The *lysP* Gene Encodes the Lysine-Specific Permease

CHRIS STEFFES, JERI ELLIS, JIANHUA WU, AND BARRY P. ROSEN*

Department of Biochemistry, Wayne State University School of Medicine, Detroit, Michigan 48201

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*Corresponding author.

**METHODS**

**MATERIALS AND METHODS**

Strains, plasmids, phages, and media. The *E. coli* strains, phages, and plasmids used in this study are listed in Table 1. Except when noted, media were prepared according to Miller (16). YT medium consisted of 4% tryptone and 3% yeast extract (24). Antibiotics were each added at final concentrations of 40 μg/ml, except when otherwise noted. Plasmid DNA preparation, endo- and exonuclease digestions, ligations, and transformations were performed as described elsewhere (30). For selection of *Ts* strains, medium E was supplemented with 100 μg of thiostrepton per ml and a mixture of amino acids and vitamins, as described by Novick and Maas (18), except that 40 μg of arginine per ml was substituted for lysine. Cultures were incubated at 37°C, except as otherwise noted.

Isolation of *TnphoA* mutants. The procedure of Manoil and Beckwith (14) was used for *TnphoA* transposition. Transposon *TnphoA* was introduced into *E. coli* CC118 by infection with phage λ*TnphoA*. The cells were spread on lysine-free enriched medium containing thiostrepton, kanamycin, and 20 μg of XP (5-bromo-4-chloro-3-indolyl phosphate) per ml. Blue *Ts* colonies were analyzed for lysine transport activity.

Genetic mapping. Hfr mapping was performed as described elsewhere (19) with a collection of linked *Tn10* insertions in Hfr donor strains (33). P1 transduction was performed by using a set of linked *Tn10* insertions as donors (33) and a *lysP-phoA* strain as recipient. Transconjugants and transductants were selected for Sm* and *Ts* and screened for Km* and *Ts*.

Southern and dot blot hybridization. A 1.5-kb *BamHI*-*DraI* fragment containing the *lysP-phoA* fusion was radiolabeled with [α-32P]dATP by using a random primer kit from Bethesda Research Laboratories according to the manufacturer's instructions. Southern and dot blot hybridizations of λ phage DNA were performed with a Hybrid nylon membrane according to the manufacturer's protocol.

Cloning of the *lysP* gene. The *lysP-phoA* fusion was cloned by digesting total DNA from *E. coli* BPR2 with *BamHI* and ligation into the unique *BamHI* site of plasmid pBR322.
BamHI fragment digests of positive plasmids BamHI screened for the dGTP) were used for hybridization. Taq nase method of genetic on XP

Transformants were selected for Km' and screened for blue on XP plates. The resulting plasmid, pBBR2, contained 1.2 kb of E. coli DNA and 4.9 kb from TnphoA. From the results of genetic mapping, several λ clones containing inserts of E. coli chromosomal DNA in the region of 46.2 to 47.2 min were screened for the lysP gene by Southern and dot blot hybridization. Positive clones were digested with BamHI. The resulting mixture was ligated into the BamHI site of plasmid pUC19 and transformed in E. coli TG1. Plasmids with inserts were screened by dot blot hybridization with the radiolabeled lysP probe. Southern blot hybridization of BamHI digests of positive plasmids demonstrated the presence of a 2.7-kb BamHI fragment which hybridized to the probe. One plasmid, pLYSP, was selected for further study.

DNA sequencing. DNA sequencing was done by the dideoxyn method of Sanger et al. (31) with 32P-dATP and Sequenase (United States Biochemical Corp.). To resolve compressions, Taq polymerase and 7-deaza dGTP (in place of dGTP) were used according to the directions of the manufacturer (United States Biochemical Corp.). The 2.7-kb BamHI fragment was cloned into the BamHI site of M13mp18 (39) in both orientations. Exonuclease III was used to prepare deletion clones, with sequencing by using universal primer. Sequencing primers complementary to additional regions of lysP were synthesized as necessary. Computer analyses of DNA sequences were performed by the GENEPRO program (Riverside Scientific).

Transport assays. For routine transport assays, cells were isolated in the exponential phase of growth, washed three times with medium E lacking a carbon source, and suspended at 23°C at approximately 30 mg of cell protein per ml of the same medium. For induction studies, cells were grown in a medium consisting of 1% peptone, 0.6% yeast extract, and 1% glucose buffered to pH 5.5 with 50 mM 2-(N-morpholino)ethanesulfonate (MES), to pH 6.8 with 50 mM 3-(N-morpholino)propanesulfonate (MOPS), or to pH 8.0 with 50 mM Tris (2). The buffers were adjusted to the appropriate pH values with concentrated HCl or KOH, as required. As noted, the medium was supplemented with 0.5% lysine. The cells were grown by a 10-fold dilution of an overnight culture in medium either aerobically with shaking or anaerobically in filled tightly capped tubes without shaking. The cells were grown at 37°C for 2 h for aerobic cultures

<table>
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<tr>
<th>Strain, plasmid, or phage</th>
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<td>HB101</td>
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<tr>
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<td>K12 Δ(lac-pro) supE F' traD36 proAB lacI ΔlacZM15 araD139 Δ ara-araE17097 ΔlacX74 galE galK ΔphaO220 thi rpsE rpoB argE(Aam) recA1</td>
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<td>BPR2</td>
<td>CC118 with TnphoA insertion in lysP (Ts')</td>
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<tr>
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<td>F' supE supE hsdR galK trpR metB lacY</td>
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<td>Cloning vector (Cm' and Tc')</td>
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<td>pLYSP</td>
<td>pUC19 with 2.7-kb BamHI lysP insert</td>
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<td>T7 phage RNA polymerase gene under control of λP₇ promoter and λP₄ (Km')</td>
<td>Tabor and Richardson (36)</td>
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<td>Kohara et al. (12)</td>
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<td>Kohara et al. (12)</td>
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and for 3 to 4 h for anaerobic cultures and prepared for transport assays as described above.

Transport assays were performed as described previously (23) in 1 ml of medium E containing 0.2% glucose and washed cells (approximately 1 mg of cell protein). The assays were initiated by the addition of [3H]lysine or [3H]leucine to a final concentration of 10 μM. Portions were withdrawn at the indicated intervals, filtered on nitrocellulose filters (0.45 μm-pore size), and washed once with 5 ml of medium E lacking glucose. Radioactivity was determined by liquid scintillation counting.

Identification of the lysP gene product. The T7 expression system was used as described previously (36). Plasmid pBPR20 or pBPR30 was transformed into strain K38 (pGP1.2) containing the T7 RNA polymerase gene under the control of a temperature-sensitive repressor on plasmid pGP1.2. To enable labeling of cloned gene products, cells were grown at 30°C in Luria-Bertani medium to an optical density at 600 nm of 0.5. Cells from 1 ml of these cultures were washed four times each with 5 ml of M9 medium (16). The cells were suspended in 2 ml of M9 medium lacking methionine but supplemented with 0.4% glucose, 20 μg of thiamine per ml, and a mixture of 19 amino acids, each at a concentration of 50 μg/ml. This culture was incubated at 30°C for 60 min. The T7 RNA polymerase was induced by transferring the culture to 42°C for 15 min. Rifampin was added to 0.2 mg/ml to inhibit E. coli RNA polymerase, and the incubation was continued for 10 min. The temperature was then shifted to 37°C for 60 min. The cells were labeled with 20 μCi of [35S]methionine (1,000 Ci/mmol) for 10 min at 37°C. The labeled cells were pelleted by centrifugation and suspended in sodium dodecyl sulfate (SDS) sample buffer for SDS-polyacrylamide gel electrophoresis (PAGE).

Reagents. Sequenase was purchased from United States Biochemical Corp. Oligonucleotides were synthesized in the Wayne University Macromolecular Core Facility. Isotopes were purchased from New England Nuclear Corp. Thiosine, XP, and 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal) were obtained from Sigma Chemical Corp. All other chemicals were purchased from commercial sources.

Nucleotide sequence accession number. The lysP nucleotide sequence has been assigned GenBank accession number M89774.

RESULTS

Induction of lysine transport. In a preliminary communication, we reported that the two lysine permeases of E. coli responded differentially to growth conditions (24, 25). When grown aerobically in a basal salts medium at neutral pH, the two systems contributed about equally to lysine uptake. When the cells were transferred to a YT medium, the activity of the LysP system increased, with a concomitant decrease in the activity of the LAO system. One factor which correlated with increased lysine transport was a decrease in medium pH, usually to a pH of less than 6. When cells were grown in buffered medium at pH 6, lysine transport was increased.

To investigate this phenomenon further, cells were grown in media with different combinations of low pH, anaerobic, and exogenous lysine. Lysine transport increased dramatically when the pH of the medium was shifted from 7.5 to 5.5, when the cells were grown under anaerobic conditions, and when 0.5% lysine was added to the medium (Fig. 1).

Isolation and characterization of lysP-phoA gene fusions.

![FIG. 1. Lysine transport as a function of growth conditions.](http://jb.asm.org/)

Translational fusions with the phoA gene have been useful in identifying a membrane protein (14). Ts' mutants of CC118 were created by TnphoA mutagenesis. Isolates which were blue on XP plates were considered candidates for gene fusions into the lysP gene, which would be expected to encode an inner membrane protein. One such fusion strain, BPR2, was examined in more detail. This strain was light blue on XP plates but was definitely more colored than phoA mutant strains. In cells grown in YT medium, in which the majority of lysine uptake occurs via the lysine-specific system (24), lysine transport was nearly absent in the phoA mutant, compared with the wild type, CC118 (Fig. 2). As a control, leucine transport was shown to be identical in both strains (data not shown).

Genetic mapping of the lysP gene in the E. coli chromosome. The location of the lysP gene on the E. coli chromosome was mapped by Hfr crosses by using a collection of linked Tn10 insertions (33). A streptomycin-resistant derivative (LE392S), the rec+ E. coli strain LE392, was generated by nitrosoguanidine mutagenesis (16). The lysP-phoA fusion was introduced into LE392S by P1 transduction to produce strain BPR392S by selection for resistance to thiosine, streptomycin, and kanamycin. This strain was used as a recipient in mating experiments with the donors from a set of strains having linked Tn10 insertions (33). Streptomycin- and tetracycline-resistant recombinants were isolated and scored for loss of kanamycin and thiosine resistance. The results indicated that the mutation is located between 45 and 62 min on the chromosomal map. Since other Ts' mutants had mapped...
Cloning of the \( \text{lysP} \) gene. A 1.5-kb \( \text{BamHI-DraI} \) fragment was used as a probe to identify the \( \text{lysP} \) gene. \( \lambda \) phage clones containing overlapping inserts of \( E. \) coli chromosomal DNA covering the region 46.2 to 47.2 min (12) were used for dot blot hybridization with the \( \text{BamHI-DraI} \) fragment. Positive hybridization was observed with the following four phage clones: 7F1, 7H12, 5H11, and 4F2. DNA from each was digested with \( \text{BamHI} \), ligated into the \( \text{BamHI} \) site of \( \text{pUC19} \), and transformed into strain TG1. Plasmids with inserts were screened by dot blot hybridization with the radiolabeled \( \text{lysP-phaOA} \) \( \text{BamHI-DraI} \) probe. Two positively hybridizing clones were shown by Southern analysis to have a 2.7-kb \( \text{BamHI} \) fragment which hybridized to the \( \text{BamHI-DraI} \) probe. One, termed \( \text{pLYSP} \), originally derived from \( \lambda \) phage clone 7H12, was used for further study (Fig. 3).

**Complementation of the \( \text{lysP-phaOA} \) mutation.** Plasmid \( \text{pLYSP} \) was introduced into strain \( \text{BPR2} \) by electroporation. Lysine transport in \( \text{BPR2(pLYSP)} \) was increased approximately 100-fold above the level in the mutant and about 10- to 20-fold above wild-type levels (Fig. 2).

For phenotypic complementation, the 2.7-kb \( \text{BamHI} \) fragment was cloned into the \( \text{BamHI} \) site of plasmid \( \text{pACYC184} \) to produce plasmid \( \text{pNE1} \), which was transformed in strain \( \text{BPR2} \). The Ts' phenotype of strain \( \text{BPR2} \) was transformed to Tc' sensitivity by plasmid \( \text{pNE1} \). These results show that the \( \text{lysP} \) gene is contained within the 2.7-kb \( \text{BamHI} \) fragment.

**Nucleotide sequence of the \( \text{lysP} \) gene.** Sequence data were obtained for all 2,661 bp of the \( \text{BamHI} \) fragment from both strands with overlaps between sequenced regions. From computer analysis, three potential open reading frames were identified (Fig. 4). The last reading frame, starting at bp 2286 and extending to the end of the fragment, is the 5' region of the \( \text{cir} \) gene, which encodes the colicin I receptor protein (17). The first potential open reading frame (orf1) extended from an ATG at bp 76 to a TAA at bp 413. This could code for a 13-kDa hydrophilic protein of 112 residues. A GAAG at bp 67 could serve as a ribosome-binding site for orf1. The second reading frame extended from bp 522 to bp 1989, preceded by a putative ribosome-binding site, GGATAG, at bp 508. Sequencing of the fusion junction in \( \text{pBPR2} \) located the site of fusion at bp 1205, demonstrating that it is within the \( \text{lysP} \) gene. Following the termination codon, a region of dyad symmetry between bp 2011 and 2034 is observed, as well as a 16-bp region from bp 2034 to bp 2049 with 10 T and 3 A residues which could serve as a transcriptional termination structure (21). No promoter sequences upstream of the first or second reading frames were obvious. There are two

![FIG. 2. Amino acid transport in wild-type and mutant cells and expression of the cloned gene. Cells were grown aerobically in YT medium. Transport assays were performed with 10 \( \mu \)M \( [\text{H}] \)lysine in \( E. \) coli C118 (wild type) (A), BPR2 (mutant \( \text{lysP} \)) (B), or BPR2 (p\( \text{LYSP} \)) (C).](image)

at 46 min, this region of the chromosome was analyzed by P1 transduction. P1 phage was prepared from strains having \( \text{Tn}10 \) insertions at 45.75, 46.5, and 47.75 min. The phage was used to locate the \( \text{lysP} \) gene. With strain \( \text{BPR392S} \) as recipient, \( \text{Tc}' \) transductants were selected and screened for cotransduction of thiosine sensitivity. Only with phage grown on strain \( \text{CAG12098} \), which contains \( \text{Tn}10 \) at 46.5 min, was cotransduction of the thiosine phenotype with \( \text{Tc}' \) observed. The data demonstrate that the \( \text{lysP} \) gene is located near 46.5 min.

**Cloning of the chromosomal \( \text{lysP-phaOA} \) gene fusion.** Since there is no \( \text{BamHI} \) site between the site of fusion in \( \text{TnphoA} \) and the kanamycin phosphotransferase gene and there is a \( \text{BamHI} \) site immediately following the 3' end of the kanamy- cin phosphotransferase gene (11), a \( \text{BamHI} \) digest of chromosomal DNA from strain \( \text{BPR2} \) was used to clone that portion of the \( \text{lysP} \) gene proximal to the fusion junction. The \( \text{BamHI} \) digest was ligated into the unique \( \text{BamHI} \) site of \( \text{pBR322} \), selecting for \( \text{Km}' \) and screening for loss of \( \text{Tc}' \). The resulting plasmid, \( \text{pBPR2} \), contained a 6.1-kb \( \text{BamHI} \) insert composed of 1.2 kb of \( E. \) coli chromosomal DNA and 4.9 kb from \( \text{TnphoA} \).

![FIG. 3. Cloning of the \( \text{lysP} \) gene. The \( \lambda \) phage clone 7H12 was confirmed to contain the \( \text{lysP} \) gene by hybridization with \( \text{pBPR2} \). DNA from \( \lambda \)7H12 was digested with \( \text{BamHI} \) and ligated into the \( \text{BamHI} \) site in the polylinker region of \( \text{pUC19} \). Restriction sites according to Kohara et al. (12) were B, \( \text{BamHI} \); D, \text{HindIII}; E, \text{EcoRI}; F, \text{EcoRV}; G, \text{BglII}; O, \text{KpnI}; S, \text{FstI}; and V, \text{PvuII}.](image)
potential ~10 sequences at bp 410 and 440, but the corresponding ~35 sequences are not obvious (8). If those sequences are the start of transcription for lpsP, then orf1 would not be included in the transcript. These data would place lpsP between nfo (6) and cir (17) on the physical map of the E. coli chromosome.

Similarity of LysP and amino acid permeases. The predicted translation product of the lpsP gene is a protein of 489 residues with a predicted mass of 53,660 Da. From analysis of the hydrophobicity profile, the protein would be expected to be extremely hydrophobic, with 12 predicted membrane-spanning regions (Fig. 5). The primary amino acid sequence exhibits similarity to a family of amino acid permeases, including the aromatic amino acid (33% similarity) (5) and phenylalanine-specific permeases (34% similarity) (20) of E. coli, the arginine (34% similarity) (10), histidine (30% similarity) (37), and proline (31% similarity) (38) permeases of S. cerevisiae, and the proline permease of A. nidulans (27% similarity) (34) (Fig. 6). The similarities of these proteins are more apparent at the structural level, as shown by the alignment of the hydropathy profiles of the lysine-specific permease and the aromatic amino acid permease (Fig. 5).

Expression of the lpsP gene. To identify gene products, the BamHI fragment was put under control of the T7 phase promoter in plasmid pBP20 and induced in the presence of rifampicin and [35S]methionine (36). An insert-specific band corresponding to a mass of about 40 kDa was observed (Fig. 7). Although this is less than the predicted 53.7 kDa, many membrane proteins migrate anomalously fast on SDS-PAGE. To confirm its identity, plasmid pBP20 was digested with PstI and relaxed, removing a 1,258-bp fragment, including the last 787 bp of the lpsP gene and the cir sequence. The resulting construct would be expected to encode a truncated LysP polypeptide of 24.4 kDa, as was observed (Fig. 7). Two smaller labeled polypeptides, of approximately 14 kDa each, a sharper upper band, and a less

FIG. 4. Nucleotide sequence and deduced amino acid sequence of the lpsP gene of E. coli. Three potential open reading frames are identified, with the single letter code for amino acids under the nucleotide sequence. Putative ribosome-binding sites (RBS) are indicated in boxes. The second reading frame is the lpsP gene. The third reading frame was identified as a portion of the cir gene. Following the lpsP gene sequence is a region of dyad symmetry and a T-rich sequence, which is proposed to be a transcriptional terminator.
The protein was adjusted according to the alignment in Fig. 6.

distinct lower band were observed. These are approximately the sizes of the potential orf1 product and the product of the truncated cir gene. The upper band was not observed in the subclone and may be the cir product. The less distinct lower band was still observed. Its relation to orf1 is under investiga-

**DISCUSSION**

We have shown that the lysine-specific transport system is induced to its highest levels in anaerobic media of low pH containing lysine (Fig. 1). The inducers of lysine decarboxylase, the enzyme which converts lysine to cadaverine, the product of the cadA gene, similarly include a combination of low pH, high concentration of lysine in the medium, and anaerobiosis (2, 7, 28). An increase in the uptake and decarboxylation of lysine is an adaptive response to medium acidification which results in formation of the intracellular base cadaverine and alkalization of the cytosol. An ability to respond and adapt to such environmental changes is a tremendous survival advantage, perhaps essential for survival under certain conditions.

As a basis for future studies of regulation of the lysine-specific permease, it was necessary to identify the gene for the permease. We had previously isolated mutants defective in lysine-specific transport by resistance to the lysine analog thioisole (24). Popkin and Maas (22) isolated similar mutants. Their lysP mutation, which mapped at 46 min, had a pleiotropic effect, decreasing lysine-specific transport but increasing lysine decarboxylase. Tabor and coworkers (35) isolated similar mutants that also mapped at 46 min. They called the locus cadR, which differs from the proposed model of regulation of the cadA gene for lysine decarboxylase, which maps at 93.7 min. From their work, it could not be decided whether the effect of mutation in the lysP (cadR) locus on the cad operon was direct, which would have required a regulatory gene to be encoded by lysP; on the other hand, the cad operon could respond indirectly to secondary metabolic effects resulting from decreased uptake of lysine.
of the applied samples derived from 0.2
ylase original gene is only proportional
SDS-15% PAGE gel and Richardson (36).
mutation of various transposon density and
plates. The fusions pBPR30 tRNA K38(pGP1.2)
bearing protein in addition such other E.
lysine-specific transporters, which could be
determined, so a cytoplasmic location of this loop is not
established. In the mutant BPR2, the chimeric protein
would have alkaline phosphatase fused to residue 228 of LysP.
Since BPR2 is \( \text{phoA}^+ \), residue 228, must have a periplasmic
location when it is fused to alkaline phosphatase. However,
strain BPR2 is only light blue on XP plates, a phenotype
which could result from an intramembranal location of residue 228 in the wild-type LysP protein. The orientation of the helices and the placement of the extramembranal loops must await a detailed topological analysis.

Although there are no data on the role of any residues in the homologs shown in Fig. 6, the multiple alignment yields some intriguing similarities. Most of the identities are glycy1 or prolyl residues. These are probably located in turns, and replacement by bulkier residues would disrupt the structure. Many of the membrane-spanning regions have interchangeable leucines, isoleucines, or valines, again reflecting structural elements. There are insertions in the fungal permeases in the linker regions between helices II and III, between helices V and VI, and between helices VII and VIII. With the assumption that the N terminus is located in the cytosol, these insertions would be in the first cytoplasmic loop and in the third and fourth periplasmic loops.

Of note are the two positions with either conserved glutamates (E71, E154, E156, E222, and E230 in LysP) or conserved aspartate (D113) and four positions with conserved basic amino acids (R20, R270, R330, and R337). Since all of these are amino acid transporters, the conserved acidic residues are potential candidates for recognition of the \( \alpha \)-amino group, and the conserved basic residues may be involved in recognition of the carboxyl group of the substrate. For example, in the bacterial asparagine receptor, the carboxyl-binding site uses hydrogen binding with arginyl residues (15).

Three of the permeases transport basic amino acids, and four transport neutral amino acids. In the three proteins which transport positively charged amino acids, there is an acidic residue at the position corresponding to E16 in LysP. In the four proteins which transport neutral amino acids, this residue is a glycine. This is the only position in every protein in which the charge of the residue correlates with the charge of the substrate. E16 would therefore be a candidate for a recognition site for the \( \varepsilon \)-amino group of lysine. The conserved acidic residues would be postulated to be in or near the first three cytoplasmic loops, the conserved basic residues in or near the fourth cytoplasmic loop, and the common acidic residues of the basic amino acid permeases near the
cytosolically located N terminus. This may indicate that the substrate recognition sites for the permeases are closer to the cytosolic than the periplasmic side of the membrane, even though topologically, initial recognition takes place outside the cell. By analogy, the aspartate-binding site of the aspartate receptor is buried deep within the protein (15). Thus, the permeases may have a single binding site that is topologically external but physically located near the cytosol.

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