JOURNAL OF BACTERIOLOGY, May 1992, p. 3242–3249
0021-9193/92/103242-08$02.00/0

Vol. 174, No. 10

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

Received 27 December 1991/Accepted 6 March 1992

Escherichia coli **transports lysine by two distinct systems, one of which is specific for lysine (LysP) and the other of which is inhibited by arginine or ornithine. The activity of the lysine-specific system increases with growth in acidic medium, anaerobiosis, and high concentrations of lysine. It is inhibited by the lysine analog *S*-(β-aminoethyl)-L-cysteine (thiosine). Thiosine-resistant (Ts^r) mutants were isolated by using transpositional mutagenesis with Tn*phoA*. A Ts^r mutant expressing alkaline phosphatase activity in intact cells was found to lack lysine-specific transport. This *lysP* mutation was mapped to about 46.5 min on the *E. coli* chromosome. The *lysP-phoA* fusion was cloned and used as a probe to clone the wild-type *lysP* gene. The nucleotide sequence of the 2.7-kb *Bam*HI fragment was determined. An open reading frame from nucleotides 522 to 1989 was observed. The translation product of this open reading frame is predicted to be a hydrophobic protein of 489 residues. The *lysP* gene product exhibits sequence similarity to a family of amino acid transport proteins found in both prokaryotes and eukaryotes, including the aromatic amino acid permease of *E. coli* (*aroP*) and the arginine permease of *Saccharomyces cerevisiae* (CAN1). Cells carrying a plasmid with the *lysP* gene exhibited a 10- to 20-fold increase in the rate of lysine uptake above wild-type levels. These results demonstrate that the *lysP* gene encodes the lysine-specific permease.**

Three systems for the transport of the basic amino acids arginine, lysine, and ornithine exist in *Escherichia coli*. One, the arginine-specific system, transports only arginine (23, 25, 26). A second, the lysine-arginine-ornithine (LAO) system, transports lysine and ornithine (23, 25, 26) and is inhibited by arginine, although arginine is not a substrate of the LAO system (23, 25). The third system, termed the lysine-specific permease (LysP) system, is inhibited by the lysine analog *S*-(β-aminoethyl)-L-cysteine (thiosine) but not by ornithine or arginine (23). The activity of the LysP system is increased in cells grown on enriched medium, with a concomitant decrease in the activity of the LAO system (24, 25). Here, we show that a combination of growth conditions known to induce lysine decarboxylase also induces lysine-specific transport, including low pH, anaerobiosis, and lysine concentration in medium.

Thiosine has been used to select for mutants with alterations in lysine metabolism. Mutations which produce Ts^r have been mapped at approximately 46 min on the *E. coli* chromosome. Both the *lysP* (22) and *cadR* (35) mutations produced pleiotropic phenotypes, decreasing lysine transport activity and increasing lysine decarboxylase activity. From those studies, it was not clear whether the *cadR* (*lysP*) locus is regulatory or also contains the gene(s) for the lysine permease. We reasoned that Tn*phoA* mutagenesis, which has been used for topological analysis of membrane proteins (14), should also be useful to identify the genes for membrane proteins. In this paper, we report the isolation of *lysP-phoA* fusions with extracellular localized alkaline phosphatase activity. One such fusion was mapped to about 46.5 min on the *E. coli* chromosome. The *lysP* gene was cloned and sequenced and shown to have similarity to a family of amino acid transport proteins including the aromatic amino acid (5) and phenylalanine-specific (20) permeases of *E. coli*, the arginine (10), histidine (37), and proline permeases (38) of *Saccharomyces cerevisiae*, and the proline permease of *Aspergillus nidulans* (34).

* Corresponding author.

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^r strains, medium E was supplemented with 100 μg of thiosine 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 with aeration at 37°C, except as otherwise noted.

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

Genetic mapping. Hfr mapping was performed as described elsewhere (19) with a collection of linked Tn*10* insertions in Hfr donor strains (33). P1 transduction was performed by using a set of linked Tn*10* insertions as donors (33) and a *lysP-phoA* strain as recipient. Transconjugants and transductants were selected for Sm^r and Tc^r and screened for Km^s and Ts^s.

Southern and dot blot hybridization. A 1.5-kb *Bam*HI-*Dra*I fragment containing the *lysP-phoA* fusion was radiolabeled with [α-^{32}P]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 Hybond 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 *Bam*HI and ligation into the unique *Bam*HI site of plasmid pBR322.

TABLE 1. Strains, plasmids, and phages

Strain, plasmid, or phage	Genotype or description	Source (reference)
Strains		
HB101	F⁻ *hsdR hsdM supE44 ara-14 galK2 lacY1 proA2 rspL20 xyl-5 mtl-1 recA13 mcrB*	Sambrook et al. (30)
TG1	K12 Δ(*lac-pro*) *supE* F′ *traD36 proAB lacI*^{q} Δ*lacZM15*	Amersham Corp.
CC118	*araD139* Δ(*ara-leu*)*7697* Δ*lacX74 galE galK* Δ*phoA20 thi rpsE rpoB argE*(Am) *recA1*	Manoil and Beckwith (14)
BPR2	CC118 with Tn*phoA* insertion in *lysP* (Ts^r)	This study
LE392	F⁻ *supF supE hsdR galK trpR metB lacY*	Manoil and Beckwith (14)
LE392S	Sm^r derivative of LE392	This study
BPR392S	P1 transduction of *lysP-phoA* fusion into LE392S	This study
K38	HfrC (λ)	Russell and Model (27)
CAG12179	MG1655 *mgl-500*::Tn*10*	Singer et al. (33)
CAG12098	MG1655 *zeg-3130*::Tn*10*	Singer et al. (33)
CAG12177	MG165 *zeh-298*::Tn*10*	Singer et al. (33)
CAG5051	HfrH *nadA57*::Tn*10*	Singer et al. (33)
CAG5052	KL227 *btuB3191*::Tn*10*	Singer et al. (33)
CAG5053	KL208 *zbc-280*::Tn*10*	Singer et al. (33)
CAG5054	KL96 *trpB83*::Tn*10*	Singer et al. (33)
CAG5055	KL16 *zed-3069*::Tn*10*	Singer et al. (33)
CAG8209	KL228 *zgh-3075*::Tn*10*	Singer et al. (33)
CAG8160	KL14 *thi-39*::Tn*10*	Singer et al. (33)
Plasmids		
pBR322	Cloning vector (Ap^r and Tc^r)	Bolivar et al. (3)
pUC19	Cloning vector (Ap^r)	Yanisch-Perron et al. (39)
pACYC184	Cloning vector (Cm^r and Tc^r)	Chang and Cohen (4)
pBPR2	pBR322 with 6.1-kb *Bam*HI *lysP-phoA* insert	This study
pLYSP	pUC19 with 2.7-kb *Bam*HI *lysP* insert	This study
pNE1	pACYC184 with 2.7-kb *Bam*HI *lysP* insert	This study
pGP1.2	T7 phage RNA polymerase gene under control of λp_L promoter and λ*cI*^{ts} (Km^r)	Tabor and Richardson (36)
pT7-5	Expression vector with the T7 promoter (Ap^r)	Tabor and Richardson (36)
pBPR20	*Bam*HI fragment of pLYSP containing the *lysP* gene cloned into pT7-5	This study
pBPR30	pBPR20 with deletion of *Pst*I fragment	This study
Phages		
λTn*phoA*	Tn*5* IS*50*$_L$::*phoA* (Km^r)	Manoil and Beckwith (14)
λ7F1	*E. coli* chromosomal clone	Kohara et al. (12)
λ7H12	*E. coli* chromosomal clone	Kohara et al. (12)
λ5H11	*E. coli* chromosomal clone	Kohara et al. (12)
λ4F2	*E. coli* chromosomal clone	Kohara et al. (12)
M13mp18	Sequencing phage	Yanish-Perron et al. (39).

Transformants were selected for Km^r and screened for blue on XP plates. The resulting plasmid, pBPR2, contained 1.2 kb of *E. coli* DNA and 4.9 kb from Tn*phoA*. 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 *Bam*HI. The resulting mixture was ligated into the *Bam*HI 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 *Bam*HI digests of positive plasmids demonstrated the presence of a 2.7-kb *Bam*HI fragment which hybridized to the probe. One plasmid, pLYSP, was selected for further study.

DNA sequencing. DNA sequencing was done by the dideoxy method of Sanger et al. (31) with ^{35}S-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 *Bam*HI fragment was cloned into the *Bam*HI 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

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 [^{3}H]lysine or [^{3}H]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 [^{35}S]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 State 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, anaerobiosis, 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.

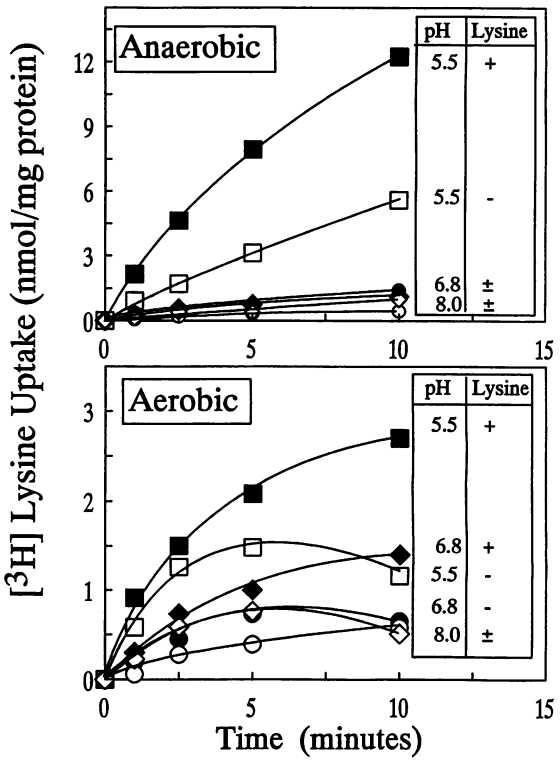

FIG. 1. Lysine transport as a function of growth conditions. Cells were grown in modified Falkow medium adjusted to pH 5.5 (squares), 6.8 (circles), or 8.0 (diamonds) anaerobically (top) or aerobically (bottom) with (filled symbols) or without (empty symbols) 0.5% lysine. Uptake assays were performed as described in Materials and Methods.

Translational fusions with the *phoA* gene have been useful in identifying a membrane protein (14). Ts^r mutants of CC118 were created by Tn*phoA* 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 Tn*10* 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 Tn*10* 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^r mutants had mapped

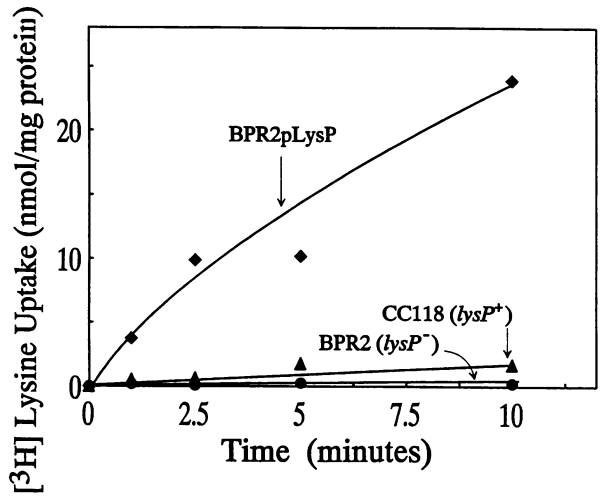

FIG. 2. Amino acid transport in wild-type and mutant cells and expression of the cloned gene. Cells were grown aerobically in YT medium. Transport assays were performed with 10 μM [^{3}H]lysine in *E. coli* CC118 (wild type) (▲), BPR2 (mutant *lysP*) (●), or BPR2 (pLysP) (◆).

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

Cloning of the chromosomal *lysP-phoA* gene fusion. Since there is no *Bam*HI site between the site of fusion in Tn*phoA* and the kanamycin phosphotransferase gene and there is a *Bam*HI site immediately following the 3′ end of the kanamycin phosphotransferase gene (11), a *Bam*HI digest of chromosomal DNA from strain BPR2 was used to clone that portion of the *lysP* gene proximal to the fusion junction. The *Bam*HI digest was ligated into the unique *Bam*HI site of pBR322, selecting for Km^r and screening for loss of Tc^r. The resulting plasmid, pBPR2, contained a 6.1-kb *Bam*HI insert composed of 1.2 kb of *E. coli* chromosomal DNA and 4.9 kb from Tn*phoA*.

Cloning of the *lysP* gene. A 1.5-kb *Bam*HI-*Dra*I fragment was used as a probe to identify the *lysP* gene. λ 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 *Bam*HI-*Dra*I fragment. Positive hybridization was observed with the following four phage clones: 7F1, 7H12, 5H11, and 4F2. DNA from each was digested with *Bam*HI, ligated into the *Bam*HI site of pUC19, and transformed into strain TG1. Plasmids with inserts were screened by dot blot hybridization with the radiolabeled *lysP-phoA Bam*HI-*Dra*I probe. Two positively hybridizing clones were shown by Southern analysis to have a 2.7-kb *Bam*HI fragment which hybridized to the *Bam*HI-*Dra*I probe. One, termed pLYSP, originally derived from λ phage clone 7H12, was used for further study (Fig. 3).

Complementation of the *lysP-phoA* mutation. Plasmid pLYSP was introduced into strain BPR2 by electroporation. Lysine transport in 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 *Bam*HI fragment was cloned into the *Bam*HI site of plasmid pACYC184 to produce plasmid pNE1, which was transformed in strain BPR2. The Ts^r phenotype of strain BPR2 was transformed to sensitivity by plasmid pNE1. These results show that the *lysP* gene is contained within the 2.7-kb *Bam*HI fragment.

Nucleotide sequence of the *lysP* gene. Sequence data were obtained for all 2,661 bp of the *Bam*HI 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 *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 pBPR2 located the site of fusion at bp 1205, demonstrating that it is within the *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

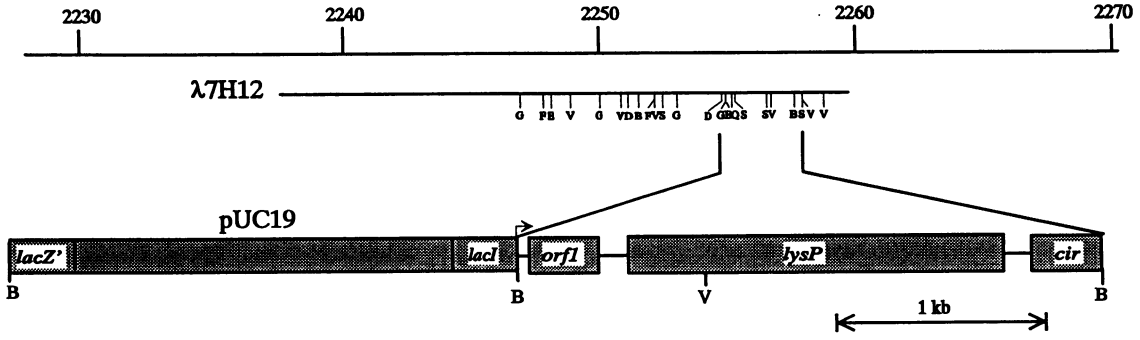

FIG. 3. Cloning of the *lysP* gene. The λ phage clone 7H12 was confirmed to contain the *lysP* gene by hybridization with pBPR2. DNA from λ7H12 was digested with *Bam*HI and ligated into the *Bam*HI site in the polylinker region of pUC19. Restriction sites according to Kohara et al. (12) were B, *Bam*HI; D, *Hin*dIII; E, *Eco*RI; F, *Eco*RV; G, *Bgl*I; O, *Kpn*I; S, *Pst*I; and V, *Pvu*II.

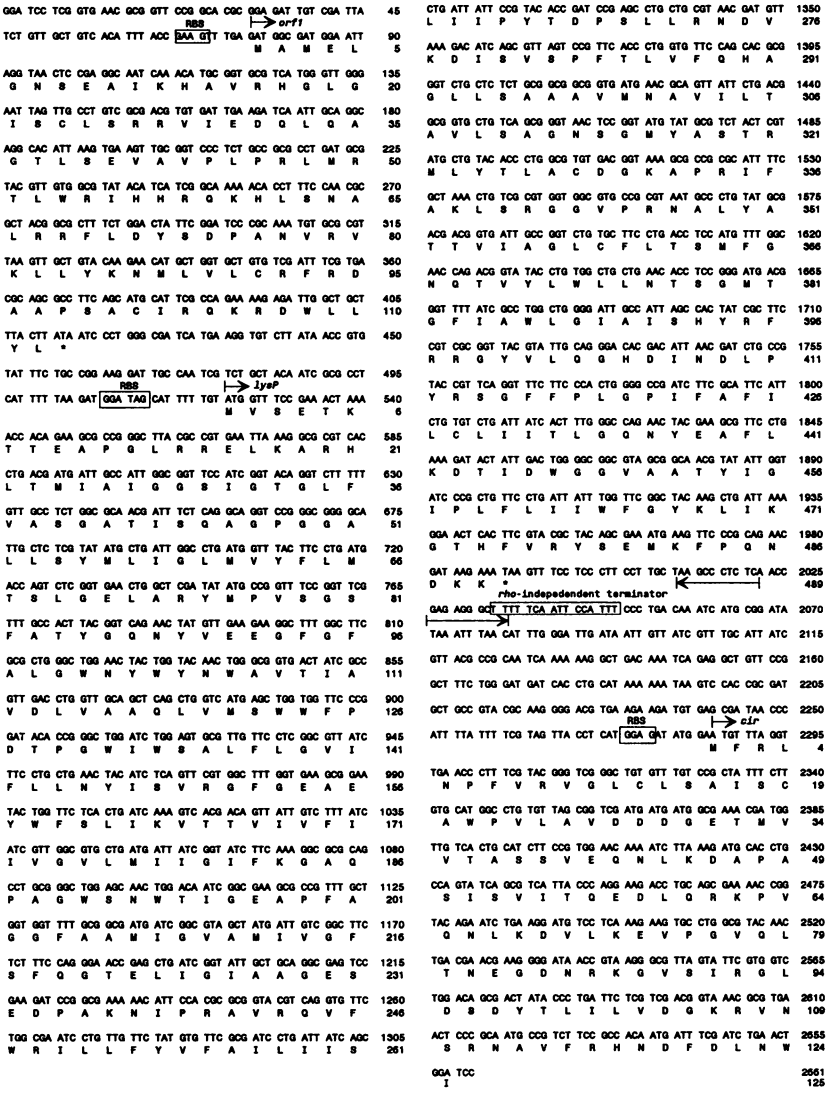

FIG. 4. Nucleotide sequence and deduced amino acid sequence of the *lysP* gene of *E. coli*. Three potential open reading frames are identified, with the single letter code for amino acids under the nucleotide sequence. Putative ribosome-binding sites (RBS) are indicated in boxes. The second reading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

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 *lysP*, then *orf1* would not be included in the transcript. These data would place *lysP* 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 *lysP* gene is a protein of 489 residues with a predicted mass of 53,660 Da. From analysis of the hydropathy 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 *lysP* gene. To identify gene products, the *Bam*HI fragment was put under control of the T7 phage promoter in plasmid pBPR20 and induced in the presence of rifampin and [^{35}S]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 pBPR20 was digested with *Pst*I and religated, removing a 1,258-bp fragment, including the last 787 bp of the *lysP* 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

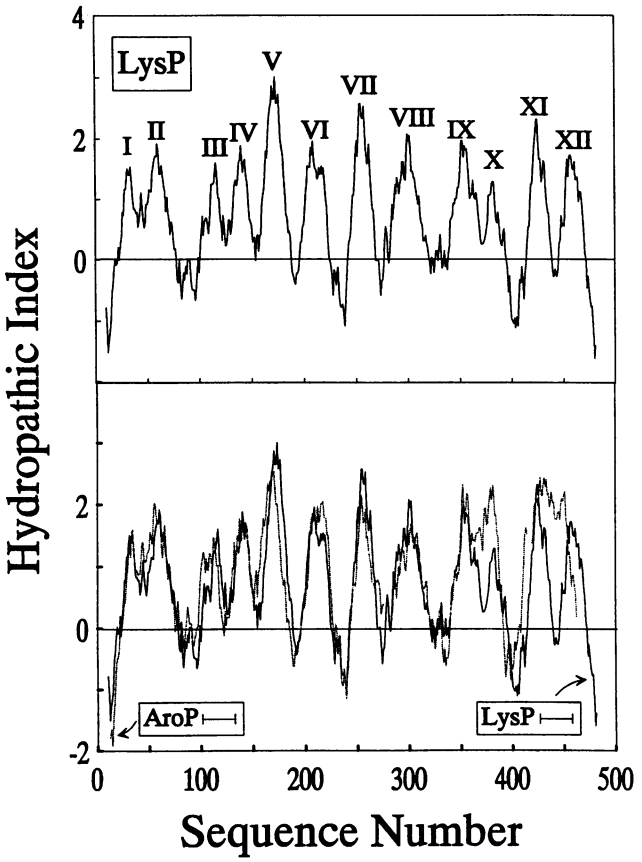

FIG. 5. Hydropathy profiles of the LysP and AroP proteins. Hydropathy indices were calculated by using GENEPRO with a window of 19 amino acids. Twelve regions of sufficient hydrophobicity to produce membrane-spanning α-helices in the LysP protein are identified (top). The hydropathy profiles of the LysP (unbroken line) and AroP (dotted line) proteins are superimposed (bottom). The placement of residues in the AroP protein relative to the LysP protein was adjusted according to the alignment in Fig. 6.

FIG. 6. Multiple alignment of the amino acid sequences of a family of amino acid permeases. Indicated are residues in all seven proteins which are identical (*) or conservative (:) replacements. Conserved acidic and basic residues are enclosed in boxes. The arrow indicates an acidic residue in the basic amino acid permeases and a neutral residue in the neutral amino acid permeases. LysP, lysine-specific permease of *E. coli*; AroP, aromatic amino acid permease of *E. coli* (5); PheP, phenylalanine-specific permease of *E. coli* (20); ArgP, arginine permease of *S. cerevisiae* (10); HisP, histidine permease of *S. cerevisiae* (37); ProP1, proline permease of *S. cerevisiae* (38); ProP2, proline permease of *Aspergillus nidulans* (34).

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 investigation.

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 alkalinization 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 thiosine (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* because the mutation resulted in loss 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.

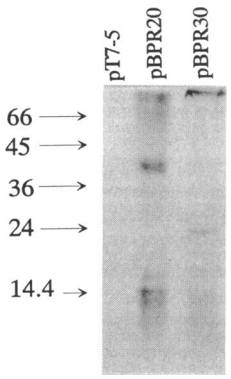

FIG. 7. Identification of the *lysP* gene product. Cells of *E. coli* K38(pGP1.2) bearing plasmid pT7-5 (lane 1), pBPR20 (lane 2), or pBPR30 (lane 3) were labeled with [^{35}S]methionine, as described by Tabor and Richardson (36). Whole-cell lysates were separated by SDS-15% PAGE gel followed by autoradiography. To each lane were applied samples derived from 0.2 ml of cell culture (optical density at 600 nm of 0.8).

To resolve this question, we selected Ts^r mutants by transposon mutagenesis using Tn*phoA*. Ts^r could result from mutation of various genes, including the *lysU* gene for lysyl tRNA synthetase (9). However, only cells with translational fusions to proteins with extracellular domains such as the lysine-specific permease would give blue colonies on XP plates. The mutant lacks lysine-specific transport (Fig. 2) and exhibits approximately fivefold higher lysine decarboxylase (2a). The mutation was mapped to 46.5 min on the *E. coli* chromosome. It is therefore similar in phenotype to the original *lysP* and *cadR* mutations. From sequence analyses of the cloned *lysP* gene and the *lysP-phoA* fusion gene, it is clear that (i) the mutation is within the coding sequence for the lysine-specific permease and that (ii) there are no genes downstream of *lysP* which could be within the same transcript as the permease gene. Thus, the loss of regulation of *cadA* results directly from loss of function of the *lysP* gene product. Whether the LysP protein acts as a regulatory protein in addition to its transport function must be determined.

The LysP protein is a member of a family of basic and aromatic amino acid permeases found in both prokaryotes and eukaryotes (Fig. 6). Phylogenetic analysis suggests that the lysine-specific permease is most closely related to the other *E. coli* permeases, but it is nearly as closely related to fungal permeases for basic amino acids (Fig. 8). The *lysP* gene is only distantly related to other secondary amino acid permeases, such as the transporters for specific aromatic amino acids (32). It is also unrelated to basic amino acid antiporters, such as *arcD* (13), and to the other lysine permease, the LAO system, which belongs to a different family, the osmotic-shock-sensitive amino acid permeases (1).

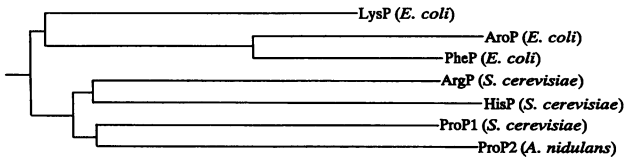

FIG. 8. Phylogenetic relationship among amino acid permeases. A series of pairwise alignments of the sequences were made by using GENEPRO. The pairwise scores were used to align by computer all of the sequences against one another by the neighbor-joining method of Saitou and Nei (29). The branch lengths in the phylogenetic tree are proportional to the numbers of amino acid substitutions separating each pair.

The similarity of the LysP family of amino acid permeases is more apparent at the structural level, as demonstrated by superimposition of hydropathy plots of LysP with its homolog AroP (Fig. 5). Putative α-helices I to IX are quite similar. Helices X to XII are not equivalent; the degree of similarity in the C-terminal regions of all of the homologs is weak (Fig. 6). Of interest is the central loop (approximately residues 230 to 250 in LysP), which is present in all of the permeases. Almost all secondary porters, whether related or not, show a motif of two groups of six membrane-spanning α-helices separated by a central highly charged cytoplasmic loop. The significance of this motif is not known, but almost certainly there is an important structural reason. The topological arrangements of the residues in LysP have not been 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 *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 glycyl 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 six 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 permeases are amino acid transporters, the conserved acidic residues are potential candidates for recognition of the α-amino group, and the conserved basic residues may be involved in recognition of the carboxyl group of the substrate. For example, in the bacterial asparate 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 ε-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.

ACKNOWLEDGMENTS

We are indebted to Joan L. Slonczewski for suggestions. Thanks are due to George N. Bennett for lysine decarboxylase assays and to Michael Stanhope for computer analysis of the phylogenetic relationship of the permeases. We thank Wei-Ping Shi, Etienne Ngoumgna, and Jiyang Liu for assistance.

This work was supported by NSF grant DCB-8796318.

REFERENCES

1. **Ames, G. F.-L.** 1990. Energetics of periplasmic transport systems. The Bacteria **12**:225–246.
2. **Auger, E. A., K. E. Redding, T. Plumb, L. C. Childs, S.-Y. Meng, and G. N. Bennett.** 1989. Construction of *lac* fusions to the inducible arginine and lysine decarboxylase genes of *Escherichia coli* K12. Mol. Microbiol. **3**:609–620.

2a. **Bennett, G.** Personal communication.

3. **Bolivar, F., R. L. Rodriguez, P. J. Green, M. C. Betlach, H. L. Heyeneker, H. W. Boyer, J. H. Crosa, and S. Falkow.** 1977. Construction and characterization of new cloning vehicles. II. A multipurpose cloning system. Gene **2**:95–113.
4. **Chang, A. C. Y., and S. N. Cohen.** 1978. Construction and characterization of amplifiable multicopy DNA cloning vehicles derived from the P15A cryptic miniplasmid. J. Bacteriol. **134**: 1141–1156.
5. **Chye, M. L., J. R. Guest, and J. Pittard.** 1986. Cloning of the *aroP* gene and the identification of its product in *Escherichia coli* K-12. J. Bacteriol. **167**:749–753.
6. **Cunningham, R. P., and S. M. Saporito.** 1988. Nucleotide sequence of the *nfo* gene of *Escherichia coli* K-12. J. Bacteriol. **170**:5141–5145.
7. **Gale, E. F.** 1946. The bacterial amino acid decarboxylases. Adv. Enzymol. **6**:1–32.
8. **Harley, C. B., and R. P. Reynolds.** 1987. Analysis of *E. coli* promoter sequences. Nucleic Acids Res. **15**:2343–2361.
9. **Hirshfield, I. N., and P. C. Zamecnik.** 1972. Thiosine-resistant mutants of *Escherichia coli* K-12 with growth-medium-dependent lysyl-tRNA synthetase activity. Biochim. Biophys. Acta **259**:330–343.
10. **Hoffmann, W.** 1985. Molecular characterization of the *CAN1* locus in *Saccharomyces cerevisiae*. J. Biol. Chem. **260**:11831–11837.
11. **Jorgensen, R. A., S. J. Rothstein, and W. S. Reznikoff.** 1979. A restriction enzyme cleavage map of Tn5 and location of a region encoding neomycin resistance. Mol. Gen. Genet. **177**:65–72.
12. **Kohara, Y., K. Akimama, and K. Isono.** 1987. The physical map of the whole *E. coli* chromosome: application of a new strategy for rapid analysis of a large genomic library. Cell **50**:495–508.
13. **Luthi, E., H. Baur, M. Gamper, F. Brunner, D. Villeval, A. Mercenier, and D. Haas.** 1989. The *arc* operon for anaerobic arginine catabolism in *Pseudomonas aeruginosa* contains an additional gene, *arcD*, encoding a membrane protein. Gene **87**:37–43.
14. **Manoil, C., and J. Beckwith.** 1985. Tn*phoA*: a transposon probe for protein export signals. Proc. Natl. Acad. Sci. USA **82**:8129–8133.
15. **Milburn, M. V., G. G. Privé, D. L. Milligan, W. G. Scott, J. Yeh, J. Jancarik, D. E. Koshland, Jr., and S.-H. Kim.** 1991. Three-dimensional structures of the ligand-binding domain of the bacterial aspartate receptor with and without a ligand. Science **254**:1342–1347.
16. **Miller, J.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
17. **Nau, C. D., and J. Konisky.** 1989. Evolutionary relationship between the TonB-dependent outer membrane transport proteins: nucleotide and amino acid sequences of the *Escherichia coli* colicin I receptor gene. J. Bacteriol. **171**:1041–1047.
18. **Novick, R. P., and W. K. Maas.** 1961. Control by endogenously synthesized arginine of the formation of ornithine transcarbamylase in *Escherichia coli*. J. Bacteriol. **81**:236–290.
19. **Peruski, L. F.** 1990. Rapid Hfr mapping of Mu dlac fusions. BioTechniques **9**:40–41.
20. **Pi, J., P. J. Wookey, and A. J. Pittard.** 1991. Cloning and sequencing of the *pheP* gene, which encodes the phenylalanine-specific transport system of *Escherichia coli*. J. Bacteriol. **173**:3622–3629.
21. **Platt, T.** 1986. Transcriptional termination and the regulation of gene expression. Annu. Rev. Biochem. **55**:339–372.
22. **Popkin, P. S., and W. K. Maas.** 1980. *Escherichia coli* regulatory mutation affecting lysine transport and lysine decarboxylase. J. Bacteriol. **141**:485–492.
23. **Rosen, B. P.** 1971. Basic amino acid transport in *Escherichia coli*. J. Biol. Chem. **246**:3653–3662.
24. **Rosen, B. P.** 1971. Regulation of lysine transport in *Escherichia coli*. Fed. Proc. **30**:1061.
25. **Rosen, B. P.** 1973. Basic amino transport in *Escherichia coli*. II. Purification and properties of an arginine-specific binding protein. J. Biol. Chem. **148**:1211–1218.
26. **Rosen, B. P.** 1973. Basic amino acid transport in *Escherichia coli*. III. Properties of canavanine-resistant mutants. J. Bacteriol. **116**:627–635.
27. **Russell, M., and P. Model.** 1984. Replacement of the *fip* gene of *Escherichia coli* by an inactive gene cloned on a plasmid. J. Bacteriol. **159**:1034–1039.
28. **Sabo, D. L., E. A. Boeker, B. Byers, H. Waron, and E. H. Fisher.** 1974. Purification and physical properties of inducible *Escherichia coli* lysine decarboxylase. Biochemistry **13**:662–670.
29. **Saitou, N., and M. Nei.** 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. **4**:406–425.
30. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
31. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74**:5463–5467.
32. **Sarsero, J. P., P. J. Wookey, P. Gollnick, C. Yanofsky, and A. J. Pittard.** 1991. A new family of integral membrane proteins involved in transport of aromatic amino acids in *Escherichia coli*. J. Bacteriol. **173**:3231–3234.
33. **Singer, M., T. A. Baker, G. Schnitzler, S. M. Deischel, M. Goel, W. Dove, K. J. Jaacks, A. D. Grossman, J. W. Erickson, and C. A. Gross.** 1989. A collection of strains containing genetically linked alternating antibiotic resistance elements for genetic mapping of *Escherichia coli*. Microbiol. Rev. **53**:1–24.
34. **Sophianopoulou, V., and C. Scazzocchio.** 1989. The proline transport protein of *Aspergillus nidulans* is very similar to amino acid transporters of *Saccharomyces cerevisiae*. Mol. Microbiol. **3**:705–714.
35. **Tabor, H., E. W. Hafner, and C. W. Tabor.** 1980. Construction of an *Escherichia coli* strain unable to synthesize putrescine, spermidine, or cadaverine: characterization of two genes controlling lysine decarboxylase. J. Bacteriol. **144**:952–956.
36. **Tabor, S., and C. C. Richardson.** 1985. A bacterial T7 RNA polymerase/promoter system for controlled exclusive expression of specific genes. Proc. Natl. Acad. Sci. USA **82**:1074–1078.
37. **Tanaka, J., and G. Fink.** 1985. The histidine permease gene (HIP1) of *Saccharomyces cerevisiae*. Gene **38**:205–214.
38. **Vandenbol, M., J. C. Jauniaux, and M. Grenson.** 1989. Nucleotide sequence of the *Saccharomyces cerevisiae* PUT4 proline-permease-encoding gene: similarities between CAN1, HIP1 and PUT4 permeases. Gene **83**:153–159.
39. **Yanisch-Perron, C., J. Vieira, and J. Messing.** 1985. Improved M13 phage cloning vectors and their host strains: nucleotide sequences of the M13mp18 and pUC19 vectors. Gene **33**:103–119.