lpd}^l\text{-ace}^l$) and labelling with pulses of 3 or 6 min over a period of 36 min (Fig. 6). All the components were detectable from the earliest time and their rates of synthesis increased steadily for 9 min before levelling off and then declining after 18–24 min. The molar ratios were approximately 1:1:1 (E1:E2:E3) in the early stages but after 9 min there appeared to be less E1 and more E3 relative to the sum of the main E2 components (M_r 80 000 and 83 000) thus: 0.5:1.0:1.3 (E1:E2:E3). It was also clear that the components of the multienzyme complexes accounted

Fig. 7. Peptide mapping of the large polypeptide (M_r 190 000) and other components of the pyruvate dehydrogenase complex. The products of digestion of the ^{35}S -labelled polypeptides were separated by electrophoresis in a 15% polyacrylamide gel containing SDS and located autoradiographically. The tracks correspond to polypeptides of the following sizes: 1, M_r 190 000; 2, 100 000 (E1); 3, 83 000 (E2 minor component); 4, 80 000 (E2 major component); 5, 56 500 (E3). The positions of the untreated polypeptides in the same gel are indicated by the horizontal bars.

for the greater part of the newly synthesized protein. These findings were confirmed by parallel cumulative labelling studies, which showed a continuous increase in the amounts of the three gene products up to 30 min after infection. In addition to the heterogeneous E2 region and its presumptive degradation products, a very large polypeptide (M_r 190 000) was observed. From its size this could conceivably represent an E1–E2 fusion product translated from the *aceE*–*aceF* messenger. This possibility was explored by subjecting the excised band to limited proteolysis with *S. aureus* V8 protease followed by electrophoresis and autoradiography (Fig. 7). Well-separated samples of the other major components were analysed by the same method. The major components generated characteristic proteolytic fragmentation patterns which were identical to those obtained previously with stained preparations of the E1, E2 and E3 components of the *E. coli* pyruvate dehydrogenase complex (Jeyaseelan *et al.*, 1980; Jeyaseelan, 1980). The two main forms of the E2 component (M_r 80 000 and 83 000) were indistinguishable, indicating that they have a common origin. The very large polypeptide (M_r 190 000) seemed to be rather resistant to proteolysis and the few fragments which were detected resembled the products of E1 digestion but not E2. This does not totally exclude the presence of E2 sequences but it does indicate that the M_r 190 000 material contains E1 sequences. This material could arise by aggregation of the polypeptides of a dimer followed by incomplete resolution in the denaturing conditions used for preparing samples for SDS–polyacrylamide gel electrophoresis. In this connection, even larger molecules (M_r 270 000 and 310 000) were occasionally observed. These could represent higher aggregates of E1 components with or without other components of the

multienzyme complex, detectable due to the high sensitivity of the autoradiographic method. No precursor-product relationships between any of the E2 components or for the very large polypeptides were apparent from the labelling studies.

DISCUSSION

The existence of λ transducing phages containing the pyruvate dehydrogenase complex genes provided an excellent opportunity for studying *ace* and *lpd* gene expression over short periods of time in vivo. Using appropriate phages, the *aceE*, *aceF* and *lpd* gene products were identifiable as labelled polypeptides. Their synthesis in a λ -immune host indicated that these genes are accompanied by their own promoters, one for the two *ace* genes and another for the *lpd* gene. This confirmed earlier observations on the expression of the *ace* gene (Henning *et al.*, 1966) and the independence of the *lpd* gene (Langley & Guest, 1978; Guest & Stephens, 1980). By analysing the early- and late-labelled polypeptides synthesized after infecting a non-lysogenic host, the polarities of expression of the cloned genes could be defined relative to the phage promoter. Thus the *ace* and *lpd* genes cloned in the phages depicted in Fig. 1 were found to be expressed with *rightward* (G90 and G82L) and *leftward* (G83L, G93 and G105) orientation. This showed for the first time that the *lpd* gene is expressed with the same polarity as the *ace* operon (Fig. 8): clockwise with respect to the *E. coli* linkage map. Because they have the same polarity it is conceivable that transcription originating at P_{ace} could continue through the *lpd* gene without termination near P_{lpd} . If so, independent transcription from P_{lpd} may normally be important only for the synthesis of the 2-oxoglutarate dehydrogenase complex. However, this function would be essential when the *ace* genes are repressed. It is anticipated that the nature of the transcripts generated from this region will emerge during further studies of the expression of the cloned genes.

The results confirmed and extended previous correlations of the restriction and genetic maps (Fig. 8). The central *EcoRI* target is clearly situated in the *aceE* gene because the original λ *lpd* phages (G83L), containing the 8.2 kb *EcoRI* fragment, expressed an intact *aceF* product (the acetyltransferase component, E2) and a considerable portion of the *aceE* product, as well as lipoamide dehydrogenase, when coupled to a λ promoter. Furthermore, the 5.4 kb sub-fragment of phage G105 encoded a functional *lpd* gene. The λ *nadC* phages expressed only one detectable bacterial product (M_r 36500). This could be quinolinate phosphoribosyltransferase or the general aromatic amino acid permease, the only functions known to be cloned in these phages. The segment of DNA represented by the two *EcoRI* fragments (18.3 kb) encodes only five known genes and the four detectable products have an approximate coding requirement of only 8.5 kb. This considerable excess of coding capacity indicates that there are several undetected genes in this region or that it contains a significant proportion of unexpressed DNA. It is known that the entire *nadC*–*lpd* region can be deleted without loss of any essential functions other than the five genes already mentioned (Langley & Guest, 1978).

Quantitative densitometry of the labelled pyruvate dehydrogenase complex components, expressed from the λ -cloned genes in a lysogenic host, clearly indicates that the E1 and E2 components are synthesized in almost equal molar proportions whereas there is a molar excess of E3 components. Under these conditions the genes are expressed solely from their own promoters. Consequently, there would not seem to be any inherent modulation of E1 and E2 synthesis encoded in the *ace* operon. Complexed and uncomplexed components were not resolved in the present analysis but the results are consistent with the early 1:1 (E1:E2) stoichiometries reported for the isolated pyruvate dehydrogenase complex (Eley *et al.*, 1972) and the core complex (Vogel *et al.*, 1972). It would appear that the isolated complexes reported to contain a molar excess of E1 components could be due to post-translational events such as differential assembly or stability of the components or to some translational

Fig. 8. Diagram showing the transcriptional polarities of the pyruvate dehydrogenase complex genes relative to the genetic and physical maps of the *nadC*–*lpd* region (2.5 to 2.8 min). The diagram is drawn to scale; the positions of the *ace* and *lpd* genes relative to the restriction map are based on previous work (Guest & Stephens, 1980), present results and unpublished observations. The sizes of the *ace* and *lpd* genes are derived from the molecular weights of their products, and the corresponding promoters are denoted P_{ace} and P_{lpd} . The restriction targets for *Hind*III (▽) and *Eco*RI (▲) are indicated and the fragment sizes are in kilobase pairs (kb).

modulation or attenuation mechanism that could be geared to the physiological state of the organism.

It is not possible to relate the labelling of the E3 component to complex structures because two complexes are involved. However, the 1.4–2.3-fold excess of E3 components relative to the *ace* gene products confirms the presence of an *lpd* promoter that functions independently, at least when providing components for assembly into the 2-oxoglutarate dehydrogenase complex. Transcription of the *ace* and *lpd* genes in a non-lysogenic host can originate from both bacterial and phage promoters and the labelling patterns in the early phase of infection exhibited a 1:1:1 (E1:E2:E3) stoichiometry. Most of the newly synthesized protein was represented by the three components and it would appear that the results reflect the overriding influence of the powerful *leftward* phage promoter P_L . At a later stage, deviations from this stoichiometry were observed but these could be due to the temporal changes in phage-directed transcription causing differential effects on the progress of *ace* and *lpd* gene expression.

The ability to label the gene products with a relatively high specific radioactivity and then analyse the polypeptides of the whole cells within minutes of their synthesis led to the discovery of considerable heterogeneity amongst the polypeptides designated *aceF* products, as well as a very large polypeptide (M_r 190 000). The E2 component was almost invariably represented by two bands, M_r 80 000 and 83 000, and in some cases these were accompanied by a family of minor components of approximately M_r 86 000, 89 000 and 91 000. The double-banded nature of the E2 component has been observed with preparations of the isolated *E. coli* complex (unpublished observations) and with the corresponding complex of *Pseudomonas aeruginosa* (Jeyaseelan *et al.*, 1980). If it is not an artefact it may have some structural significance with respect to the assembly of the complex. The extended heterogeneity could also correspond to stages in the processing of a large primary gene product into the smaller polypeptides of the complex. This component is noted for its instability and it may be significant that using a faster purification, Gebhardt *et al.* (1978) have indicated that the native acetyltransferase is larger (M_r 89 000) than is generally reported but decays via a fragment of M_r 82 000. Unfortunately, no precursor–product relationships could be detected for the labelled polypeptides. The same was true for the M_r 190 000 polypeptide, which contained E1 sequences; its nature and origin remain obscure.

We are indebted to Dr N. E. Murray for helpful discussions and advice. This work was supported by the Science Research Council (GR/B35543) and K.J. acknowledges a postgraduate studentship from the Commonwealth Commission in the United Kingdom.

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