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Genetic Map of the *opp* (Oligopeptide Permease) Locus of *Salmonella typhimurium*

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The uptake of peptides by *Salmonella typhimurium* is mediated by three apparently independent transport systems. One of these systems, the oligopeptide permease, is encoded by a genetic locus (*opp*) which has been mapped at 34 min on the *S. typhimurium* chromosomal map. We accurately mapped the location of *opp* by cotransduction frequencies and by deletion analysis and show that the gene order for this region of the chromosome is *cysB-trp-tonB-opp-galU-tdk*. All *opp* mutants, independently isolated by a variety of means, mapped at this one locus, between *tonB* and *galU*. Spontaneous and transposon Tn*10*-generated deletions were used to construct a fine-structure genetic map of *opp*. Evidence is presented which indicates that *opp* covers a 5- to 6-kb segment of DNA and is therefore likely to consist of more than one gene.

Peptides are able to serve efficiently as both sole carbon and sole nitrogen source for the growth of enteric bacteria (11). The rate-limiting step in peptide utilization is generally the uptake into the cell (25). Three apparently independent and genetically distinct transport systems serve to mediate peptide transport in both *Escherichia coli* and *Salmonella typhimurium*; these systems are peptide specific and do not handle free amino acids (25). The dipeptide permease is relatively specific for peptides of only two amino acid residues. The oligopeptide permease on the other hand will efficiently handle both dipeptides and oligopeptides containing up to five amino acids (27). Both systems show little specificity for the nature of the amino acid residues which comprise the peptide (5, 23). A third peptide transport system, handling both di- and oligopeptides but with a rather restricted specificity toward the composition of the peptide has also been identified in both *S. typhimurium* and *E. coli* (4, 15, 21), although as yet this system remains poorly characterized.

Although a considerable amount is known about their substrate specificities, these three transport systems are poorly characterized genetically. Mutants defective in the dipeptide permease (*dpp*) have been mapped at about 13 min on the *E. coli* chromosome (2), and the restricted oligopeptide transport system is believed to map between 84 and 88 min (R. Alves and J. W. Payne, personal communication). The gene(s) encoding the oligopeptide permease (*opp*) has been mapped near the *trp* locus at 27 min in *E. coli* (2) and at 34 min in *S. typhimurium* (29), within the chromosomal segment which is inverted in *S. typhimurium* with respect to *E. coli* (29). However, there has been some disagreement as to the precise location of *opp*; it has been variously positioned on either side of *trp* and at different distances from this locus (3, 10, 19). Recently, Lenny and Margolin obtained evidence positioning *opp* between *tonB* and *galU* in both *E. coli* and *S. typhimurium* (19). We here confirm this location and show that all *opp* mutations map at this one locus. A detailed genetic map of the *opp* locus in *S. typhimurium* is presented which will be extremely useful in further genetic investigations into the mechanisms and regulation of oligopeptide transport in bacteria.

MATERIALS AND METHODS

Bacterial strains. All strains used in this study were derivatives of *S. typhimurium* LT2. All *opp* mutations were isolated either from the wild type or from the proline auxotroph CH2. These strains are identified in Fig. 1. The genotypes and sources of all other strains used in this study are listed in Table 1. Where necessary, strains harboring an *opp* mutation were transduced to proline auxotrophy by using P22 grown on strain TT184 (*pro662*::Tn*10*). *galE* derivatives were constructed by a two-step procedure involving transduction to Tet^r with JL2688 (*bio203*::Tn*10*) as donor followed by transduction to Bio$^+$ with CH20 as donor (*gal* and *bio* are about 15% linked). *recA* derivatives were constructed by a similar two-step process with strains TT520 (*srl*::Tn*10*) and TR2951 (*recA*$^-$) as donors, making use of the linkage between *srl* and *recA*.

Media. Bacteria were routinely grown at 37°C with aeration in LB medium (20). Crosses and screening for auxotrophic requirements were performed on minimal

agar plates (28); where necessary, amino acid (10 μmol) or peptide (2 μmol) supplements were spread on each plate immediately before use. Tetracycline was used at 25 μg ml^{-1} in LB medium and LB agar plates and at 10 μg ml^{-1} in minimal agar plates.

Identification of genotypes and phenotypes. Amino acid auxotrophy was determined by radial streaking on minimal glucose plates around filter paper disks impregnated with the appropriate amino acid (1 μmol). The Opp^- phenotype was screened by resistance to 400 μM triornithine and also, in the case of strains which were auxotrophic for proline, by their inability to grow on minimal agar plates supplemented with 80 μM L-prolylglycylglycine (Pro-Gly-Gly; see below). Mutations in the *tonB* locus were detected by their sensitivity to chromium ions (7) and by slow growth on LB agar (made with Oxoid tryptone) which is stimulated to normal growth by adding 100 μM $FeCl_3$ (34). A *galU* deficiency was identified by inability to ferment galactose on MacConkey or tetrazolium indicator plates (20) and by the poor efficiency with which they plaque phage P22 (35). The deoxythymidylate kinase phenotype was identified according to the procedure of Igarashi et al. (14).

Isolation of *opp* mutations. Most *opp* mutations were isolated by resistance to the toxic peptide triornithine (3). Cells (2×10^8) were spread on a minimal agar plate, and colonies growing within the zone of inhibition around a filter paper disk containing 1 μmol of triornithine were selected. That triornithine-resistant mutants were indeed oligopeptide transport-deficient was determined by screening for the loss of ability to utilize various oligopeptides as a source of a required amino acid while retaining the ability to utilize dipeptides. Certain Opp^- derivatives (see below) were isolated by penicillin selection (20) for proline auxotrophs unable to grow on Pro-Gly-Gly as a source of proline.

Tn*10*-induced deletions were isolated as Tet^s derivatives by the fusaric acid selection procedure of Bochner et al. (6). Deletions extending from a *trp*::Tn*10* insertion into *opp* were sometimes isolated by a double selection on minimal fusaric acid plates (6) supplemented with 400 μM triornithine. That such isolates were indeed deletions (rather than a double event or an inversion) was ascertained by screening for the simultaneous loss of *tonB* function.

Tn*10* insertions into *opp* were obtained by simultaneous transduction to Tet^r and triornithine resistance with P22 grown on a mixed population of bacterial cells containing random insertions of Tn*10* throughout the chromosome. Such a population was made as described previously (16).

Genetic crosses. Transductions were carried out by using a high-transducing derivative of phage P22 *int-4* (30). Because the efficiency of P22 infection of *galU* strains of *S. typhimurium* is very poor (35; unpublished results), P1 *clr* was used for mapping this locus. P1 was grown as described (28) on *galE* derivatives of appropriate *S. typhimurium trp* and *opp* mutants (the *galE* lesion is necessary to allow P1 infection of *S. typhimurium* [22]) and used to transduce SL4507 ($galU^-$) to Gal^+. Transductants were then screened for the nonselected marker. The *trp*, *cysB*, and *tonB* alleles used for determining cotransduction frequencies were those carried by strains CH19, CH273, and PM784, respectively.

Proline auxotrophs are able to satisfy their amino acid requirement by utilizing Pro-Gly-Gly. However, in strains carrying an *opp* mutation, this peptide is no longer able to serve as a source of proline; Pro-Gly-Gly is not transported at an adequate rate by the other peptide transport system. Thus, Opp^+ recombinants can be selected in a proline auxotroph by growth on minimal plates with Pro-Gly-Gly as the sole source of proline. Crosses were normally conducted in the presence of tetracycline to prevent Pro^+ derivatives arising by Tn*10* excision. Where possible, strains carrying deletions were used as recipients to eliminate the possibility of opp^+ revertants arising. However, many *opp* point mutations also show a very low rate of spontaneous reversion to opp^+. In all crosses where negative results (no recombinants) were obtained, conditions were such that at least 1,000 recombinants would be obtained between point mutations lying at opposite ends of a gene. Certain partially defective *opp* mutations still allowed the utilization of Pro-Gly-Gly (see below). Progeny arising from crosses with these mutations as donors fell into two categories: true opp^+ recombinants and recombinants in which the recipient had received the partially defective *opp* allele from the donor. These could be distinguished by colony size and by screening the progeny for triornithine sensitivity or resistance.

RESULTS

Frequency of spontaneous *opp* mutations. It has been reported previously that *opp* mutations in *E. coli* arise at rather high frequencies (3, 25). We determined the frequency with which *opp* mutations arise by plating 10^8 cells on a minimal glucose plate spread with triornithine (10 μmol). Frequencies of 5.0×10^{-6} and 2.5×10^{-6} mutations to opp^- per cell per generation were obtained with strains of *E. coli* (strain 294) and *S. typhimurium* (LT2), respectively. Otherwise isogenic *recA* derivatives of these strains showed similar frequencies of mutation.

Location of *opp* with respect to nearby genes. The frequencies of cotransduction of various genetic markers located at around 34 min on the *S. typhimurium* chromosome are given in Table 2. In no case was interference by a nonselected marker observed. Different alleles of *opp* consistently gave different frequencies of cotransduction with *trp* and *tonB*. Some of these alleles turned out to be spontaneous deletions. However, most were shown to behave as point mutations (see below). Subsequent mapping of these mutations showed excellent correlation between the frequency of cotransduction with *tonB* and the location of the mutation within *opp* (i.e., the greater the frequency of cotransduction with *tonB* the closer it mapped to the *tonB* end of *opp*; see Fig. 1). Only two selected examples are given in Table 2: *opp-231*, which maps toward the end of *opp* nearest *galU*, and *opp-242*, which maps at the *tonB* end of *opp*. These results suggest that *opp* is a large locus. Calculation of the distance between *opp-231* and *opp-242* from

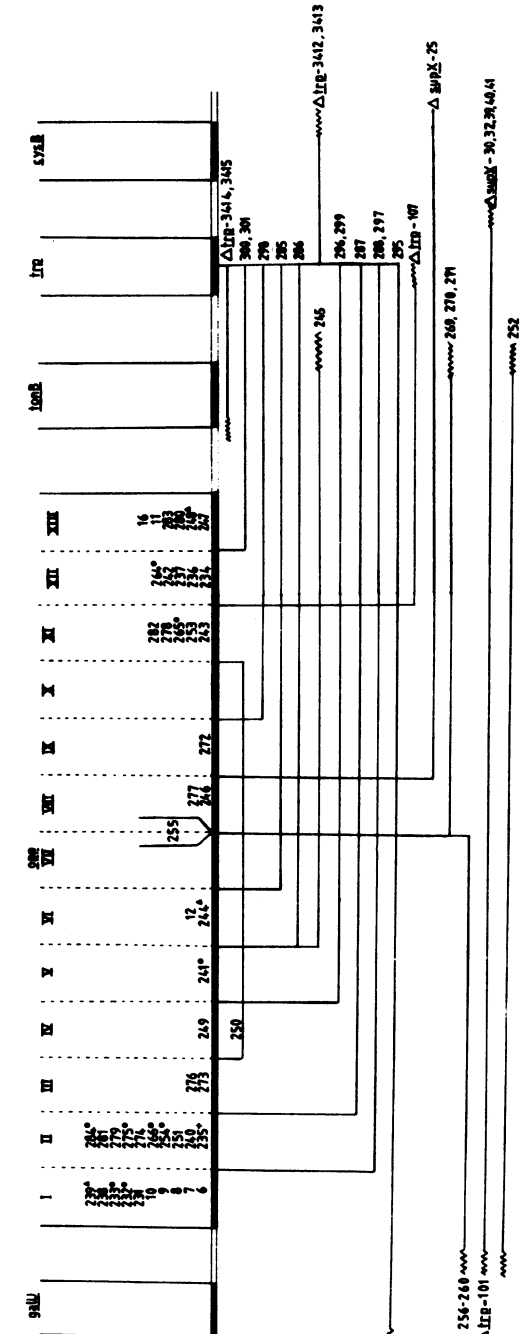

FIG. 1. Deletion map of *opp*. The thick solid horizontal line represents the chromosome. The thinner horizontal lines below the chromosome represent deleted regions. Deletion endpoints are indicated by a vertical line extending to the chromosome; in some cases the endpoints of two or more deletions have not been separated. A wavy line at the end of a deletion indicates that the endpoint has not been precisely defined. Roman numerals indicate deletion intervals of *opp*. Each number within a given deletion interval represents a different *opp* allele mapping within that interval. Deletions *Δtrp-101*, *Δtrp-107*, *ΔsupX-25*, *ΔsupX-30*, *ΔsupX-32*, *ΔsupX-39*, *ΔsupX-40*, and *ΔsupX-41* were isolated and have been described previously (19). *opp-6*, *opp-7*, *opp-8*, and *opp-9* were isolated by resistance to trilysine; *opp-10*, *opp-11*, and *opp-12* by resistance to norleucylglylclyglycine; and *opp-16* by resistance to glycylglycyl-histidinol phosphate ester as described previously (1). All other mutations were isolated in this study. Mutation *opp-255*, indicated by an arrow, is a Tn*10* insertion. Deletions with one endpoint at this point of insertion were derived from *opp-255* during Tn*10* excision. Deletions *Δopp-250*, *Δopp-245*, and *Δopp-252* arose spontaneously. Deletions with one endpoint in *trp* arose during Tn*10* excision from strain CH56. Symbols: +, a fast-reverting allele (see text); ▲, penicillin selected for nongrowth on Pro-Gly-Gly; ●, triornithine-resistant alleles still able to grow on Pro-Gly-Gly as a source of proline (see text).

TABLE 1. Genotypes of strains used in this study[a]

Strain	Genotype	Source
S. typhimurium		
CH56	*pro-594 trp-1012*::Tn*10*	This study
JL2688	*bio-203*::Tn*10*	G. Ferro-Luzzi Ames
PM784	*tonB51*[b]	P. Margolin
SL4507	*galU455 leu-1051 cysl175 hisC1150*	B. A. D. Stocker
TR2951	*recA1 his-63 strA*	G. Ferro-Luzzi Ames
TT520	*srl-202*::Tn*10*	G. Ferro-Luzzi Ames
TT97	*trp-1012*::Tn*10*	J. Roth
TT184	*pro-662*::Tn*10*	J. Roth
CH273	*cysB517*	N. M. Kredich
CH20	*galE503*	G. Ferro-Luzzi Ames
CH2	*pro-594*	G. Ferro-Luzzi Ames
CH19	*trpC109*	G. Ferro-Luzzi Ames
E. coli		
294	*endoI thi endA hsdR*	G. Ferro-Luzzi Ames

[a] Opp$^-$ mutants isolated in this study and obtained from other sources are described in the legend to Fig. 1.

[b] The original *chr* designation (7) is now referred to as *tonB*.

their respective cotransduction frequencies with *tonB*, according to the methods of Langley and Guest (18) and Wu (36), indicated that the *opp* locus covers 5 to 6 kilobases of the chromosome. The cotransduction data shown in Table 2 indicate that the gene order in this region of the chromosome is *cysB-trp-tonB-opp-galU*. This gene order was supported by deletion mapping data (see below).

Deletion mapping of the *opp* locus. The extent of deletions isolated in and around *opp* are shown in Fig. 1. Deletions extending from *trp* toward *opp* were isolated by selecting Tet^s derivatives of CH56, a strain carrying a Tn*10* insertion in *trp*. That Tet^s derivatives were deletions rather than inversions (17) was confirmed by crosses with point mutations in *trp* located on either side of the point of insertion. All deletions extending from *trp* into *opp* were also TonB$^-$. We were, however, able to isolate *trp-tonB* deletions which remained Opp$^+$ (Δ*trp-3414*; Δ*trp-3415*). One *trp-opp* deletion (Δ*opp-295*) also extended into *galU*, though not as far as *tdk*. In addition, two spontaneous deletions (Δ*opp-245* and Δ*opp-252*), as well as three deletions (Δ*opp-269*;Δ*opp-270*;Δ*opp-271*) extending from a Tn*10* insertion within *opp* (*opp255*::Tn*10*), also removed the *tonB* locus, yet remained Trp$^+$ GalU$^+$. None of these deletions affected *cysB*; deletions extending from *trp* which affected the *cysB* locus remained Opp$^+$ TonB$^+$ (Δ*trp-3412* and Δ*trp-3413*). This unambiguously demonstrated that the gene order *cysB-trp-tonB-opp-galU-tdk*, indicated by cotransduction frequencies, was correct.

Fine-structure map of *opp*. Figure 1 shows a fine-structure genetic map of *opp*. The locus is divided into 13 deletion intervals, into which each point mutation was mapped. We not only mapped *opp* mutations isolated in this study but also some *opp* point mutations and deletions described previously by Ames et al. (1) and Lenny and Margolin (19), respectively. All *opp* mutations isolated mapped at this one locus. This included mutations isolated by resistance to the toxic peptides, triornithine, trilysine, norleucylglycylglycine, and glycylglycyl-histidinol phosphate ester, as well as those isolated by penicillin selection for Pro-Gly-Gly nongrowers (see legend to Fig. 1). Four independent selections for a Tn*10* insertion in *opp* each apparently resulted in the same insertion (not separable by recombination). Hot spots for Tn*10* insertions have often been observed (12). Only one of these insertions (*opp-255*::Tn*10*) was used further. Whereas most triornithine-resistant mutants were completely unable to grow on Pro-Gly-Gly

TABLE 2. Frequency of cotransduction of genetic markers around the *opp* locus

Selected marker	Nonselected marker	Frequency of cotransduction (%)[a]
trp	*cysB*	43
cysB	*trp*	51
cysB	*tonB*	21
trp	*tonB*	55
trp	*opp-231*	8
trp	*opp-242*	21
opp-231	*tonB*	50
opp-242	*tonB*	78
opp-231	*trp*	5
opp-242	*trp*	19
cysB	*opp-242*	6
opp-242	*cysB*	4
galU[b]	*opp-231*	90 (65)
galU[b]	*trp*	45 (<5)

[a] Between 85 and 300 transductants were scored for each cross.

[b] Transductions were performed with phage P1 (see text). The cotransduction frequencies given are those obtained with P1. Since the cotransduction frequencies obtained with P1 are not directly comparable with those obtained with P22, equivalent cotransduction frequencies which would be expected if P22 had been used were calculated from the P1 frequencies according to Langley and Guest (18) and are given in parentheses (the possible inaccuracies of such calculations must, however, be borne in mind).

as a source of proline (although still able to utilize Pro-Gly, which presumably enters the cell via the dipeptide permease), certain strains did show some growth on Pro-Gly-Gly (see Fig. 1). Different mutations allowed different degrees of growth on Pro-Gly-Gly (results not presented). Two of these mutations (*opp-254* and *opp-284*) also remained partially sensitive to triornithine. Although about one in five of the point mutations mapped possessed this intermediate phenotype, we specifically selected these mutations for mapping. They normally arise at a frequency of about 1 in 15 of all triornithine-resistant mutations. Interestingly, many such mutations cluster within two deletion intervals (I and II) at one end of *opp* (Fig. 1).

A further interesting *opp* mutation is *opp-235*. This mutation reverted to wild type (triornithine sensitive, Pro-Gly-Gly grower) at a frequency of about 10^{-3}. Reversion was *recA* independent; the lesion behaved like a point mutation for mapping purposes and was highly polar (B. Hogarth and C. F. Higgins, manuscript in preparation). This suggests that this mutation may be due to an insertion event. No insertion sequence has yet been characterized in *S. typhimurium*, although a mutation in the histidine biosynthetic operon (*hisD984*) is believed to be due to a *Salmonella*-specific insertion sequence (J. R. Roth, personal communication); nor do any of the known *E. coli* insertion sequences occur in *S. typhimurium*.

DISCUSSION

We showed, both by cotransduction frequencies and by deletion analysis, that the gene order around 34 min on the *S. typhimurium* chromosome linkage map is *cysB-trp-tonB-opp-galU-tdk*. This confirms the gene order proposed by Lenny and Margolin (19). However, in *E. coli* there has been some disagreement as to the precise location of *opp*. Two groups originally placed *opp* on the *cysB* side of *trp* (3, 10), although these data are open to other interpretations (19). More recently, Lenny and Margolin (19) presented data indicating that the gene order in *E. coli* is the same as that in *S. typhimurium*. We reached the same conclusions by deletion mapping in *E. coli* (N. H. Smith, L. M. Powell, and C. F. Higgins, unpublished data). Thus, it now seems clear that in both *S. typhimurium* and *E. coli* the location of *opp* is between *tonB* and *galU*; all *opp* mutants map in this region, and deletions extending from *trp* to *cysB* have no effect on peptide transport. Two other genes, *leuT* (leucine transport [31]) and *aroT* (aromatic amino acid transport [32]), have also been reported as mapping on the *tonB* side of *trp* in *S. typhimurium*. As these genes are both apparently removed by *Δtrp-107* (31, 32), which has one endpoint in *trp* and the other, as we now show, in *opp*, these two loci must presumably also map between *trp* and *opp*.

It has often been reported that triornithine-resistant mutations occur spontaneously at high frequency (3, 25). We obtained figures of 2.5×10^{-6} and 5×10^{-6} for *S. typhimurium* and *E. coli*, respectively, which are not unusually high. If one considers that *opp* is a large locus, covering several kilobases of DNA (see above), this value is even less unusual. In addition, the majority of these mutations are due to different events, showing that no high-frequency, site-specific events occur. However, spontaneous deletions in *opp* seem common (about 10% of all spontaneous mutations; results not presented). A high proportion of deletions in other genes located in this region of the chromosome has also been reported (8, 33). As the frequency of such deletions is significantly affected by genetic background (8), it seems probable that the high frequency of *opp* mutations reported by some investigators is not so much a function of *opp* itself but of the genetic background in which the mutations were isolated. In this context, however, it is interesting that we obtained an *opp* mutation (*opp-235*) which seems to be related to an insertion event.

Despite the possible inaccuracies inherent in calculating physical distances from cotransduction frequencies, it is clear that *opp* covers a relatively large segment of DNA, probably 5 to 6 kilobases. This is sufficient to encode several proteins, and indeed we have obtained evidence that the locus consists of at least four cistrons (Hogarth and Higgins, in preparation). Bacterial transport systems, other than those in which the substrate is modified during transport, can broadly be divided into two groups: (i) unicomponent, ion gradient-driven systems such as the lactose permease system; and (ii) multicomponent, periplasmic binding protein-dependent systems which are energized by hydrolysis of a high-energy phosphate bond. Two lines of evidence have been presented previously which suggest that the oligopeptide permease might fall into this latter class. Peptide uptake is sensitive to osmotic shock, indicative of a requirement for a periplasmic binding protein, although no binding activity was detected in the shock fluid (9). In addition, it seems that phosphate bond energy rather than an ion gradient is the driving force for peptide transport (9, 24, 26). All binding protein-dependent systems that have been well characterized seem to require the function of several genes located adjacent to each other on the chromosome, unlike ion gradient-driven systems which require only a single gene product (13). Thus, evidence which indicates that *opp* occupies several kilobases of DNA and is there-

fore likely to consist of more than one gene is further support for the view that the oligopeptide permease is a binding protein-dependent transport system.

The genetic analysis of *opp*, together with the series of mapped deletions described here, is important for the further characterization of this transport system. This study provides a basis for the isolation and analysis of mutants which will enable us to investigate the mechanisms and regulation of transport, to identify the protein(s) involved, and to characterize the cloned *opp* genes.

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