Molecular Cloning of the Pyruvate Dehydrogenase Complex Genes of *Escherichia coli*

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The three components of the pyruvate dehydrogenase complex of *Escherichia coli* are encoded by three linked genes, *aceE* (pyruvate dehydrogenase, E1), *aceF* (dihydrolipoamide acetyltransferase, E2) and *lpd* (lipoamide dehydrogenase, E3), situated close to the *nadC* (quinolinate phosphoribosyltransferase) and *aroP* (general aromatic amino acid permease) genes with the gene order: *nadC-aroP-aceE-aceF-lpd*. Several types of transducing phages, λ *nadC* and λ *lpd*, carrying the *nadC* and *lpd* genes were isolated from populations of artificially constructed transducing phages containing R.*Hind*III or R.*Eco*RI fragments of bacterial DNA, by selecting for their ability to complement the metabolic lesions of the corresponding mutants. The cloned fragments were extended to include a functional *ace* operon by *in vivo* methods involving prophage insertion into the *nadC-lpd* region and aberrant excision to yield λ *nadC-lpd* and λ *lpd-ace* phages. These contained overlapping segments of bacterial DNA capable of expressing the *aceE*, *aceF* and *lpd* genes. A physical map of a 20 kilobase pairs (kb) segment of bacterial DNA encoding the entire *nadC-lpd* region, bounded by R.*Hind*III and R.*Eco*RI targets and possessing several internal restriction targets, R.*Hind*III (3) and R.*Eco*RI (2), was constructed. Using a combination of nutritional and enzymological studies with dilyso gens and genetic analysis with *ace* mutants the approximate positions of the genes specifying the pyruvate dehydrogenase complex were traced to a 9.5 kb segment of the restriction map. The cloned *lpd* gene was expressed in the complete absence of a functional *ace* operon and when the major λ promoters were repressed. This confirms that the *lpd* gene can be independently transcribed from its own promoter.

INTRODUCTION

The pyruvate dehydrogenase complex of *Escherichia coli* catalyses the overall conversion of pyruvate to acetyl-CoA

through a series of enzyme-bound intermediates (Reed, 1974; Guest, 1978). It is a very large complex containing multiple copies of three different enzymic components: pyruvate dehydrogenase or decarboxylase (E1), dihydrolipoamide acetyltransferase (E2) and lipoamide dehydrogenase (E3). The molecular weights have been estimated as 96 000 to 100 000 (E1), 70 000 to 89 000 (E2) and 56 500 (E3) for the individual chains and up to 6.1×10^6 for the complex (Eley *et al.*, 1972; Vogel & Henning, 1971; Vogel *et al.*, 1971; Gebhardt *et al.*, 1978; Vogel, 1977; Danson *et al.*, 1979). There appears to be a disproportionate content of enzymic components in the native complex but the polypeptide stoichiometry, if constant, is still controversial. The structural genes, *aceE* and *aceF*, for the dehydrogenase and acetyltransferase components constitute an operon with *aceEF* polarity, very close to the *nadC* (quinolinate phosphoribosyltransferase) and *aroP* (general aromatic amino acid permease) genes at 2.6 min in the *E. coli* linkage map (Langley & Guest, 1977, 1978; Bachmann & Low,

1980). The lipoamide dehydrogenase structural gene, *lpd*, is linked to the *ace* genes thus: *nadC-aroP-aceE-aceF-lpd* (Guest, 1974). The amount of DNA required to code for single copies of each polypeptide component approximates to 6.5 kilobase pairs (kb) and this is consistent with the size of the *aceE-lpd* region that can be deduced from P1 cotransduction frequencies (Guest, 1974). The specific dehydrogenase (E1) and dihydrolipoamide succinyltransferase (E2) components of the analogous 2-oxoglutarate dehydrogenase are encoded by the *sucA* and *sucB* structural genes, which constitute an operon with the same functional polarity (*sucAB*) at 16.4 min in the *E. coli* linkage map (Guest, 1978). The lipoamide dehydrogenase component of the 2-oxoglutarate dehydrogenase complex is encoded by the same *ace*-linked *lpd* gene and this poses the problem of how the expression of a single *lpd* gene is controlled and geared to the expression of the *ace* and *suc* operons. Biochemical studies with polar and deletion mutants indicate that the *ace*, *suc* and *lpd* genes are independently regulated, although expression of the *lpd* gene is normally coupled to the synthesis of both complexes (Langley & Guest, 1977, 1978). This suggests that the *lpd* gene has its own promoter and regulatory elements and, in consequence, need not be expressed with the same polarity as the closely linked *ace* genes. An autoregulatory mechanism based on the dehydrogenase (E1) and uncomplexed lipoamide dehydrogenase (E3) components functioning as negatively acting repressors at the corresponding operator sites has been proposed (Guest, 1978). Other outstanding problems associated with the two complexes include: the biochemical basis for the reaction specificities of the components, the subunit interactions involved in the assembly of each complex and the evolutionary relationships between the corresponding E1 and E2 components and between different regions of the dihydrolipoamide acetyltransferase chain. The emergence of *in vitro* recombination methods and DNA-sequencing techniques offers new approaches for their investigation as well as opportunities for the rapid solution of some of these problems. With this aim in view the cloning of the genes encoding the pyruvate dehydrogenase complex of *E. coli* in lambda vectors, using a combination of *in vivo* and *in vitro* methods, is reported.

METHODS

Bacterial strains. The characteristics and sources of the strains of *Escherichia coli* are listed in Table 1. Most of these have been described previously: *ace* mutants and deletion strains by Langley & Guest (1977) and *lpd* mutants by Guest & Creaghan (1973). The parental strains H, K1-1 and W1485E were used as controls in the enzymological studies.

Bacteriophages. The characteristics of the three vector phages are shown in Fig. 1. Phage NM540 (*srI* λ 1-2 Δ *shn* λ 3⁺ *att*⁺ *imm*²¹ *nin*5 *shn* λ 6⁺) is an insertion vector for R.*Hind*III (Borck *et al.*, 1976). Phage NM761 (*srI* λ 1-2 Δ *shn* λ 3⁺ *att-red* Δ *imm*²¹ *nin*5 *shn* λ 6⁺) is a replacement vector for R.*Hind*III with a fragment of *E. coli* DNA containing the *supF* gene inserted at the *shn* λ 3 site; it is a derivative of NM742 (Murray *et al.*, 1977). Phage NM781 (*srI* λ 1-3 Δ *cI*857 *nin*5) is a replacement vector for R.*Eco*RI with a *supE*-containing fragment inserted between *srI* λ 1 and *srI* λ 3 (Murray *et al.*, 1977). Pools of recombinant phages containing the corresponding restriction fragments of *E. coli* CR63 DNA were kindly provided by Dr N. E. Murray. Other phages - λ *imm*⁴³⁴, λ *imm*²¹, λ *bio*1 (*int-red* Δ *imm*²¹), λ *b2c imm*²¹, λ *b2c imm* λ and λ *vir* - were used as hetero-immune helper phages, for curing and routine tests for immunity and phage-sensitivity.

Media and general methods. The rich medium was L broth (Lennox, 1955) with glucose (0.1 %, w/v) for routine subculture of *ace* and *lpd* mutants but without glucose for other organisms and for the preparation of phage stocks. Phage assays were made on BBL medium containing (g l⁻¹): BBL Trypticase, 10; NaCl, 5; Difco Bacto agar, 10 for plates and 6.5 for top layers.

The minimal media (E) with carbon sources D-glucose (10 mM), potassium acetate (50 mM) or potassium succinate (50 mM) used for nutritional tests and genetic selections have been described previously (Langley & Guest, 1977). Supplements were added as required: acetate (2 mM with glucose as substrate, or 4 mM with succinate), succinate (2 mM), L-amino acids (30 μ g ml⁻¹), thymine (50 μ g ml⁻¹), vitamins (10 μ g ml⁻¹). Selective media were sometimes enriched with Difco Bacto nutrient broth (0.2 %, v/v) or Difco Casamino acids (vitamin-free; 0.2 g l⁻¹).

The general methods of Borck *et al.* (1977) were used for the preparation of phage stocks, phage assays and other routine procedures. The nutritional characterization of mutants and their derivatives have been described previously, as have the methods for testing resistance to azide and aromatic amino acid analogues

Table 1. *Strains of Escherichia coli* K12

Strain	Relevant genotype	Source
H	<i>thi azi relA?</i> (Hfr)	W. Hayes
K1-1	<i>metB relA thyA tsx pps azi ton?</i>	H. L. Kornberg
W1485E	<i>supE iclR</i>	C. Yanofsky
C600	<i>thr leu thi supE tonA</i>	N. E. Murray
C600(P2)	<i>thr leu thi supE tonA</i> (P2)	P2 lysogen of C600
CR63	<i>supD</i> λ^R	N. E. Murray
KS302	(<i>gal-bio</i>) Δ	K. Shimada
T94A	<i>mutT1 bio-1 phe-1</i> (λ)	K. A. Stacey
JRG73	<i>aceE1</i>	Formerly W1485A1
JRG260	<i>aceE131</i>	Formerly WGAaceE131
JRG354	<i>lpd-7</i>	Formerly Hlpd7
JRG355	<i>lpd-8</i>	Formerly Hlpd8
JRG582	(<i>nadC-aroP</i>) Δ 2	Formerly H Δ 2
JRG590	(<i>nadC-aceF</i>) Δ 10	Formerly H Δ 10
JRG593	(<i>aroP-aceE</i>) Δ 13	Formerly H Δ 13
JRG599	(<i>aroP-lpd</i>) Δ 18	Formerly K Δ 18
JRG603	(<i>nadC-aceE</i>) Δ 22	Formerly K Δ 22
JRG613	(<i>nadC-aceE</i>) Δ 32	Formerly K Δ 32
JRG615	(<i>nadC-aceE</i>) Δ 34	Formerly K Δ 34
JRG616	(<i>nadC-aceE</i>) Δ 35	Formerly C Δ 35
JRG620	(<i>nadC-aceE</i>) Δ 39	Formerly C Δ 39
JRG1173	<i>mutT1</i>	λ^S derivative of T94A
JRG1174	<i>aceE2</i>	λ^S derivative of A2T3
JRG1175	<i>aceE6</i>	λ^S derivative of A6
JRG1176	<i>aceF10</i>	λ^S derivative of A10

(Langley & Guest, 1977, 1978). Mutator activity was tested by replica-plating or direct application of drops of stationary phase cultures on L agar containing rifampicin (100 $\mu\text{g ml}^{-1}$) or, better, streptomycin (100 $\mu\text{g ml}^{-1}$).

Transduction. Spot transductions were performed by placing serially diluted lysates on to plates of selective media with seeded overlayers (2.5 ml) containing 2×10^8 recipient bacteria and MgSO_4 (10 mM). Quantitative transductions were performed by incubating 2×10^8 recipient bacteria in 0.2 ml MgSO_4 (10 mM) with 0.1 ml of serially diluted phage lysate [containing 10^2 to 10^{10} plaque-forming units (p.f.u.)] for 15 min at 20 °C before plating as an overlay on appropriate selective media using soft water agar. The selective conditions were based on the nutritional characteristics of the recipients and have been described in detail previously (Langley & Guest, 1978). Incubation was at 30 or 37 °C depending on the phage, and transductants were scored daily for up to 5 d. The frequency of transduction was determined by comparison with the plaque-forming activity determined in parallel assays with *E. coli* C600 as indicator and BBL medium.

Phage lysates and DNA preparation. Phage preparations for DNA extraction were made by infecting exponentially growing cultures of *E. coli* C600 (200 ml) in L broth containing MgSO_4 (10 mM); lysis was completed with CHCl_3 (0.5 ml l^{-1}) and NaCl (40 g l^{-1}) was added. The crude lysate was then treated with RNAase and DNAase (1 $\mu\text{g ml}^{-1}$ each, 1 h at room temperature), clarified by centrifuging (15 min at 5000 g) and the phages were concentrated with polyethylene glycol 6000 according to Yamamoto *et al.* (1970). Purification was achieved by centrifuging the suspension (9 ml) into a discontinuous gradient (5 ml) of buffered CsCl solutions (densities 1.3, 1.5 and 1.7 g ml^{-1}) for 2 h at 33 000 rev. min^{-1} and 25 °C in the 6 $\times$ 14 ml rotor of a PrepSpin 50 centrifuge (MSE). The phage band was collected and dialysed against Tris/HCl (10 mM; pH 8.0) plus EDTA (1 mM) and the DNA was extracted using phenol and dialysed against the same buffer (Kaiser & Hogness, 1960).

Restriction endonuclease techniques and agarose gel electrophoresis. The methods used for DNA digestion with restriction endonucleases and the analysis of restriction fragments by electrophoresis in agarose gels have been described previously (Cole & Guest, 1980). Standards covering the range 23.7 to 0.59 kb, obtained by digesting lambda (*Ac1857*) DNA with *R.Hind*III, *R.Eco*RI and *R.Hind*III plus *R.Eco*RI, were included in several tracks on all gels to provide a calibration for fragment size relative to mobility. The sizes of the standard fragments are based on the lambda restriction map of P. Philippsen & R. W. Davis (personal communication, 1978). The enzymes *R.Hind*III, *R.Eco*RI, *R.Bam*HI, *R.Bgl*II, *R.Kpn*I and *R.Xho*I were kindly provided by Professor K. and Dr N. E. Murray, and *R.Sma*I (Boehringer), *R.Sst*I, *R.Sal*I and *R.Ava*I (Uniscience) were commercial samples.

Electron microscopy. Heteroduplex molecules were made and observed by the methods of Davis *et al.*

Fig. 1. Diagram illustrating the characteristics of the vector phages: NM540, *R.HindIII* insertion vector; NM761, *R.HindIII* replacement vector; NM781, *R.EcoRI* replacement vector. The map at the top shows some of the genetic markers in the λ genome. The filled arrows represent *R.EcoRI* targets, open arrows the *R.HindIII* targets; the cloning sites are marked *RI* and *HIII*, respectively. ● marks the position of the λ attachment site. Gaps indicate deletions, dotted lines denote the deletion associated with the substitution of the immunity region and the double lines identify the bacterial restriction fragments present in replacement vectors.

(1971) in solutions containing (final concentration) Tris/HCl (100 mM, pH 8.6), EDTA (10 mM), formamide (48 %, v/v) and cytochrome *c* (0.18 mg ml⁻¹). Molecules were mounted on parlodion films, stained with uranyl acetate, rotary shadowed with platinum and viewed in a Siemens Elmiskop 101 electron microscope at accelerating voltage of 80 kV and 20000 $\times$ magnification. The micrographs were enlarged photographically and contour lengths were determined with a map measurer.

Enzymology. Cultures were grown aerobically in glucose minimal medium supplemented with acetate and succinate, harvested and ultrasonic extracts were prepared for enzymology according to Langley & Guest (1977). The methods for assaying overall pyruvate dehydrogenase complex, pyruvate dehydrogenase (E1; EC 1.2.4.1) plus pyruvate oxidase (when present), dihydrolipoamide acetyltransferase (E2; EC 2.3.1.12) and lipoamide dehydrogenase (E3; EC 1.6.4.3) have also been described previously (Langley & Guest, 1977). Enzyme activities in ultrasonic extracts are expressed as μ mol substrate transformed (mg protein)⁻¹ h⁻¹.

RESULTS

Isolation of λ nadC and λ lpd transducing phages

Populations of artificially constructed recombinant phages made by the incorporation of *R.HindIII* and *R.EcoRI* fragments of *E. coli* DNA in the λ vectors NM540, NM761 and NM781 (Fig. 1) were screened for transducing phages which complemented the metabolic lesions of *nadC*, *aceE*, *aceF* and *lpd* mutants using appropriate nutritional selections. This multi-faceted approach was adopted because the estimated size for the coding sequence of the pyruvate dehydrogenase complex, based on the sizes of its three components, is large (6.5 kb approx.). It was reasoned that the complete sequence was likely to be disrupted by the presence of internal targets for the two restriction endonucleases, whereas the individual

genes, or the neighbouring *nadC* gene, may be released intact. If so, the combination of *in vitro* methods plus *in vivo* extension of a cloned segment could be used to isolate the entire *ace-lpd* region. Accordingly, several mutants deleted for the *nadC-aroP* ($\Delta 2$), *nadC-aceE* ($\Delta 22$), *nadC-aceF* ($\Delta 10$) and *aroP-lpd* ($\Delta 18$) regions as well as *aceE* (JRG73) and *lpd* (JRG354, JRG355) point mutants were used as recipients. No transduction of the *ace* genes was detected with any of the three populations of recombinant phages, but they all transduced the *nadC* gene and lysates derived from the R.*EcoRI* vector (NM781) transduced the *lpd* gene. Representative λ *nadC* and λ *lpd* phages were isolated from transduction plaques or, in the case of λ *nadC* derivatives of NM540, induced from transductant colonies. Those to be described include (with the corresponding vector): λ *nadC* – G75N (NM540), G76N (NM761) and G78N (NM781) – and λ *lpd* – G81L, G82L and G83L (all NM781 derivatives) – where N and L denote their initial selection as λ *nadC* and λ *lpd* transducing phages, respectively. Two distinct and independent types of λ *lpd* phages were detected on the basis of the rate at which the transductant plaques developed. With phages represented by G81L and G83L the initial size of the transductant plaque was reproducibly greater than with those of the G82L type. Preliminary genetic studies using a variety of recipients deleted in the *nadC-lpd* region and selecting for *Nad*⁺ or *Lpd*⁺ transductants indicated that all the λ *nadC* phages, but none of the λ *lpd* phages, expressed the wild-type *aroP* gene. This was confirmed with lysogens and dilysogens (with λ *imm*⁴³⁴) which had been isolated without any nutritional selection. Likewise, it appeared that none of the λ *nadC* or λ *lpd* phages expressed functional *aceE*⁺ or *aceF*⁺ genes capable of complementing the nutritional lesions of the corresponding mutants. This indicated that the *ace* region probably contains restriction targets for both R.*EcoRI* and R.*HindIII*.

Isolation of λ nadC and λ lpd phages with extended transducing ranges

Plaque-forming phages containing the entire *ace-lpd* region were selected after *in vivo* extension of the cloned *nadC-aroP* and *lpd* fragments in opposite directions to incorporate the *ace* genes and generate phages with overlapping sequences in the *aroP-aceE-aceF-lpd* region. The λ *nadC* and λ *lpd* phages were established as prophages by recombination close to, or within, the host *nadC* and *lpd* genes, and λ *nadC-lpd* and λ *ace-lpd* phages, generated by excision errors, were selected following induction of the respective prophages (see examples in Fig. 2). All the starting phages except G75N are integration-deficient so that lysogeny by recombination in the region of shared chromosomal homology was favoured. In addition, a host (KS302) lacking the normal lambda attachment site was used in order to increase the probability of the same type of integration occurring with G75N. The lysates obtained by u.v.-irradiation or thermal induction (NM781 derivatives only) of the lysogens were used to transduce several *lpd* and *ace* mutant recipients with appropriate nutritional selections. The frequencies of *Lpd*⁺ and *Ace*⁺ transduction were about 10⁻⁷ transductants per p.f.u. All types of transductant were included: colonies, clusters of colonies (galaxies) and transductant plaques.

The lysogens should contain the lambda genome incorporated in one of two orientations dictated by the orientation of the cloned segment of bacterial DNA within the phages. Furthermore, aberrant excisions should extend the segments of bacterial DNA to incorporate the *ace-lpd* region or the *aceE,F* genes with alterations of the phage genotype which are diagnostic of the orientations of both the prophages and the cloned DNAs relative to the bacterial chromosome. Thus, extensions in one direction are likely to generate defective transducing phages as a consequence of deleting essential phage genes in the left arm (e.g. *J*). By contrast, extensions in the opposite direction can generate transducing phages which lack the *red* and *gam* genes and these are easily recognized by their plating ability on P2 lysogens, the *Spi*⁻ phenotype (Zissler *et al.*, 1971). In order to investigate the orientations of the cloned segments the sub-populations of *Spi*⁻ phages in the induced lysates were enriched

Fig. 2. Diagrammatic representation of the extension of the transducing ranges of λ *nadC* and λ *lpd* phages by integration in the region of bacterial DNA homology and aberrant excision of the transducing prophages. The orientations of the inserted genes (in boxes) correspond to the situations presented by G78N (λ *nadC*) and G81L (λ *lpd*) based on the gene order in the bacterial chromosome (thick line) and the enrichment of the respective phage derivatives (G90 and G91–G94) by Spi^- selection.

on lawns of *E. coli* C600(P2) to see whether or not their transducing activities were simultaneously enriched. The frequencies of Lpd^+ transduction were increased 500-fold (G78N) and 5000-fold (G75N and G76N) after Spi^- enrichment indicating that bacterial genes are inserted with a clockwise orientation relative to the vegetative phage: λ *nadC*–*lpd* ($\lambda A \cdots J \cdot \textit{nadC} - \textit{ace} - \textit{lpd} \cdots R$). Likewise, the frequency of Ace^+ transduction was increased 400-fold after Spi^- enrichment of the G81L-derived lysate and this is diagnostic of an anti-clockwise orientation of the bacterial genes: λ *lpd*–*ace* ($\lambda A \cdots J \cdot \textit{lpd} - \textit{aceF} - \textit{aceE} \cdots R$). The origin of these phages from G78N and G81L is shown diagrammatically in Fig. 2. By contrast, no increase in Ace^+ transduction was observed after Spi^- selection of the phages induced from the lysogen of G82L. This suggests that the *lpd* region of G82L is inserted with the opposite polarity to that in G81L. In fact, no plaque-forming λ *ace*–*lpd* phages were ever obtained by extending the G82L phage. Presumably the lysates contained defective Ace^+ transducing phages (λ *dace*–*lpd*) and this would be consistent with the predicted orientation of the *lpd* region in G82L.

Plaque-forming phages with extended transducing ranges were purified from the Spi^- -enriched lysates and representative phages were selected for further studies. The λ *nadC*–*lpd* phages were G87 (from G75N), G88 (from G76N) and G90 (from G78N). Of these G87 and G88 formed clear plaques and needed a helper phage (usually NM540) for successful transduction. Phage G90 was less defective, it produced speckled plaques and did not need a helper phage for transduction. Several λ *lpd*–*ace* phages, G91 to G94, all retained the temperature-sensitive immunity of G81L but transduction of some recipients was improved by presence of a helper (λ^+).

Fig. 3. Physical map of the *nadC-lpd* region of *Escherichia coli* deduced from the structures of the genomes of the transducing phages used in this investigation; drawn to scale. Vector DNA is indicated by single lines with the arrowheads pointing leftwards towards the head gene region of the phage DNA. Bacterial DNA is indicated by the double lines and the dashed lines denote regions where the transition from bacterial to phage DNA is uncertain. ● indicates the phage attachment site. The segments of bacterial DNA have been aligned and, where necessary, the orientation of the vector arms have been reversed. R.*Eco*RI targets are denoted by filled arrows and R.*Hind*III targets by open arrows; the numbers refer to fragment lengths in kilobase pairs (kb). Approximate positions of the genes in the bacterial chromosome (2.4 to 2.8 min) are indicated in the segment of genetic map at the bottom.

Restriction analysis of λ *nadC*, λ *lpd* and derivative phages

DNA from each of the 13 phages and the three vector phages was digested with restriction endonucleases R.*Hind*III and R.*Eco*RI, both separately and in combination. The resulting fragments were separated by electrophoresis in agarose gel and calibrated using standard digests of lambda (λ cI857) DNA. A physical map of the *nadC-lpd* region of the *E. coli* chromosome was then deduced from the restriction maps of the transducing phage genomes (Fig. 3).

The bacterial DNA in all three λ *nadC* phages was inserted with the same orientation. In the NM540 derivative (G75N) a fragment estimated at between 5.7 and 9.0 kb was fused to the left arm of the vector. The NM761 (G76N) and NM781 (G78N) derivatives contained overlapping R.*Hind*III (10.5 kb) and R.*Eco*RI (10.1 kb) fragments comprising a common segment of 9.0 kb adjacent to 1.8 kb and 1.2 kb sub-fragments, respectively (Fig. 3).

The λ *lpd* phages contained identical R.*Eco*RI insertions of 8.2 kb and sub-fragments of 0.9, 2.0 and 5.4 kb generated after further digestion with R.*Hind*III. However, single digestion with R.*Hind*III showed that the bacterial DNA is inserted with opposing polarities in G81L and G83L relative to G82L (Fig. 3). Since the isolation of these λ *lpd* transducing phages relied on the complementation of *lpd* mutants, expression of the cloned *lpd* must occur regardless of its orientation in the phage genome, but there must be some orientation-dependent effect on expression to account for the observed differences in transducing activity.

The three extended λ *nadC* phages (G87, G88 and G90; λ *nadC-lpd*) had gained the two R.*Hind*III targets responsible for the 0.9 and 2.0 kb sub-fragments of the λ *lpd* phages. In

Fig. 4. Scale map of the *nadC-lpd* region of the bacterial chromosome based on the fragmentation of λ *nadC* (G76N, G78N), λ *lpd* (G83L) and λ *nadC-lpd* (G88) transducing phages by the corresponding restriction endonucleases. The number of targets for each enzyme is shown in parentheses. The orientation (left to right) corresponds to a *nadC-lpd* (clockwise) orientation of the bacterial chromosome. The R.*HindIII* and R.*EcoRI* targets are indicated by open and filled arrows, respectively. The sizes are shown in kilobase pairs (kb) for key fragments (other fragments are listed in Table 2). The segments of bacterial DNA contained by specific phages are shown below the map.

each case the extensions had modified the structures of the right arms of the parental phages: *srI* λ 3 was deleted in G87 and the *srI* λ 3-4 (G87), *shn* λ 3-*srI* λ 4 (G88), and *srI* λ 3-*shn* λ 4 (G90) fragments were replaced by hybrid fragments containing bacterial and phage DNA. This confirms the conclusion based on the Spi⁻ selection, that incorporating the *lpd* gene is accompanied by an aberrant excision affecting the right arm. One fragment (2.1 kb) in all the R.*HindIII* digests was clearly identifiable as linking the double-digestion products (1.2 and 0.9 kb) of the NM781-derived λ *nadC* and λ *lpd* phages (G78N and G81L, G82L and G83L). This not only shows that the R.*EcoRI* fragments of the λ *nadC* and λ *lpd* phages are adjacent in the *E. coli* chromosome, it further defines their orientation relative to the *E. coli* linkage map because the *lpd* gene is located clockwise to *nadC* (Fig. 3). The λ *nadC-lpd* phages each contained hybrid fragments of 6.1 kb (G87), 5.2 kb (G88) and 6.8 kb (G90) in which the extended bacterial segment is fused to a segment of phage DNA to the left of the nearest vector target (*srI* λ 4, *srI* λ 4 and *shn* λ 4, respectively).

Studies with three λ *lpd-ace* phages (G91, G93 and G94) showed that each had incorporated an extra R.*HindIII* target 1.2 kb from the parental R.*EcoRI* target and hybrid fragments of 6.2 kb (G91), 5.5 kb (G93) and 4.2 kb (G94) between this new R.*HindIII* target and the R.*HindIII* target (*shn* λ 4) in the immunity region of G81L, the parental λ *lpd* (Fig. 3). This confirmed both the polarity of the extensions, originally predicted from the Spi⁻ selection, and the DNA structure between *nadC* and *lpd*.

The fragments generated by R.*EcoRI* and R.*HindIII*, separately and in combination, unambiguously define the organization and orientation of DNA containing the *nadC*, *aroP*, *aceE*, *aceF* and *lpd* genes of *E. coli* (Fig. 3).

Table 2. Restriction fragments derived from the *nadC-lpd* region of the *E. coli* chromosome

DNA fragments of bacterial origin were generated by digesting four transducing phages (G76N, G78N, G83L and G88) with the indicated enzyme alone or in conjunction with *R.HindIII* or *R.EcoRI*. A consistent and unambiguous map based on the fragmentation patterns is shown in Fig. 4. The fragments are listed according to their terminal sequences, and in order to identify the positions of double-digestion products in the physical map, fragments possessing targets for the variable enzyme at the left-hand extremity are shown in italics. No targets for *R.XhoI* or *R.SalI* were detected. Average values derived from several independent determinations are given in kilobase pairs (kb). Fragments of less than 0.3 kb were not detected but their existence can be predicted and this is indicated by an asterisk. Fragments overlapping bacterial and vector DNA are not included.

Restriction endonuclease	No. of targets	Fragments of bacterial origin (kb) generated by the specified restriction endonuclease		
		Alone	+ <i>R.HindIII</i>	+ <i>R.EcoRI</i>
<i>R.SstI</i>	1	—	8.1, 2.7	9.2, 0.98
<i>R.KpnI</i>	2	11.85	10.7, 1.95, 0.5, *	7.6, 1.31, 0.98
<i>R.BamHI</i>	2	6.8	6.3, 4.7, 1.35, 0.76	2.9, 0.51
<i>R.BglII</i>	4	8.7, 1.5, 0.4	7.2, 2.02, 1.93, 1.82, 0.32, *	8.0, 7.1, 0.52, *
<i>R.SmaI</i>	3	8.9, 1.27	1.82, 1.12, 0.95, *	8.8, *, *
<i>R.AvaI</i>	4	6.4, 2.65, 1.27	1.82, 1.08, 0.95, *	6.3, *, *

A detailed physical map of the *nadC-lpd* region of the bacterial chromosome was constructed by analysing the cleavage fragments obtained by digesting transducing phage DNAs using restriction endonucleases *BamHI*, *BglII*, *KpnI*, *SalI*, *XhoI*, *SstI*, *AvaI*, *SmaI* with and without *HindIII*, *EcoRI* and *HindIII* plus *EcoRI*. Four phages were used: three recombinant phages (G76N, G78N and G83L) and one derivative (G88, λ *nadC-lpd*) to provide segments of DNA overlapping the *R.EcoRI* and *R.HindIII* fragments cloned in recombinant phages. No targets for *R.SalI* and *R.XhoI* were detected but targets for the other enzymes were located at positions defined by the sizes of the digestion products (Table 2; Fig. 4). A completely self-consistent and unambiguous order of restriction targets was established. Half of the restriction targets were clustered in a 2.5 kb region containing the 2.1 kb *R.HindIII* fragment, but no targets were found in the 5.4 kb *R.HindIII* plus *R.EcoRI* double-digestion product of the λ *lpd* phages.

Electron microscopic heteroduplex studies

The opposing polarities of the *lpd* regions in G82L and G83L were confirmed by electron microscopic analysis of heteroduplex molecules (Fig. 5a). The regions of single-strand non-homology were the same length and originated at the appropriate position in the phage genome. From photographs of 14 independent heteroduplex molecules, each accompanied by at least one ϕ X174 marker, the region of non-homology was estimated as 7.7 ± 0.42 kb flanked by homologous regions of 21.9 ± 0.5 and 14.65 ± 0.61 kb. This is in reasonable agreement with values of 8.2 kb for the inserted DNA and 21.8 and 13.9 kb for the vector DNA, based on restriction analysis. The λ *nadC-lpd* and λ *lpd-ace* phages should possess a common segment of bacterial DNA containing the *aceE*, *aceF* and *lpd* genes. To locate this region and estimate its size, heteroduplex molecules of two phages (G88 and G94) with oppositely polarized inserts and differing immunity were examined (Fig. 5b, c). Most of the molecules contained a single and unequal region of non-homology (13.4 ± 0.6 and 18.6 ± 0.9 kb) flanked by homologous regions representing the left and right extremities of the lambda genome, 21.26 ± 0.76 and 7.04 ± 0.02 kb, respectively (Fig. 5b). No separate region corresponding to the different immunities was detected indicating that the segment of homology between the cloning site and the immunity region had been deleted, in one or

Fig. 5. Electron micrographs of heteroduplex molecules of lambda transducing phages containing oppositely polarized segments of bacterial DNA. The extremities of the left (A) and right (B) arms of the phage genomes are indicated. Phage $\phi X174$ replicative (5.386 kb) was included as a standard length marker. (a) Two λpd derivatives, G82L and G83L; (b) $\lambda nadC-lpd$ (G88) and $\lambda pd-ace$ (G94); (c) the same phages as in (b) with the ends identifiable as A, B ($\lambda nadC-lpd$, G88) and A', B' ($\lambda pd-ace$, G94).

Table 3. *Specific activities of the pyruvate dehydrogenase complex and components in dilysogenic derivatives of ace and lpd mutants*

Ultrasonic extracts of cultures grown on glucose-based media were prepared and assayed as described in Methods. The activities of the dilysogens are compared with the original mutant containing the helper prophage and its parental strain. The series of strains derived from K1-1 all lack pyruvate oxidase, which is present in the other strains and is active in the assay for the E1 component. Specific activities are expressed as μmol substrate transformed $(\text{mg protein})^{-1} \text{ h}^{-1}$. Abbreviations: pdhc, overall pyruvate dehydrogenase complex; E1, pyruvate dehydrogenase, plus oxidase when present; E2, dihydroliipoamide acetyltransferase; E3, liipoamide dehydrogenase.

Strain	Relevant genotype	Specific activity			
		pdhc	E1	E2	E3
K1-1	<i>ace</i> ⁺ <i>lpd</i> ⁺	3.37	1.51	6.69	2.51
JRG599(λi^{434})	<i>(ace-lpd)</i> $\Delta 18$	0.02	0.02	<0.01	0.02
JRG599(λi^{434} λ G82L)	<i>(ace-lpd)</i> $\Delta 18$, λlpd ⁺	0.07	0.02	<0.01	2.57
JRG599(λi^{434} λ G83L)	<i>(ace-lpd)</i> $\Delta 18$, λlpd ⁺	0.04	0.02	<0.01	2.38
JRG599(λi^{434} λ G93)	<i>(ace-lpd)</i> $\Delta 18$, λlpd - <i>ace</i> ⁺	2.23	0.88	4.62	1.58
JRG599(λi^{434} λ G78N)	<i>(ace-lpd)</i> $\Delta 18$, $\lambda nadC$ ⁺	0.03	0.03	<0.01	0.02
JRG599(λi^{434} λ G90)	<i>(ace-lpd)</i> $\Delta 18$, $\lambda nadC$ - <i>lpd</i> ⁺	1.76	1.24	4.50	1.57
W1485E	<i>ace</i> ⁺	2.88	1.82	5.78	1.82
JRG73(λi^{434})	<i>aceE1</i>	0.03	0.80	11.06	1.50
JRG73(λi^{434} λ G82L)	<i>aceE1</i> , λlpd ⁺	0.14	1.30	7.90	1.69
JRG73(λi^{434} λ G83L)	<i>aceE1</i> , λlpd ⁺	0.10	1.26	8.00	2.18
JRG73(λi^{434} λ G93)	<i>aceE1</i> , λlpd - <i>ace</i> ⁺	1.83	1.88	8.88	1.58
JRG73(λi^{434} λ G78N)	<i>aceE1</i> , $\lambda nadC$ ⁺	0.02	0.80	9.33	1.68
JRG73(λi^{434} λ G90)	<i>aceE1</i> , $\lambda nadC$ - <i>lpd</i> ⁺	3.03	1.46	6.52	1.65
H	<i>lpd</i> ⁺	5.66	1.56	8.23	1.81
JRG355(λi^{434})	<i>lpd</i> -8	0.07	0.80	10.98	0.07
JRG355(λi^{434} λ G82L)	<i>lpd</i> -8, λlpd ⁺	2.24	3.66	7.65	1.76
JRG355(λi^{434} λ G83L)	<i>lpd</i> -8, λlpd ⁺	2.16	1.67	6.93	1.91
JRG355(λi^{434} λ G93)	<i>lpd</i> -8, λlpd - <i>ace</i> ⁺	2.06	3.87	8.76	2.06
JRG355(λi^{434} λ G78N)	<i>lpd</i> -8, $\lambda nadC$ ⁺	0.08	1.26	5.77	0.06
JRG355(λi^{434} λ G90)	<i>lpd</i> -8, $\lambda nadC$ - <i>lpd</i> ⁺	2.06	3.14	8.38	2.26

both phages, during extension of the incorporated bacterial DNA. The larger of the two single-stranded loops probably contains the *nadC-lpd* region of G88. A few of the molecules were paired so that the shared segment of bacterial DNA formed a duplex flanked by single-stranded ends containing bacterial and vector DNA (Fig. 5c). The size of the common region, estimated from 10 independent molecules, was 11.35 ± 0.34 kb and the flanking regions were 28.4 ± 0.66 and 8.0 ± 0.34 kb (A, B; G88) and 22.42 ± 0.64 and 9.25 ± 0.23 kb (A', B'; G94). This confirms that the phages containing oppositely polarized extensions share a continuous segment of homologous DNA overlapping in the *aceE-aceF-lpd* region. However, this segment is still about 4.8 kb larger than the size estimated for the coding sequence of the pyruvate dehydrogenase complex.

Enzymological studies with dilysogens

The transducing phages were isolated by their ability to complement the nutritional deficiencies of *lpd*, *aceE*, *aceF* and *nadC* mutants. To establish whether the *ace* and *lpd* genes are expressed from bacterial promoters, the nutritional and enzymological properties of a series of mutant and lysogenic strains were investigated. In the prophage state, transcription of the cloned genes is entirely dependent on bacterial promoters because initiation from phage promoters is repressed. Several mutants, JRG599 (*aceE-lpd*) $\Delta 18$, JRG73 (*aceE1*) and JRG355 (*lpd*-8), were infected with representative λimm^{λ} phages, G82L (λlpd), G83L (λlpd), G93 (λlpd -*ace*), G78N ($\lambda nadC$) and G90 ($\lambda nadC$ -*lpd*) together with an integration-proficient helper (λimm^{434}). The products with dual-immunity were expected to be dilysogens in which

the helper and transducing phages were integrated in tandem within the attachment site in the bacterial chromosome. This outcome was confirmed by showing that, in each case, both of the resident prophages were cured by superinfection with a heteroimmune phage (λimm^{21}) but not with an excision-defective heteroimmune phage ($\lambda bio1 int-red_{\Delta} imm^{21}$). Growth tests were performed on appropriately supplemented minimal media with glucose, succinate and acetate as the major substrates. All the dilysogens carrying G90 and G93 possessed a wild-type nutritional phenotype, as did the dilysogenic derivatives of the *lpd-8* strain carrying λpd prophages (G82L and G83L). The latter prophages enabled the deletion strain (*ace-lpd*) $\Delta 18$ to use acetate as substrate but growth on glucose and succinate still required supplementary acetate (i.e. only the Lpd⁻ component of the combined Ace⁻Lpd⁻ phenotype was reversed). None of the other combinations, including G78N and λimm^{434} alone, had any effect on the nutritional phenotypes of the mutants. In every case curing was accompanied by the restoration of the original mutant phenotype.

Enzymological studies with dilysogenic derivatives of the deletion strain (JRG599, $\Delta 18$) established that the pyruvate dehydrogenase complex and its three component activities are restored in the presence of G90 ($\lambda nadC-lpd$) and G93 ($\lambda pd-ace$) prophages (Table 3). In the same host only the lipoamide dehydrogenase (E3) component was expressed from the λpd prophages, G82L and G83L, and no significant orientation-dependent difference was detectable with these phages established as prophages. As expected, no functional components of the pyruvate dehydrogenase complex were expressed from the $\lambda nadC$ prophage (G78N). Similar studies with dilysogenic derivatives of the *aceE1* (JRG73) and *lpd-8* (JRG355) mutants confirmed that only the *lpd* gene is expressed from the λpd prophages, whereas the *aceE* and *lpd* genes are expressed from the $\lambda nadC-lpd$ and $\lambda pd-ace$ prophages (Table 3). No significant gene dosage effects were observed in the dilysogens which are partial diploids.

The results provide conclusive evidence that the intact *lpd* gene has been cloned with its own promoter and that it can be expressed independently of the *ace* operon. They show that the intact *aceE* and *aceF* genes together with their promoter are present in phages with extended transducing ranges (G90 and G93). It can also be deduced that the R.*EcoRI* target situated between the *nadC* and *lpd* genes resides within the *aceE* gene or the *ace* promoter and not the *aceF* gene. If it were in the *aceF* gene or outside the *ace* operon then the pyruvate dehydrogenase component (*aceE* gene product) should have been expressed from the $\lambda nadC$ or λpd prophages, respectively. It follows that the λpd phages contain an intact *aceF* gene which is not functional because it is divorced from the *ace* promoter.

Genetic studies

The total amount of bacterial DNA cloned in the $\lambda nadC$ and λpd phages is 20 kb and this could code for several genes in addition to the *nadC*, *aroP*, *aceE*, *aceF* and *lpd* genes. However, only two genes, *azi* and *mutT*, are known to be within reasonable proximity of the *nadC* gene (Fig. 3; Bachmann & Low, 1980).

Linkage tests with all of 100 NadC⁺ transductants of the deletion strain JRG582 (*azi*, *nadC-aroP* Δ) obtained with each of the $\lambda nadC$ transducing phages (G75N, G76N and G78N) retained the Azi^R phenotype of the recipient, suggesting that the wild-type *azi* gene is not encoded by these phages.

The *mutT1* mutation is known to be recessive (Cox & Yanofsky, 1969). However, the mutant phenotype (high frequency of spontaneous mutation to streptomycin resistance and rifampicin resistance) was unaffected by the presence of any of the $\lambda nadC$ prophages. It is concluded that the $\lambda nadC$ phages do not contain a functional *mutT* gene, nor the *azi* gene, because *mutT* is located between *azi* and *nadC*. In this context, nine strains with deletions extending anticlockwise beyond the *nadC* gene ($\Delta 3$, $\Delta 10$, $\Delta 17$, $\Delta 22$, $\Delta 27$, $\Delta 32$, $\Delta 35$, $\Delta 39$ and $\Delta 42$; Langley & Guest, 1978) were tested for increased rates of spontaneous mutation.

Table 4. Mapping studies with transducing phages and *ace* mutants

Ace⁺ transductants were selected on enriched succinate minimal media after infecting recipient bacteria (2×10^8) with serial dilutions of the transducing phage lysates starting at a multiplicity of infection of 2. Transduction frequencies are expressed as numbers of *Ace*⁺ colonies per 10^6 phages added and the values obtained without phages (reversion frequencies) are expressed as numbers per 10^6 recipient bacteria.

Recipient	Genotype	Frequency of <i>Ace</i> ⁺ transduction with phage:					
		G76N	G78N	G82L	G83L	G93	None
JRG260	<i>aceE131</i>	<0.20	200	<0.20	<0.20	1800	0.15
JRG1174	<i>aceE2</i>	<0.20	250	<0.20	<0.20	1300	0.04
JRG1175	<i>aceE6</i>	<0.20	<0.20	37	52	770	0.04
JRG73	<i>aceE1</i>	<0.01	<0.01	36	15	300	0.12
JRG1176	<i>aceF10</i>	<0.20	<0.20	850	1800	1900	0.03
JRG620	(<i>nad-aceE</i>) Δ 39	<0.05	54	<0.03	<0.02	27	0.05
JRG616	(<i>nad-aceE</i>) Δ 35	<0.05	58	<0.03	<0.02	90	0.18
JRG613	(<i>nad-aceE</i>) Δ 32	<0.01	120	<0.01	<0.01	110	<0.01
JRG593	(<i>aroP-aceE</i>) Δ 13	<0.01	3	<0.01	<0.01	53	0.05
JRG615	(<i>nad-aceE</i>) Δ 34	<0.01	<0.01	<0.01	<0.01	19	<0.01
JRG603	(<i>nad-aceE</i>) Δ 22	<0.01	<0.01	<0.01	<0.01	16	<0.01
JRG590	(<i>nad-aceF</i>) Δ 10	<0.01	<0.01	<0.01	<0.01	120	<0.01
JRG599	(<i>aroP-lpd</i>) Δ 18	<0.01	<0.01	<0.01	<0.01	120	<0.01

Such a phenotype could arise by deletion of the *mutT* gene but no evidence supporting this possibility was obtained with any of the strains.

The *aroP* gene codes for the general aromatic amino acid permease, it is situated between *nadC* and *aceE* and it is inactivated by total or partial deletion in strains JRG590 (Δ 10; *nadC-aceF* Δ) and JRG599 (Δ 18; *aroP-lpd* Δ). The growth of strains lacking the *aroP* function is resistant to the inhibitory analogues 5-methyltryptophan and β -thienylalanine. All the transducing phages were investigated for the presence of a functional *aroP* gene by several methods. These showed that all of 500 *NadC*⁺ transductants from the Δ 10 strain obtained with six λ *nadC* and λ *nadC-lpd* phages had acquired the wild-type phenotype indicating that these phages carry the *aroP* gene. Comparable tests with *Lpd*⁺ transductants of the Δ 18 strain indicated that neither the λ *lpd* phages nor the four λ *lpd-ace* phages (G90 to G94) carry a functional *aroP* gene. Further tests with a series of lysogenic and dilyogenic (plus *limm*⁴³⁴) derivatives of both the Δ 10 and Δ 18 strains confirmed that the intact *aroP* gene is only present in the six phages that carry *nadC*.

Genetic tests were also performed in order to correlate the physical and genetic maps of the *nadC-lpd* region, particularly with respect to the position of the *ace* operon (Fig. 3). The *ace* genes appear to be situated in the segment containing the three *R.HindIII* targets and the single *R.EcoRI* target. Representative transducing phages carrying bacterial DNA terminated by two of the critical restriction targets – *R.HindIII* (G76N, λ *nadC*) and *R.EcoRI* (G78N, λ *nadC*; G82L and G83L, λ *lpd*) – were used to transduce a collection of genetically defined *ace* point and deletion mutants (Table 4). Phage G93 (λ *lpd-ace*) was used as a positive control and acetate-independent (*Ace*⁺) transductants were selected. The failure of phage G76N to transduce any *ace* mutants indicated that the corresponding *R.HindIII* target is probably located outside and to the left of the *ace* operon. The other λ *nadC* phage (G78N) produced *Ace*⁺ transductants with *aceE* mutants possessing lesions in, or extending into, the promoter-proximal end of the *aceE* gene (Table 4). The same mutants were not transduced by the λ *lpd* phages (G82L, G83L), nor were any of the deletion mutants, because their deletions all enter the *ace* operon from the proximal end. However, *aceE* and *aceF* point mutants with distal lesions were transduced (Table 4). The corresponding *R.EcoRI* target would thus appear to be located in the *aceE* gene between the *aceE2*

Fig. 6. Correlation of the physical and genetic maps in the *ace* region (not to scale). The positions of the fragments of bacterial DNA included in the transducing phages relative to the genetic map of the *ace-lpd* region of *E. coli* (Langley & Guest, 1978) are based on the transduction frequencies obtained with *ace* point and deletion mutants (Table 4). The approximate positions of point mutations and deletion end-points are shown by vertical bars, the deleted regions are shown by dotted lines. The vertical zig-zag lines denote possible extremities of the corresponding structural genes. The data locate one target for *R.Hind*III very close to the operator proximal end of the *aceE* gene and a target for *R.Eco*RI in the *aceE* gene between the *aceE2* mutation and the end of deletion $\Delta 34$.

mutation and the end of deletion $\Delta 34$. The results are consistent with the marker order defined previously by P1-transduction (Langley & Guest, 1978).

The segments of bacterial DNA contained by some of the transducing phages and the correlation between physical and genetic maps at two of the restriction targets are shown in Fig. 6.

DISCUSSION

Biochemical, genetic and physical evidence is presented for the incorporation of the genes coding for the pyruvate dehydrogenase complex of *E. coli* in λ vectors. Consistent with the large size of the polypeptide components and the corresponding coding sequence (approximately 6.5 kb), the genes were found to be distributed between two types of phages, λ_{nadC} and λ_{lpd} . In fact, two large *R.Eco*RI fragments or at least three *R.Hind*III fragments, containing a common segment of 9.5 kb, were needed to express a functional complex. The results suggest that the *aceE* and *aceF* genes each contain a *R.Hind*III target. Thus, the *aceE* gene should be encoded by the two small *R.Hind*III fragments (2.1 and 2.0 kb) with the *aceF* gene spanning the 2.0 kb fragment and the 5.4 kb double-digestion product (Fig. 3 and 6). It is probable that the 5.4 kb fragment contains a functional *lpd* gene and up to 3.0 kb of unrelated DNA. The *R.Eco*RI fragment (8.2 kb) cloned in the λ_{lpd} phages extended from a point in the *aceE* gene to a point beyond the *lpd* gene (Fig. 6). This fragment should contain an intact *aceF* gene that is presumably not expressed because it is a distal gene divorced from its promoter. Nevertheless, the λ_{lpd} phages might have been expected to generate *Ace*⁺ transduction plaques at a high frequency with *aceE*⁺*aceF*⁻ recipients (e.g. the *aceF10* mutant) by expressing their *aceF* gene from a phage promoter and thus enabling all the components of a functional pyruvate dehydrogenase complex to be synthesized. The relatively low frequencies observed could indicate that successful expression of the *aceF* gene requires simultaneous transcription or translation of the proximal *aceE* gene. Alternatively, the frequencies of *Ace*⁺ transduction were equally low (<0.002 transductants per p.f.u.) with phages containing the entire *aceE-aceF-lpd* region so the results

may be a direct consequence of the specific selection procedures employed. By contrast, the *lpd* gene was cloned in the λ *lpd* phages in a form that is expressible in the prophage state. This confirms that the *lpd* gene is not an obligatory component of the *ace* operon but an independent gene that can be transcribed from its own promoter. The direction of *lpd* transcription was not investigated but the existence of λ *lpd* transducing phages with both orientations of the *lpd* gene should facilitate the identification of the coding strand.

The *nadC* and *aroP* genes were expressed by all of the λ *nadC* phages and this places them in the 8.9 kb double-digestion product that is common to these transducing phages (Fig. 3). These genes would not be expected to occupy more than 3 kb so the cloned region contains an additional coding capacity equivalent to approximately 7.5 kb of DNA with unidentified functions. The *azi* and *mutT* genes appeared not to be included in the cloned region.

It is anticipated that the existence of transducing phages containing the genetic determinants for the pyruvate dehydrogenase complex will provide the basis for a speedy solution of many of the outstanding problems connected with the genetic organization and expression of the *ace* and *lpd* genes as well as the primary structures and assembly of the gene products.

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