JOURNAL OF BACTERIOLOGY, Feb. 1985, p. 484–492
0021-9193/85/020484-09$02.00/0

Vol. 161, No. 2

Genetic Analysis of *Escherichia coli* Oligopeptide Transport Mutants

JOHN C. ANDREWS AND STEVEN A. SHORT*

Department of Microbiology, Wellcome Research Laboratories, Research Triangle Park, North Carolina 27709

Received 30 July 1984/Accepted 6 November 1984

The composition of the outer membrane channels formed by the OmpF and OmpC porins is important in peptide permeation, and elimination of these proteins from the *Escherichia coli* outer membrane results in a cell in which the primary means for peptide permeation through this cell structure has been lost. *E. coli* peptide transport mutants which harbor defects in genes other than the *ompF/ompC* genes have been isolated on the basis of their resistance to toxic tripeptides. The genetic defects carried by these oligopeptide permease-negative (Opp$^-$) strains were found to map in two distinct chromosomal locations. One *opp* locus was *trp* linked and mapped to the interval between *att*$^{\phi 80}$ and *galU*. Complementation studies with F'123 *opp* derivatives indicated that this peptide transport locus resembles that characterized in *Salmonella typhimurium* as a tetracistronic operon (B. G. Hogarth and C. F. Higgins, J. Bacteriol. 153:1548–1551, 1983). The second *opp* locus, which we have designated *oppE*, was mapped to the interval between *dnaC* and *hsd* at 98.5 min on the *E. coli* chromosome. The differences in peptide utilization, sensitivity and resistance to toxic peptides, and the L-[*U*-^{14}C]alanyl-L-alanyl-L-alanine transport properties observed with these Opp$^-$ *E. coli* strains demonstrated that the transport systems encoded by the *trp*-linked *opp* genes and by the *oppE* gene(s) have different substrate preferences. Mutants harboring defects in both peptide transport loci defined in this study would not grow on nutritional peptides except for tri-L-methionine, were totally resistant to toxic peptides, and would not actively transport L-[*U*-^{14}C]alanyl-L-alanyl-L-alanine.

Gram-negative bacteria such as *Escherichia coli* and *Salmonella typhimurium* utilize small oligopeptides as both carbon and energy sources for growth. For these bacteria to grow on oligopeptides in the absence of periplasmic or extracellular peptidases (17, 34, 41), the peptides must be able to cross the permeability barriers posed by both the outer membrane and the cytoplasmic membrane. Permeation through the outer membrane is purportedly restricted only by molecular size (10, 40), whereas peptide passage through the cytoplasmic membrane requires the presence of specific transport systems (37, 41). These peptide-specific transport systems appear to be constitutively synthesized, periplasmic binding protein-dependent systems capable of catalyzing the accumulation of their peptide substrates against significant concentration gradients (9, 20, 38, 39). Other properties which have been ascribed to the bacterial peptide transport systems have been reviewed in detail by Payne (37).

In *E. coli* and *S. typhimurium*, the number of distinct oligopeptide transport systems and the genetic relationship between oligopeptide transport-defective mutants isolated in different laboratories remains to be determined. Results presently available suggest that there is a general peptide transport system with broad specificity and a number of other transport systems that are restricted to specific substrates (6, 36, 37, 47). Mutants with defects in the general transport system have most commonly been isolated by resistance to the toxic tripeptide, tri-L-ornithine (Orn_3), (7, 14). The genetic locus defective in Orn_3-resistant strains has been designated *opp* for oligopeptide permease and has been localized near *trp* at 27 min on the *E. coli* chromosome (4, 5) and at 34 min on the *S. typhimurium* chromosome (29). Recently, Higgins et al. (21, 23) demonstrated that in *S. typhimurium*, *opp* is located between *trp* and *galU* and that this locus is a single operon composed of four separate genes.

* Corresponding author.

The results presented in this study demonstrate that the primary step in the transport of peptides by *E. coli* is peptide permeation through the outer membrane channels formed by the *ompF* or *ompC* gene products, or both. Moreover, a new genetic locus, designated *oppE*, which is clearly differentiable from the *trp*-linked *opp* locus, has been identified and shown to affect peptide transport in Omp$^+$ *E. coli* strains. Mutations in both *opp* loci have been mapped to distinct positions on the *E. coli* chromosome, and their effect on utilization of nutritional peptides, sensitivity to toxic peptides, and transport of [^{14}C]L-alanyl-L-alanyl-L-alanine ([^{14}C]Ala_3) has been examined.

MATERIALS AND METHODS

Bacteria and bacteriophages. A list of the *E. coli* strains used in this study is given in Table 1. Bacteriophages P1 *kc*, P1 *clm*ts, φ80, and φ80 *vir* were obtained from P. Bassford. Bacteriophage λ *c*I857 *h*80 *att*80, described by Press et al. (42), was obtained from R. Kadner. The OmpF polypeptide-specific phage K20 and the OmpC polypeptide-specific phage SS4 were obtained from T. Silhavy and C. Schnaitman, respectively.

Media and growth conditions. All *E. coli* strains were cultured in a complete minimal medium containing minimal salts described by Vogel and Bonner (49), 0.2% glucose, and the appropriate supplements of amino acids and vitamins. Other media used in this study were prepared as described by Miller (35). Solid media contained either 0.8 or 1.5% agar.

In growth experiments in which an essential amino acid was provided only in peptide linkage, cells from exponentially growing cultures were harvested, washed with minimal salts solution, and starved for the test amino acid at 37°C for 30 min. The specific growth rate (k) was determined for each strain in tripeptide-supplemented minimal medium over three generations and compared with the growth rate obtained for the same strain and the parental strain cultured in minimal medium containing free amino acids.

TABLE 1. Bacterial strains

Strain	Genotype	Source and comments
KL320	F⁻ *lacI22 lacZ pro-48 met-90 trpA trpR his-85 rpsL azi-9 gyrA* λ⁻ P1ʳ	B. Bachmann. This strain contains a cryptic P1 plasmid.
MC4100	F⁻ *araD139* Δ(*argF-lac*)*U169 relA1 rpsL150 flbB* λ⁻	P. Bassford
KLF23-KL181	F'123 *thi-1 pyrD34 his-68 trp-45 recA1 mtl-2 xyl-7 malA1 galK35 rpsL* λ⁻	B. Bachmann
DM4100	F⁻ *cysB242* λ⁻	B. Bachmann
KY895	F⁻ *tdk-1 ilv-276*	B. Bachmann
JC10240	Hfr(PO45) *srlC300*::Tn*10 recA56 thr-300 ilv-318 rpsE300*	A. J. Clark
PC1	F⁻ *leuB6 thyA47 dnaC1 deoC3 rpsL153* λ⁻	B. Bachmann
NK5525	F⁻ *pro-81*::Tn*10*, IN(*rrnD-rrnE*)*1* λ⁻	B. Bachmann
W2915	F⁻ *thr-1 leuB6 thi-1 proA2 lacY1 galK2 mtl-1 xyl-5 ara-14 supE44* λ⁻	B. Bachmann
UT5028	F⁺ *thi serB zji-1*::Tn*10*	C. Lark. *zji-1*::Tn*10* and *hsd* are 90% cotransducible by P1.
MH450	F⁻ *araD139* Δ(*argF-lac*)*U169 relA1 rpsL150 flbB ompF*::Tn*5* λ⁻	T. Silhavy
CS1230	F⁻ *zei-298*::Tn*10 ompC161 gyrA261*	C. Schnaitman
CS1253	F⁻ *zei-298*::Tn*10* Δ*ompC178 gyrA261*	C. Schnaitman
SS301	As SS320, but *ompF*::Tn*5*	By P1 transduction from MH450
SS305	As SS320, but *zei-298*::Tn*10 ompC161*	By P1 transduction from CS1230
SS306	As SS320, but *zei-298*::Tn*10* Δ*ompC178*	By P1 transduction from CS1253
SS320	As KL320, but P1ˢ	By superinfection with P1 *clm*ts
SS322	As SS320, but *trpA*⁺	By P1 transduction from MC4100
SS323	As SS320, but *tdk-2*	Spontaneous *tdk* mutation
SS324	As SS323, but (λ *c*I857 *h*80 *att*80)	
SS325	As SS322, but (λ *c*I857 *h*80 *att*80)	
SS3011	As SS301, but *zei-298*::Tn*10 ompC161*	By P1 transduction from CS1230
SS3012	As SS301, but *zei-298*::Tn*10* Δ*ompC178*	By P1 transduction from CS1253
SS3210	As KL320, but *opp-1*	Spontaneous Orn_3-resistant mutant
SS3213	As SS3210, but P1ˢ	By superinfection with P1 *clm*ts
SS3220	As KL320, but *opp-2*	Spontaneous Ala-Ala-Ala-P-resistant mutant
SS3230	As KL320, but *opp-3 oppE1*	Spontaneous Ala-Ala-Ala-P-resistant mutant
SS3222	As SS3220, but P1ˢ	By superinfection with P1 *clm*ts
SS3223	As SS3220, but *tonB* (λ *c*I857 *h*80 *att*80)	
SS3232	As SS322, but *oppE1*	
SS3233	As SS3230, but P1ˢ	By superinfection with P1 *clm*ts
SS3240	As SS320, but *zji-2*::Tn*10 oppE1*	
SS4200	As W2915, but *zji-2*::Tn*10 oppE1*	
SS4300	As PC1, but *pro-81*	By P1 transduction from NK5525, then cured of Tn*10* on fusaric acid plates

Transductants were selected and their genotypes were determined as follows: (i) $opp^{+/-}$ was defined as sensitivity or resistance to toxic tripeptides as described in the text and as growth or no growth on minimal media supplemented with 5 μg of glycyl-L-prolyl-L-alanine or L-lysyl-L-histidyl-L-lysine per ml as proline or histidine sources; (ii) the *tonB* mutation was defined by resistance to phage ϕ80 *vir* and colicin D (27); (iii) the *tdk* mutation was identified by determining sensitivity to 5-fluorodeoxyuridine, as described by Hiraga et al. (22, 24); (iv) the *trp*, *cysB*, *thr*, and *serB* mutations were identified by the usual tests for auxotrophy; (v) *deo* mutations were identified by the inability to grow on medium containing 0.2% thymidine as the sole carbon and energy source; (vi) the *dnaC*(Ts) mutation was identified by inability to grow at 39°C (50); and (vii) inheritance of transposon Tn*10* was identified by resistance to 15 μg of tetracycline per ml. The mapping data presented in this study were obtained from analysis of 100 to 300 transductants per cross.

Genetic manipulations. Lysates of P1 *kc* and P1 *clm*ts were prepared on various *E. coli* K-12 strains as described by Rosner (44). Lysates of λ *c*I857 *h*80 *att*80 and ϕ80 were prepared from an appropriate lysogen by thermal or mitomycin C induction, respectively. Transductions and conjugations were as described by Miller (35). Transposon Tn*10* was inserted randomly into the *E. coli* chromosome as described by Kleckner et al. (26). Loss of Tn*10* was selected for as described by Malloy and Nunn (33).

Isolation of peptide transport mutants. Mutants defective in peptide transport were isolated from *E. coli* K-12 strain KL320. A culture was grown in complete minimal medium to a density of 5×10^8 cells per ml. Approximately 10^8 cells were then plated in 2.5 ml of minimal top agar over complete glucose minimal agar. After the top agar layer had solidified, a sterile filter paper disk was placed in the center of each plate and saturated with either 200 μg of Orn_3 or 50 μg of L-alanyl-L-alanyl-D,L-aminoethylphosphonic acid (Ala-Ala-Ala-P). These plates were incubated for 3 to 5 days at 37°C. From within the zone of growth inhibition surrounding each disk, single colonies were isolated and purified.

Response to toxic or nutritional peptides. Toxic peptides with three different modes of activity were used in this study. Orn_3 is an inhibitor of protein synthesis (7, 14), Ala-Ala-Ala-P and L-alanyl-aminoethylphosphonic acid (Ala-Ala-P) are inhibitors of D-alanine racemase (1–3), and leucine- or valine-containing peptides inhibit branched-chain amino acid biosynthesis (12, 15, 28, 47). The ability of a toxic peptide to inhibit bacterial growth was quantitated by deter-

TABLE 2. Outer membrane porin proteins and sensitivity to toxic tripeptides[a]

Strain	OmpC/OmpF porin phenotype	Sensitivity[c] to phage:		Zone of growth inhibition (mm)[b]							
		K20	SS4	Orn_3	Ala-Ala-Ala-P	Val_3	Pro-Val-Asp	Glu-Val-Phe	Phe-Ser-Val	Pro-Val-Gly	Val-Tyr-Val
SS320	F^+C^+	S	S	24	36	30	21	16	22	28	14
SS301	F^-C^+	R	S	20	37	0	0	0	18	25	0
SS305	F^+C^-	S	R	23	33	30	ND	ND	ND	ND	ND
SS306	F^+C^-	S	R	21	36	28	22	17	24	27	18
SS3011	F^-C^-	R	R	0	0	0	ND	ND	ND	ND	ND
SS3012	F^-C^-	R	R	0	0	0	0	0	0	0	0

[a] Sensitivity to the toxic tripeptides was determined as described in the text.
[b] The amount of each peptide tested was Orn_3, 200 μg; Ala-Ala-Ala-P, 100 μg; Val_3, 100 μg; Pro-Val-Asp, 50 μg; Glu-Val-Phe, 50 μg; Phe-Ser-Val, 50 μg; Pro-Val-Gly, 50 μg; and Val-Tyr-Val, 50 μg. The numbers represent the diameter of the clear zone surrounding each disk. A value of 0 indicates that growth of the mutant was not inhibited by the peptide tested. ND, Not done.
[c] S, Sensitive; R, resistant.

mining the size of the inhibitory zone surrounding a filter paper disk saturated with the toxic peptide. For this assay, each strain was grown and plated as described above for the mutant isolation, except that the assay plates were incubated at 30°C for 18 to 20 h. Conversely, the ability of peptides to support the growth of an amino acid auxotroph was quantitated by measuring the zone of growth surrounding a disk saturated with 25 μg of a peptide containing a required amino acid.

Transport of radiolabeled Ala_3. The accumulation of radiolabeled tri-L-alanine (Ala_3) was measured at 37°C in 50-μl reaction mixtures containing 50 mM K-P_i buffer (pH 6.6), 10 mM glucose, and 6.25×10^7 buffer-washed cells harvested from a culture grown to mid-exponential phase in complete glucose minimal medium. The uptake reaction was initiated by the addition of [^{14}C]Ala_3 (specific activity, 168 μCi/μmol) to a final concentration of 20, 2, or 0.5 μM and terminated at the times indicated in Fig. 3 by washing the entire reaction mixture onto nitrocellulose membrane filters with 5 ml of 0.1 M LiCl. The filters were dissolved in 7 ml of Bray scintillation fluid, and the radioactivity of each sample was determined.

Analytical procedures. Peptidase N activity was measured as described by McCaman and Villarejo (34), using alanine-*p*-nitroanilide as substrate. The protein concentration of all samples was measured by the method of Lowry et al. (31).

Chemicals. Orn_3 and tri-L-lysine (Lys_3) were obtained from Miles-Yeda, Rehovat, Israel. Tri-L-valine (Val_3) and L-leucyl-L-tryptophanyl-L-leucine (Leu-Trp-Leu) were purchased from Bachem Inc., Torrance, Calif. The other peptides used in this study were purchased from Vega Biochemicals, Tuscon, Ariz., or Sigma Chemical Co., St. Louis, Mo. All peptides obtained from commercial sources were analyzed for purity before being used in this study. The toxic peptides Ala-Ala-P and Ala-Ala-Ala-P were synthesized by V. L. Styles and R. W. Morrison, Wellcome Research Laboratories. The L-[*U*-^{14}C]alanyl-L-alanyl-L-alanine was synthesized and purified by V. L. Styles and S. A. Short.

RESULTS

Role of the outer membrane porin proteins in peptide permeation. The influence of the *ompC* and *ompF* gene products on peptide permeation through the outer membrane was assessed by measuring the sensitivity of a collection of isogenic Omp mutants, strains SS320 (wild-type), SS301 (*ompF*), SS305 (*ompC*), SS306 (Δ*ompC*), SS3011 (*ompF ompC*), and SS3012 (*ompF* Δ*ompC*) to toxic peptides or by the ability of these mutants to use tripeptides as the sole source of a required amino acid. Because of the emergence of extragenic suppressor mutations in strains harboring *ompC* and *ompF* mutations (8, 43), all cultures for these measurements were inoculated directly from permanent frozen stocks and the sensitivity of each Omp mutant to phages K20 and SS4 was determined simultaneously with the peptide permeation assays. Passage of Val_3, Pro-Val-Asp, Glu-Val-Phe, and Val-Tyr-Val through the outer membrane required the presence of functional OmpF porin protein (Table 2). In contrast, Orn_3, Ala-Ala-Ala-P, Phe-Ser-Val, and Pro-Val-Gly penetration through the outer membrane appears to lack porin specificity and can be prevented only by the loss of both the OmpC and OmpF porin proteins from the outer membrane (Table 2). When each porin mutant was tested for its ability to grow on media in which Gly-Pro-Ala, Lys-His-Lys, Met_3, or Pro_3 served as the sole source of an essential amino acid, only the $OmpF^-$ $OmpC^-$ strains (SS3011 and SS3012) failed to utilize these peptides as amino acid sources. These results indicate that the outer membrane channels formed by the major porin proteins are required for penetration of tripeptides through the outer membrane and that tripeptide passage through these porin channels proceeds with some degree of specificity.

Characterization of tripeptide transport mutants. Derivatives of *E. coli* K-12 strain KL320 isolated as resistant to either Orn_3 or Ala-Ala-Ala-P were screened for cross-resistance to other toxic peptides and for sensitivity or resistance to phages K20 and SS4. All the mutants examined in this study were sensitive to phages K20 and SS4 (i.e., $OmpF^+$ $OmpC^+$) and could be arranged into three phenotypic classes based on their sensitivities to Orn_3, Ala-Ala-Ala-P and Val_3 (Table 3). Mutants forming Opp class 1 (66 of 84 from the Orn_3 selection; 13 of 82 from the Ala-Ala-Ala-P selection) were resistant to Orn_3, had a reduced sensitivity to Ala-Ala-Ala-P, and were sensitive to Val_3. Opp class 2 mutants (18 of 84 from the Orn_3 selection; 61 of 82 from the Ala-Ala-Ala-P selection) were completely resistant to Orn_3 and Ala-Ala-Ala-P but maintained their parental sensitivity to Val_3. Selection for resistance to Ala-Ala-Ala-P yielded a third mutant class in addition to Opp class 1 and 2 mutants. Opp class 3 mutants (8 of 82) were resistant to Orn_3, Ala-Ala-Ala-P, and Val_3. Selection for Orn_3 resistance did not yield Opp class 3 mutants. A representative strain from each Opp phenotypic class was then examined for sensitivity or resistance to two toxic leucine tripeptides and the toxic dipeptides, di-L-valine (Val_2) and Ala-Ala-P. The results obtained

TABLE 3. Growth inhibition of tripeptide transport mutants by toxic peptides[a]

Strain	Opp phenotypic class	Inhibition zone diameter (mm)[b]						
		Orn_3	Ala-Ala-Ala-P	Val_3	Leu_3	Leu-Gly-Gly	Val_2	Ala-Ala-P
KL320	Wild type	25	30	27	14	15	37	42
SS3210	1	0	13	27	12	16	37	41
SS3220	2	0	0	27	14	15	35	33
SS3230	3	0	0	0	0	0	18	0

[a] The inhibition of bacterial growth surrounding a disk saturated with the toxic peptide was measured as described in the text.

[b] The amount of each peptide tested was Orn_3, 400 μg; Ala-Ala-Ala-P, 100 μg; Val_3, 100 μg; Leu_3, 400 μg; Leu-Gly-Gly, 400 μg; Val_2, 150 μg; and Ala-Ala-P, 150 μg. The numbers represent the diameter of the clear zone surrounding each disk. A value of 0 indicates that growth of the mutant was not inhibited by the peptide tested.

with these toxic peptides are consistent with the division of the mutants into three phenotypic classes (Table 3).

The ability of each auxotrophic Opp mutant to utilize tripeptides as amino acid sources was examined by determining the diameter of the growth zone surrounding a disk containing 0.1 μmol of tripeptide. In contrast to the differences in their sensitivity to toxic tripeptides, Opp class 1, 2, and 3 mutants all failed to grow when tripeptides served as the sole source for histidine or tryptophan (Table 4). When tested with prolyl- or methionyl-containing tripeptides, Opp class 1 and 2 mutants gave growth zones which were half the size or the same size, respectively, as the growth zones obtained with the parental strain, whereas the Opp class 3 mutant would not grow with any of the prolyl or methionyl peptides examined (Table 4). When these growth studies were repeated with minimal liquid medium and a tripeptide concentration of 15 μg/ml, identical results were obtained, except for the growth of the Opp class 3 mutant, SS3230, with methionyl peptides. In the presence of 15 μg of Met_3 per ml, strain SS3230 had a specific growth rate (k = 0.200 h^{-1}) which was half that determined for the parental strain. This latter observation is in accordance with the results published by Naider and Becker (36) and indicates that Met_3 can enter *E. coli* via a system which is distinct from the uptake systems that are defective in the Opp class 1, 2, and 3 mutants.

When peptidase N activity was measured for the parental strain and a representative strain from each Opp phenotypic class, the specific activity of this peptidase was found to be essentially identical for all four strains. Moreover, the addition of Orn_3, Ala-Ala-Ala-P or Val_3 to peptidase N assay mixtures prepared with cells of the parental strain significantly inhibited alanine-*p*-nitroanilide hydrolysis (50 to 90% at a 2.5-fold molar excess of competitor; data not shown). These results indicate that the three Opp phenotypic classes described above did not arise as a result of an alteration in peptidase N activity. More importantly, the peptidase N studies indicate that if Ala-Ala-Ala-P or Val_3 could penetrate the permeability barrier posed by the Opp mutants, these peptides would be hydrolyzed, thereby liberating the toxic moiety of these tripeptides.

Mapping of the *trp*-linked *opp* mutations. Experiments were undertaken to define the number and chromosomal location(s) for the genetic lesion(s) carried by the members of each Opp phenotypic class. Transduction of either strain SS3210 (Opp class 1) or SS3220 (Opp class 2) to Trp^+ by using phage P1 grown on two genetically diverse Opp^+ donors resulted in 75 to 85% of the recombinants inheriting sensitivity to Orn_3. In contrast to the Trp-Opp transduction results obtained with strains SS3210 and SS3220 and those published elsewhere (5, 11, 21, 29), transduction of the Opp class 3 mutant, SS3230, to Trp^+ did not result in the restoration of wild-type sensitivity to all the toxic tripeptides used to define the three Opp phenotypic classes. Instead, 75 to 85% of the Trp^+ transductants assumed a novel phenotype, i.e., Orn_3 sensitive, Ala-Ala-Ala-P sensitive, but Val_3, Leu_3, Leu-Gly-Gly, Val_2, and Ala-Ala-P resistant. Strikingly, none of the SS3230 Trp^+ transductants simultaneously inherited sensitivity to this entire battery of toxic peptides. These data suggest that, although Opp class 1 and 2 mutants appear to map to a position similar to that previously reported in *E. coli* (5, 11) and *S. typhimurium* (21, 29), Opp class 3 mutants carry multiple genetic lesions, only one of which is *trp* linked (see below).

Since the Opp class 1 and 2 mutant phenotypes have essentially identical *trp* cotransduction frequencies, strain SS3220 (*opp-2*) was selected for further analysis. Examination of the recombinant classes obtained from transductions involving strains SS3220 (*trp*, *opp-2*, tdk^+), DM4100 (*cysB*, trp^+, opp^+), and KY895 (trp^+, opp^+, *tdk*) as both donors and recipients demonstrated that the *opp-2* mutation was located between *trp* and *tdk*, in contrast to results previously reported for *E. coli* (5, 11) but in agreement with the *S. typhimurium* mapping data (21, 29). The location of the *opp-2* mutation with respect to *tonB*, *att*φ80, *galU*, and *tdk* was examined next. The position of the *opp-2* mutation in relation to the *att*φ80 locus was determined by examining the effect of a prophage integrated at *att*φ80 on the *trp opp-2* cotransduction frequency. In these crosses, the *trp tonB* cotransduction frequency was unaltered, irrespective of the presence of λ *c*I857 *h*80 *att*80 integrated into the chromosome of the donor strain. In contrast, the *trp opp-2* and *trp tdk* cotransduction frequencies decreased 5- to 15-fold upon integration of this prophage at *att*φ80. When three-factor crosses were performed with Trp^- $GalU^-$ Tdk^+ and Trp^+ $GalU^+$ Tdk^- strains as both donors and recipients, a gene order of *trp*, *galU*, *tdk* was indicated. Finally, analysis of three-factor crosses involving the *opp-2* mutation, *galU*, and *tdk* indicated a gene order of *opp*, *galU*, *tdk*. A summary of the mapping data in which the *opp-1* and *opp-2* mutations have been positioned in the *tdk-cysB* region of the *E. coli* chromosome is presented in Fig. 1.

Hogarth and Higgins (23) have shown that the *S. typhimurium trp*-linked *opp* locus consists of four genes, designated *oppA*, *oppB*, *oppC*, and *oppD*, organized into a single operon. To determine the relationship of the *trp*-linked *opp* alleles defined in this study to those defined in *S. typhimurium*, we obtained from C. Higgins a collection of *E. coli* F'123 derivatives carrying characterized *oppA*, *oppB*, *oppC*, or *oppD* mutations. Results from complementation studies with these Opp^- F'123 derivatives and *recA* derivatives of each Opp mutant class demonstrated that (i) strain SS3210 (Opp class 1) harbors a mutation (*opp-1*) affecting expression of the entire *opp* operon; (ii) strain SS3220 (Opp class 2) carries a mutation (*opp-2*) affecting only *oppD* expression; and (iii) in strain SS3230 (Opp class 3), the *trp*-linked mutation (*opp-3*) affects *oppB*, *oppC*, and *oppD* expression. The *trp*-linked *opp* mutations carried by SS3210 and SS3230 do not appear to result from extensive deletions within the *opp* operon, since P1 lysates prepared on these two strains readily transduce each other and SS3220 to Opp^+.

Mapping of the *oppE* mutation. SS3230, an Opp class 3 mutant, was shown to contain a second mutation affecting

TABLE 4. Ability of the Opp mutants to grow on peptides as the sole source of an essential amino acid[a]

Strain	Diam of growth zone (mm) on:				
	Lys-His-Lys or Gly-His-Gly	Lys-Trp-Lys- or Gly-Trp-Gly	Gly-Pro-Ala or Pro-Gly-Gly	Met-Met-Met or Met-Ala-Ser	Met-Leu-Phe
SS320 (wild type)	25	27	24	27	25
SS3213 (Opp class 1)	0	0	12	26	21
SS3222 (Opp class 2)	0	0	13	26	22
SS3233 (Opp class 3)	0	0	0	0	0

[a] The ability of each strain to use a peptide as the sole source of an essential amino acid was determined as described in the text. For this determination, 0.1 μmol of amino acid or amino acid in peptide linkage was applied to each disk. These strains all gave 30 to 33-mm growth zones with L-histidine, L-tryptophan, L-proline, and L-methionine.

peptide transport in addition to the mutation preventing expression of the *trp*-linked *oppBCD* genes. The genetic locus affected by this second mutation has been designated *oppE* and is required for sensitivity to Val_3, Leu_3, Leu-Gly-Gly, Val-Leu-Ser, Thr-Val-Leu, Val_2, Pro-Val, and Ala-Ala-P, and for growth on Gly-Pro-Ala as a proline source.

Since *oppE* is not *trp*-linked and was shown to impart a unique peptide sensitivity phenotype, it was of interest to determine the location of *oppE* on the *E. coli* chromosome. To facilitate the mapping of *oppE*, transposon Tn*10* was inserted near this mutation. One Tn*10* insertion (*zji-2*::Tn*10*), which was cotransducible with *oppE* at a frequency of 0.52, was moved via P1 transduction into a collection of Hfr strains. When members of this collection were mated with appropriate recipients, the Hfr strains which transferred the *thr* region early also transferred *zji-2*::Tn*10* early.

Based on the results from the mating experiments, the location of *zji-2*::Tn*10* and *oppE* in relation to the other genes in the 98- to 100-min region of the *E. coli* chromosome was examined. Transduction of PC1 (*dnaCdeoC*) to tetracycline resistance with a P1 lysate prepared on strain SS4200 (*zji-2*::Tn*10 thr*) revealed that *zji-2*::Tn*10* was located on the *deo,thr* distal side of *dnaC*. The interval between *dnaC* and *hsd* was then examined by transduction of strains SS3232 (*oppE*) and PC1 (*dnaC*) to tetracycline resistance with a P1 lysate prepared on strain UT5028 (*zji-1*::Tn*10 serB*). On screening for the $OppE^+$, Ser^+, and temperature-resistance phenotypes, a gene order of (*hsd*) *zji-1*::Tn*10 oppE zji-2*::Tn*10 dnaC serB* was suggested. This order was supported by the recombinant classes obtained when SS4300 (*dnaC*) was transduced to temperature resistance with a P1 lysate prepared on SS4200 (*oppE zji-2*::Tn*10*). Of the temperature-resistant, $OppE^-$ transductants, the majority were tetracycline resistant, confirming the order *dnaC zji-2*::Tn*10 oppE*. A summary of the mapping data in which the *oppE* locus has been ordered within the *hsd-thr* region of the *E. coli* chromosome is presented in Fig. 2.

Influence of the *opp* mutations on Ala_3 transport. *E. coli* SS320 actively transported Ala_3 achieving an intracellular-Ala_3 concentration (11.87 mM) which was 600-fold higher

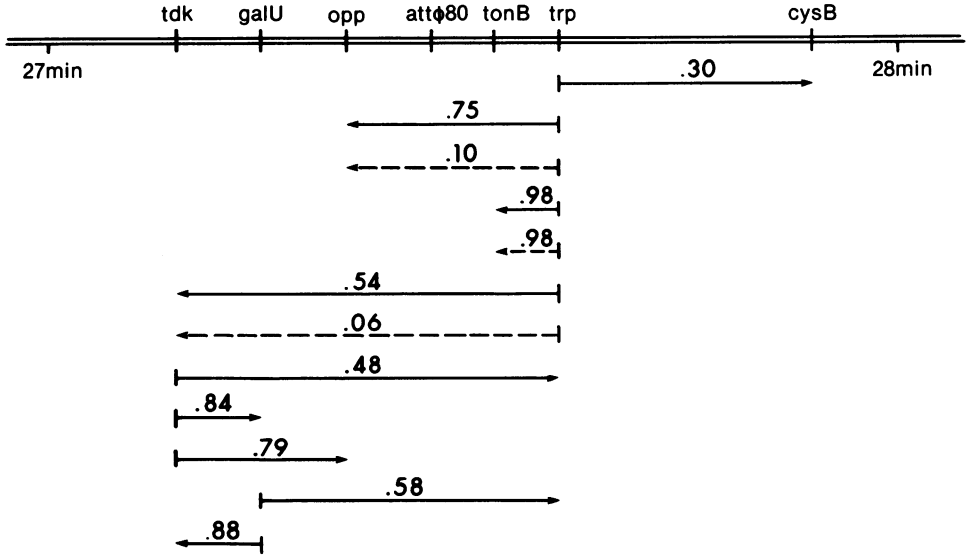

FIG. 1. Linkage of the genes in the *tdk-cysB* region of the *E. coli* chromosome. The number above each arrow represents the cotransductional frequency. For each gene pair, the arrowhead is directed toward the unselected marker of the cross. The numbers above the dashed arrows represent the cotransductional frequencies obtained when phage λ *c*I857 *h*80 *att*80 was integrated at *att*ϕ80. The distances are not drawn to scale.

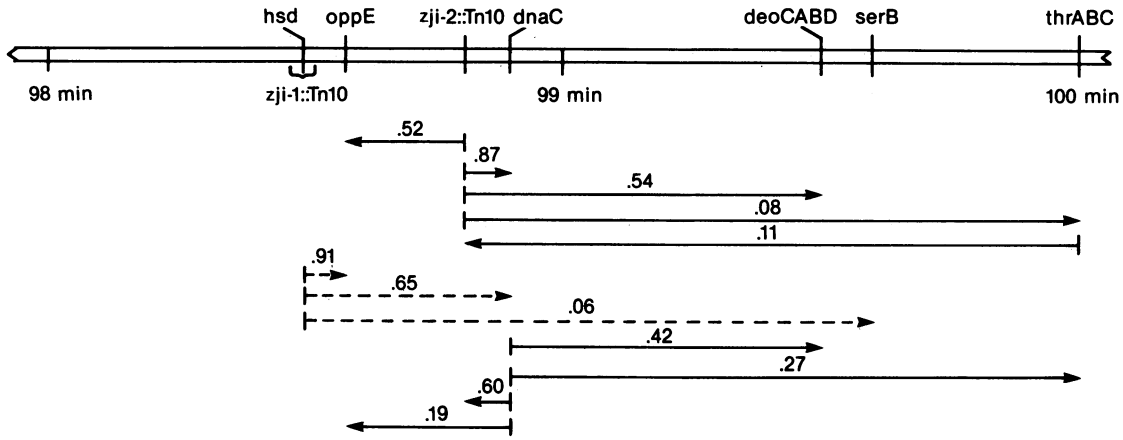

FIG. 2. Gene order determined for the *thr-hsd* region of the *E. coli* chromosome. The number above each arrow represents the cotransductional frequency. For each gene pair, the arrowhead is directed toward the unselected marker of the cross. The position of *zji-1*::Tn*10* relative to *hsd* has not been determined, although these two loci are 90% cotransducible. Therefore, the numbers above the dashed arrows represent the cotransductional frequencies obtained from crosses involving *zji-1*::Tn*10* and *oppE*, *dnaC*, and *serB*. The distances in this figure are not drawn to scale.

than the extracellular peptide concentration (0.02 mM) (Fig. 3A). This [^{14}C]Ala_3 uptake was found to be peptide specific in that only certain tripeptides, such as Val_3, Ala-Cys-Ala and Ala_2, effectively competed with [^{14}C]Ala_3 for uptake (Fig. 3A). The tripeptides Orn_3, Lys_3, Gly_3, and Ala-Ala-Ala-P or individual amino acids did not compete with [^{14}C]Ala_3 in these transport studies (Fig. 3A).

When [^{14}C]Ala_3 uptake was measured for the wild-type strain SS320 and for each peptide transport mutant, the data clearly indicated that loss of a functional *oppE* gene yielded *E. coli* strains, SS3230 [*oppA*$^+$ *oppBCD oppE*] and SS3240 [*opp(ABCD)*$^+$ *oppE*], in which the transport of [^{14}C]Ala_3 was significantly reduced compared with that measured for the wild-type strain SS320 (Fig. 3B). Both the initial rates and apparent steady-state levels of [^{14}C]Ala_3 transport observed for strains SS3230 and SS3240 were reduced to levels which were less than 5% of those measured for strain SS320. In contrast, the [^{14}C]Ala_3 accumulation measured for the Opp class 1 (SS3210) and class 2 mutant (SS3220), which are both OppE$^+$, was found to be approximately equal to the accumulation measured for SS320 (Fig. 3B). On reduction of the [^{14}C]Ala_3 concentration of the transport assay medium from 20 to 2 μM (or to 0.5 μM), the initial rates of Ala_3 uptake measured for all the mutants were now reduced compared with the wild-type strain. Strains SS3230 and SS3240, both of which harbor an *oppE* mutation, had initial rates of

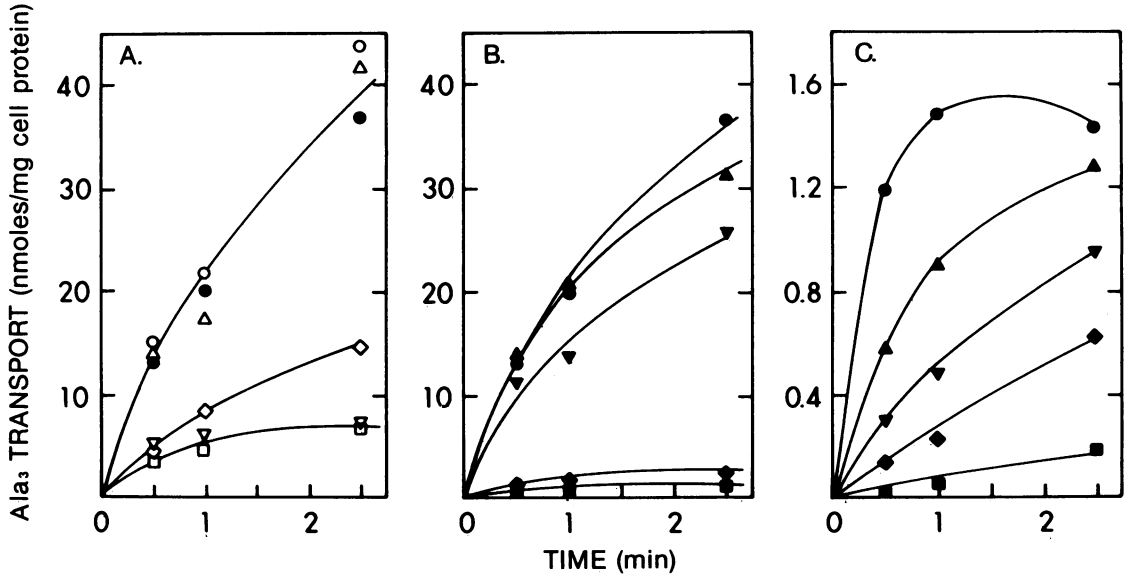

FIG. 3. Transport of Ala_3 by wild-type and Opp$^-$ *E. coli* strains. The transport of [^{14}C]Ala_3 was measured as described in the text, with *E. coli* SS320, wild-type, (●); SS3210, *opp(ABCD) oppE*$^+$, (▲); SS3220, *opp(ABC)*$^+$ *oppD oppE*$^+$, (▼); SS3230, *oppA*$^+$ *oppBCD oppE*, (■); and SS3240, *opp(ABCD)*$^+$ *oppE* (◆). A, Competition for the transport of [^{14}C]Ala_3 (20 μM) by SS320 (●) was determined in the presence of 200 μM peptide competitor: Val_3 (□); Ala-Cys-Ala (▽); Ala_2 (◇); Orn_3 (○); and Ala-Ala-Ala-P (△). B and C [^{14}C]Ala_3 uptake by the wild-type strain and each Opp$^-$ mutant was measured with extracellular [^{14}C]Ala_3 concentrations of 20 (B) and 2 μM (C).

[^{14}C]Ala_3 uptake which were 2 and 10%, respectively, of the rate determined for SS320 (Fig. 3C). Strains SS3210 (Opclass 1) and SS3220 (Opp class 2) had initial rates of Ala_3 transport which were 50 and 30%, respectively, of that measured for the wild-type strain (Fig. 3C). In control experiments, the transport of radiolabeled alanine, serine, proline, glutamine, and histidine was found to be identical for the wild-type strain and all the peptide transport mutants. This observation indicates that the defect in peptide transport shown by the Opp$^-$ mutants does not result from a defect in the general energy coupling of these strains. The [^{14}C]Ala_3 uptake studies do demonstrate, however, that the *oppE* gene product is obligatorily involved in Ala_3 transport and suggest that, at low tripeptide concentrations, mutations in the *trp*-linked peptide transport genes reduce the efficiency with which OppE$^+$ strains transport Ala_3.

DISCUSSION

The ability of *E. coli* to utilize peptides as carbon and energy sources requires the simultaneous expression of multiple genes, the products of which are components of the cell permeability barriers, the cytoplasmic and outer membranes. Growth of *E. coli*, either on peptides as the sole source of a required amino acid or in the presence of a number of toxic peptides, was drastically affected by mutations in the structural genes which encode the major outer membrane porin proteins, OmpC and OmpF. Moreover, mutations in two additional loci have been shown to affect peptide transport in *E. coli*. One locus, *oppABCD*, appears similar to that described previously in *S. typhimurium* (21, 29). The second locus which was identified and mapped in this study has been designated *oppE* and is so far unique to *E. coli*. The peptide phenotypes, [^{14}C]Ala_3 transport properties, and SS4 and K20 phage sensitivities of the strains harboring *oppABCD* or *oppE* mutations, or both, indicate that these mutations affect peptide permeation through an alteration in the periplasmic binding proteins or in the peptide transport systems of the cytoplasmic membrane.

The influence of the composition of the outer membrane porin channels on peptide uptake has been examined with a series of isogenic Omp mutants. Data obtained in this study indicate that passage of peptides such as Orn_3, Ala-Ala-Ala-P, Gly-Pro-Ala, or Lys-His-Lys through the outer membrane is independent of the OmpC-OmpF composition of the porin channels in that passage of these peptides through the outer membrane is prevented only by loss of both the *ompC* and *ompF* gene products. In contrast, Val_3, Pro-Val-Asp, and Glu-Val-Phe passage specifically requires functional OmpF protein incorporated into the outer membrane channels and will not proceed to any significant extent through channels formed only by the OmpC porin (Table 2). This observation modifies the original proposal that the role played by the OmpF and OmpC porin proteins in outer membrane permeability is interchangeable (32, 43) and that passage of a molecule through the outer membrane porin channels is dependent solely on molecular size (10, 40). The data presented in this study also indicate that elimination of both major porins from the *E. coli* outer membrane results in a cell in which the primary means for peptide permeation through the outer membrane has been lost (Table 2). The observation that strains SS3011 and SS3012 (*ompF ompC*) were unable to grow on any nutritional peptide tested and were resistant to all toxic peptides indicates that permeation of peptides through the *E. coli* outer membrane does not proceed through channels composed of peptide-specific porin proteins. If outer membrane channels composed of peptide-specific porins did exist in *E. coli*, then, by analogy to the nucleoside-specific Tsx porin (16, 32, 48) and the maltose-specific LamB protein (45, 46), strains SS3011 and SS3012 would grow on nutritional peptides and remain sensitive to toxic peptides when these molecules were present at low extracellular concentrations (Table 2). These results, however, do not preclude the possibility that peptides, when present in the medium to high concentrations, can cross the outer membrane through channels formed by other outer membrane proteins, as has been suggested from the work of Heuzenroeder and Reeves (18, 19). The data obtained with strains SS3011 and SS3012 also indicate that a mutation in *ompR* would, in a single step, lead to a cell which is phenotypically peptide transport negative but which retains completely functional *oppABCD* and *oppE* genes.

Two distinct genetic loci involved in the concentrative accumulation of peptides by *E. coli* have been identified in this study. One *opp* locus was found to be linked to the *trp* operon similar to the *S. typhimurium opp* locus, the gene order in *E. coli* being *trp tonB attϕ80 opp-2(opp-1) galU tdk* (Fig. 1). The second locus, which we have designated *oppE*, has been mapped to a position within the *dnaC-hsd* interval of the *E. coli* chromosome (Fig. 2).

A comparison of the sensitivity to toxic tripeptides determined for the wild-type strain SS320 with the sensitivities determined for strains SS3210 (*oppABCD*), SS3220 (*oppD*), and the Trp$^+$ transductants of SS3230, i.e., SS3240 (*oppE*), indicates that Orn_3 and Ala-Ala-Ala-P enter the cell primarily via the peptide transport system encoded by the *trp*-linked *oppABCD* genes. The toxic tripeptides Val_3, Leu_3, Leu-Gly-Gly, Val-Leu-Ser, and Thr-Val-Leu enter the cell primarily via the transport system encoded by the gene(s) mapping at the *oppE* locus. This assignment of different peptide transport systems for the uptake of these two families of toxic tripeptides by *E. coli* is supported by and consistent with the results from the [^{14}C]Ala_3 transport studies. When [^{14}C]Ala_3 transport was measured with wild-type *E. coli* SS320, neither the initial rate nor the apparent steady-state level of uptake was inhibited by Orn_3, Lys_3, Gly_3, or Ala-Ala-Ala-P (Fig. 3A). In contrast, Val_3, Ala-Cys-Ala, and Ala_2 significantly inhibited [^{14}C]Ala_3 transport measured with SS320 (Fig. 3A). In accord with these results, [^{14}C]Ala_3 uptake measured with *E. coli* strains harboring mutations in only the *trp*-linked *oppABCD* genes or in only the genes of the *oppE* locus indicate that a mutation in the *oppABCD* genes does not markedly affect [^{14}C]Ala_3 accumulation, whereas a mutation in the *oppE* locus yields strains in which [^{14}C]Ala_3 transport is reduced to a level of less than 5% of that measured for the wild-type strain (Fig. 3B). Apart from indicating the specificity of the peptide transport systems as described above, transport studies in which the extracellular [^{14}C]Ala_3 concentration was 2 μM (or 0.5 μM) instead of 20 μM revealed that all the peptide transport mutants isolated in this study had lower initial rates of [^{14}C]Ala_3 uptake when compared with the wild-type strain (Fig. 3C). This observation suggests that Ala_3 transport in *E. coli* may proceed via two systems which differ in their apparent K_m and V_{max} values and is not incompatible with the toxic peptide sensitivity data or the transport data obtained with 20 μM [^{14}C]Ala_3 but may simply reflect a marked difference in the velocity of peptide translocation catalyzed by each transport system.

The number of distinct peptide transport systems in *E. coli* and the relative importance of each system in peptide utilization remains to be determined. Direct evidence for the existence in *E. coli* of at least two transport systems which

recognize tripeptides is presented in this study. The distinction between these systems has been defined not only genetically but also on the basis of transport substrates preferred by each system. In addition to the systems encoded by the *oppABCD* operon and the *oppE* gene(s), data presented in this study and in the work of Naider and Becker (36) indicate that Met_3 is transported by yet another transport system. In *S. typhimurium*, Higgins and coworkers have identified another peptide transport system, designated *tppB*, which maps at 27 min in the *S. typhimurium* cotransduction gap and is induced by leucine or anaerobiosis (13, 25). With *E. coli* SS3230 (*oppBCD oppE*), the addition of leucine does not result in growth of this strain on nutritional peptides nor in sensitivity to toxic peptides (data not shown). The leucine non-responsiveness of strain SS3230 suggests that (i) the *tppB*-encoded peptide transport system may be specific to *S. typhimurium* and does not exist in *E. coli*; (ii) in *E. coli*, *oppE* is required for expression of the *tppB*-encoded system; (iii) *oppE* and *tppB* code for the same transport system but have vastly different chromosomal locations in *E. coli* and *S. typhimurium*; or (iv) SS3230 has a mutation in *tppB* in addition to mutations in the *oppABCD* operon and the *oppE* gene(s). This last option, however, appears unlikely on the basis of results obtained in the analysis of the mutations carried by strain SS3230. Therefore, as indicated above, the exact number of peptide transport systems and the substrate specificity of each system in *E. coli* remains to be determined, as well as the functional relationship of the *E. coli* transport systems to those defined in *S. typhimurium*.

ACKNOWLEDGMENTS

We thank P. Bassford, A. J. Clark, R. Kadner, C. Lark, C. Schnaitman, T. Silhavy, and B. Bachmann for bacterial strains and phages used in this study. Special thanks are due to Chris Higgins, who not only shared his *opp* mutants but also communicated results before publication.

LITERATURE CITED

1. **Allen, J. G., F. R. Atherton, M. J. Hall, C. H. Hassall, S. W. Holmes, R. W. Lambert, L. J. Nisbet, and P. S. Ringrose.** 1978. Phosphonopeptides, a new class of synthetic antibacterial agents. Nature (London) **272**:56–58.
2. **Atherton, F. R., M. J. Hall, C. H. Hassall, R. W. Lambert, W. J. Lloyd, and P. S. Ringrose.** 1979. Phosphonopeptides as antibacterial agents: mechanism of action of alaphosphin. Antimicrob. Agents Chemother. **15**:696–705.
3. **Atherton, F. R., M. J. Hall, C. H. Hassall, R. W. Lambert, W. J. Lloyd, P. S. Ringrose, and D. Westmacott.** 1983. Phosphonopeptides as substrates for peptide transport systems and peptidases of *Escherichia coli*. Antimicrob. Agents Chemother. **24**:522–528.
4. **Bachmann, B. J.** 1983. Linkage map of *Escherichia coli* K-12, edition 7. Microbiol. Rev. **47**:180–230.
5. **Barak, Z., and C. Gilvarg.** 1974. Triorinthine resistant strains of *Escherichia coli*. Isolation, definition, and genetic studies. J. Biol. Chem. **249**:143–148.
6. **Barak, Z., and C. Gilvarg.** 1975. Specialized peptide transport system in *Escherichia coli*. J. Bacteriol. **122**:1200–1207.
7. **Barak, Z., S. Sarid, and E. Katchalski.** 1973. Inhibition of protein synthesis in *Escherichia coli* by tri-L-ornithine. Eur. J. Biochem. **34**:317–324.
8. **Bavoil, P., H. Nikaido, and K. von Meyerburg.** 1977. Pleiotropic transport mutants of *Escherichia coli* lack porin, a major outer membrane protein. Mol. Gen. Genet. **158**:23–33.
9. **Cowell, J. L.** 1974. Energetics of glycylglycine transport in *Escherichia coli*. J. Bacteriol. **120**:139–146.
10. **Decad, G. M., and H. Nikaido.** 1976. Outer membrane of gram-negative bacteria. XII. Molecular-sieving function of cell wall. J. Bacteriol. **128**:325–336.
11. **DeFelice, M., J. Guardiola, A. Lamberti, and M. Iaccarino.** 1973. *Escherichia coli* K-12 mutants altered in the transport systems for oligo- and dipeptides. J. Bacteriol. **116**:751–756.
12. **DeFelice, M., M. Levinthal, M. Iaccarino, and J. Guardiola.** 1979. Growth inhibition as a consequence of antagonism between related amino acids: effect of valine in *Escherichia coli* K-12. Microbiol. Rev. **43**:42–58.
13. **Gibson, M. M., M. Price, and C. F. Higgins.** 1984. Genetic characterization and molecular cloning of the tripeptide permease (*tpp*) genes of *Salmonella typhimurium*. J. Bacteriol. **160**:122–130.
14. **Gilvarg, C., and Y. Levin.** 1972. Response of *Escherichia coli* to ornithyl peptides. J. Biol. Chem. **247**:543–549.
15. **Gollop, N., H. Tavori, and Z. Barak.** 1982. Acetohydroxy acid synthase is a target for leucine-containing peptide toxicity in *Escherichia coli*. J. Bacteriol. **149**:387–390.
16. **Hantke, K.** 1976. Phage T6-colicin K receptor and nucleoside transport in *Escherichia coli*. FEBS Lett. **70**:109–112.
17. **Hermsdorf, C. L., and S. Simmonds.** 1980. Role of peptidases in utilization and transport of peptides by bacteria, p. 301–334. *In* J. W. Payne (ed.), Microorganisms and nitrogen sources. John Wiley & Sons, Inc., New York.
18. **Heuzenroeder, M. W., and P. Reeves.** 1980. Periplasmic maltose-binding protein confers specificity on the outer membrane maltose pore of *Escherichia coli*. J. Bacteriol. **141**:431–435.
19. **Heuzenroeder, M. W., and P. Reeves.** 1981. The *tsx* protein of *Escherichia coli* can act as a pore for amino acids. J. Bacteriol. **147**:1113–1116.
20. **Higgins, C. F., and M. M. Hardie.** 1983. Periplasmic protein associated with the oligopeptide permeases of *Salmonella typhimurium* and *Escherichia coli*. J. Bacteriol. **155**:1434–1438.
21. **Higgins, C. F., M. M. Hardie, D. Jamieson, and L. M. Powell.** 1983. Genetic map of the *opp* (oligopeptide permease) locus of *Salmonella typhimurium*. J. Bacteriol. **153**:830–836.
22. **Hiraga, S., K. Igarashi, and T. Yura.** 1967. A deoxythymidine kinase-deficient mutant of *Escherichia coli*. I. Isolation and some properties. Biochim. Biophys. Acta **145**:41–51.
23. **Hogarth, B. G., and C. F. Higgins.** 1983. Genetic organization of the oligopeptide permease (*opp*) locus of *Salmonella typhimurium* and *Escherichia coli*. J. Bacteriol. **153**:1548–1551.
24. **Igarashi, K., S. Hiraga, and T. Yura.** 1967. A deoxythymidine kinase deficient mutant of *Escherichia coli*. II. Mapping and transduction studies with phage O80. Genetics **57**:643–654.
25. **Jamieson, D., and C. F. Higgins.** 1984. Anaerobic and leucine-dependent expression of a peptide transport gene in *Salmonella typhimurium*. J. Bacteriol. **160**:131–136.
26. **Kleckner, N., J. Roth, and D. Botstein.** 1977. Genetic engineering in vivo using translocatable drug-resistance elements. J. Mol. Biol. **116**:125–159.
27. **Konisky, J.** 1979. Specific transport systems and receptors for colicins and phages, p. 319–359. *In* M. Inouye (ed.), Bacterial outer membranes: biogenesis and function. John Wiley & Sons, Inc., New York.
28. **Leavitt, R. I., and H. E. Umbarger.** 1962. Isoleucine and valine metabolism in *Escherichia coli*. XI. Valine inhibition of the growth of *Escherichia coli* strain K-12. J. Bacteriol. **83**:624–630.
29. **Lenny, A. B., and P. Margolin.** 1980. Locations of the *opp* and *supX* genes of *Salmonella typhimurium* and *Escherichia coli*. J. Bacteriol. **143**:747–752.
30. **Low, K. B.** 1972. *Escherichia coli* K-12 F-prime factors, old and new. Bacteriol. Rev. **36**:587–607.
31. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **143**:265–275.
32. **Lutkenhaus, J. F.** 1977. Role of a major outer membrane protein in *Escherichia coli*. J. Bacteriol. **131**:631–637.
33. **Maloy, S. R., and W. D. Nunn.** 1981. Selection for loss of tetracycline resistance by *Escherichia coli*. J. Bacteriol. **145**: 1110–1112.
34. **McCaman, M. T., and M. R. Villarejo.** 1982. Structural and catalytic properties of peptidase N from *Escherichia coli* K-12.

Arch. Biochem. Biophys. **213**:384–394.
35. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
36. **Naider, F., and J. M. Becker.** 1975. Multiplicity of oligopeptide transport systems in *Escherichia coli*. J. Bacteriol. **122**: 1208–1215.
37. **Payne, J. W.** 1980. Transport and utilization of peptides by bacteria, p. 211–256. *In* J. W. Payne (ed.), Microorganisms and nitrogen sources. John Wiley & Sons, Inc., New York.
38. **Payne, J. W.** 1980. Regulation of peptide transport in bacteria, p. 335–358. *In* J. W. Payne (ed.), Microorganisms and nitrogen sources. John Wiley & Sons, Inc., New York.
39. **Payne, J. W., and G. Bell.** 1979. Direct determination of the properties of peptide transport systems in *Escherichia coli*, using a fluorescent-labeling procedure. J. Bacteriol. **137**:447–455.
40. **Payne, J. W., and C. Gilvarg.** 1968. Size restriction on peptide utilization in *E. coli*. J. Biol. Chem. **243**:6291–6299.
41. **Payne, J. W., and C. Gilvarg.** 1978. Transport of peptides in bacteria, p. 325–369. *In* B. P. Rosen (ed.), Bacterial transport. Marcel Dekker, Inc., New York.
42. **Press, R., N. Glansdorff, P. Miner, J. DeVries, R. Kadner, and W. K. Maas.** 1971. Isolation of transducing particles of ϕ80 bacteriophage that carry different regions of the *Escherichia coli* genome. Proc. Natl. Acad. Sci. U.S.A. **68**:795–798.
43. **Pugsley, A. P., and C. A. Schnaitman.** 1978. Identification of three genes controlling production of new outer membrane pore proteins in *Escherichia coli* K-12. J. Bacteriol. **135**:1118–1129.
44. **Rosner, J. L.** 1972. Formation, induction, and curing of bacteriophage P1 lysogens. Virology **49**:679–689.
45. **Szmelcman, S., and M. Hofnung.** 1975. Maltose transport in *Escherichia coli* K-12: involvement of the bacteriophage lambda receptor. J. Bacteriol. **124**:112–118.
46. **Szmelcman, S., M. Schwartz, T. J. Silhavy, and W. Boos.** 1976. Maltose transport in *Escherichia coli* K12. Eur. J. Biochem. **65**:13–19.
47. **Tavori, H., Y. Kimmel, and Z. Barak.** 1981. Toxicity of leucine-containing peptides in *Escherichia coli* caused by circumvention of leucine transport regulation. J. Bacteriol. **146**:676–683.
48. **van Alphen, W., N. van Selm, and B. Lugtenberg.** 1978. Pores in the outer membrane of *Escherichia coli* K12. Mol. Gen. Genet. **159**:75–83.
49. **Vogel, H. J., and D. M. Bonner.** 1956. Acetylornithinase of *Escherichia coli*: partial purification and some properties. J. Biol. Chem. **218**:97–106.
50. **Wechsler, J. A.** 1975. Genetic and phenotypic characterization of *dnaC* mutations. J. Bacteriol. **121**:594–599.