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Locations of the *opp* and *supX* Genes of *Salmonella typhimurium* and *Escherichia coli*

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The chromosomal locations of the *supX* and *opp* loci of *Salmonella typhimurium* LT2 and *Escherichia coli* K-12 were identified and found to result in the same gene sequence in both species, namely, *pyrF-cysB-supX-trpPOLEDCBA-tonB(chr)-opp*. These results differ from a previously reported location of the *opp* gene on the *E. coli* chromosome. Evidence indicates that the *opp* gene lies between *chr(tonB)* and *galU* in *S. typhimurium*.

The *supX* locus (previously designated *su-leu-500*) lies between the *trp* and *cysB* genes of *Salmonella typhimurium* (10, 16). Mutations in the *supX* locus result in suppression of certain promoter mutations, as well as other effects (9, 10). Evidence for the presence of a *supX* gene in the same general region of the *Escherichia coli* chromosome is based upon the appearance of a $SupX^+$ phenotype in a *supX* deletion mutant strain of *S. typhimurium* after introduction of an F′ carrying the *cysB*, *trp*, and *tonB* genes of *E. coli* K260 (19). That same F′ plasmid also reversed the Chr^- phenotype of *S. typhimurium* strains.

The *opp* gene apparently codes for a general oligopeptide permease in *S. typhimurium* (1) and *E. coli* (3, 8). Mutations at this locus result in resistance to certain inhibitory oligopeptides (e.g., triornithine), as well as loss of the ability of auxotrophic strains to utilize some oligopeptides containing required amino acids (1, 3, 12). Using Hfr crosses and transductions, two groups of investigators concluded that the *opp* locus of *E. coli* was located on the *cysB* side of the *trp* operon (2, 3, 8). Some linkage values reported for cotransduction of *opp* with *trp* (3) were rather high relative to the values reported for *cysB* to *trp* markers (21). This suggests that *opp* lies between *cysB* and *trp*, although other linkage values (8) allowed for a location on the other side of *cysB*.

Because of our interest in markers closely linked to the *trp* operon of *S. typhimurium*, we decided to determine the position of the *opp* locus in this organism. Examination of *S. typhimurium* LT2 strains bearing deletions of the entire region between *cysB* and *trp* revealed that they were Opp^+ (triornithine sensitive) in phenotype rather than the Opp^- (triornithine resistant) we had expected on the basis of the most recent *E. coli* map (2). Furthermore, an examination of additional *S. typhimurium* strains with deletions in this chromosome region revealed that an Opp^- phenotype was displayed only by strains in which the deletion involved the *chr* locus (previously designated *car* [7]). The *chr* locus of *S. typhimurium* appears to be the equivalent of the *E. coli tonB* locus (7, 11) and, like it, is located on the opposite side of *trp* from the *cysB* gene. These findings suggest that the *opp* locus on the *S. typhimurium* chromosome lies on the side of the *trp* operon opposite the side reported for the *E. coli opp* locus.

Although the sequences of the gene loci on the *E. coli* and *S. typhimurium* chromosomes are for the most part identical, there is a segment in which the orders of the genes in the two organisms are inverted with respect to each other (17). This segment includes the *pyrF-cysB-trp-tonB(chr)-galU* loci and represents about 10% of the chromosome (5). To determine whether the evidence that the *S. typhimurium opp* locus is not located on the *cysB* side of *trp* means that the inverted region of the chromosome harbors additional differences in the gene sequences of the two species, we decided to map the position of the *opp* locus in both *E. coli* and *S. typhimurium* and to determine more precisely the location of the *supX* locus in *E. coli*.

MATERIALS AND METHODS

Bacterial strains. All bacteria used which were derivatives of *S. typhimurium* LT2 with or without the *E. coli* F′123 plasmid are listed in Table 1. The *trp* gene designations in *S. typhimurium* have been modified to coincide with those of *E. coli* (17). The *E. coli* K-12 strain KLF23/KL181 (CGSC 4255), which was kindly provided by B. Bachmann, carries the F′123 plasmid, which includes the *trp*, *cysB*, and *pyrF* genes (14). The *chr-51* mutation was introduced into strain PM784 by transduction from strain SL1228 (*chr-51 galE706 pyrD24*), which was kindly supplied by B. A. D. Stocker.

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TABLE 1. *Description of the S. typhimurium strains*

Strain	Genotype[a]
PM153	Δ*supX24* (deletion: *cysB supX trp-POLEDCBA*) *opp-104 leu-500 ara-9*
PM236	Δ*supX26* (deletion: *cysB supX trp-POLEDCBA chr opp*) *leu-500 ara-9*
PM454	Δ*trp-101* (deletion: *trpPOLEDCBA chr opp*) *leu-500 pyrF146*
PM661	Δ*trpPOLEDCBA167 cysB529 pyrF146 leu-500*
PM767[b]	F'123/Δ*supX24* (deletion: *cysB supX trpPOLEDCBA*) *opp-104 leu-500 ara-9*
PM781	*opp-102 pyrF146 leu-500*
PM782	Δ*trpPOLEDCBA167 cysB529 pyrF146 opp-103 leu-500*
PM784	*chr-51*
PM785[b]	F'123/Δ*supX26* (deletion: *cysB supX trpPOLEDCBA chr opp*) *leu-500 ara-9*

[a] The *trp* gene designations for *S. typhimurium* have been changed to coincide with those of *E. coli* (17).

[b] *S. typhimurium* host cells bearing the *E. coli* F'123 plasmid.

Media. Minimal synthetic medium in liquid or agar form was prepared as previously described (15), with 0.2% glucose as the carbon source. When required, the following concentrations of supplements were present in the medium: L-tryptophan, 40 μg/ml; L-leucine, 40 μg/ml; L-cysteine, 80 μg/ml; uracil, 40 μg/ml. When required for replica-plate tests, 0.5 ml of a 10-mg/ml solution of triornithine was spread onto the agar of each plate (about 32 ml of agar per plate) and allowed to dry before use. Tri-L-ornithine was obtained from Miles-Yeda, Ltd. When testing for chromium sensitivity, we omitted sodium citrate from the minimal agar (Difco Laboratories) synthetic medium (7).

Complete liquid medium contained 1% (wt/vol) tryptone (Difco), 0.5% (wt/vol) yeast extract (Difco), 1% (wt/vol) NaCl, and 0.002% (wt/vol) L-cysteine. Reconstituted nutrient agar (BBL Microbiology Systems) was supplemented with 5 g of NaCl per liter and 10 mg of L-cysteine per liter.

Transductions. The bacteriophage used for transductions was HT105/1 *int* (kindly supplied by J. R. Roth), an integration-negative version of one of the high-frequency-transducing phage P22 mutants of Schmieger (18). Lysates were prepared and transductions were carried out as described previously (15), except that synthetic medium containing 0.1% glucose and 0.1% galactose was used in lysate preparation of the *galE* strain SL1228. The phenotypes of unselected markers were determined by replica-plating (13) onto appropriately supplemented medium.

Identification of phenotypes, genotypes, and deletions. The mutant states of the *pyrF*, *cysB*, and *trp* genes were identified by the usual nutritional tests for uracil (Pyr^-), cysteine (Cys^-), and tryptophan (Trp^-) auxotrophies. Absence or mutation of the *supX* locus was indicated by leucine prototrophy (Leu^+) since the *supX* condition suppresses the leucine auxotrophy imposed by the *leu-500* mutation (10, 16), which is a promoter mutation of the leucine operon (4) in the *S. typhimurium* strains used in this study. A deletion or mutation of the *chr* locus of *S. typhimurium* or its equivalent (the *tonB* locus of *E. coli*) was detected by the resultant sensitivity (Chr^-) in the absence of citrate to growth inhibition by the levels of chromium ion normally present in agar (7). A deletion or mutation of the *opp* locus was identified by resistance (Opp^-) to the growth-inhibiting capacity of triornithine (3, 8).

The simultaneous occurrence of mutations at two or more of these closely linked gene loci was interpreted to be the result of single deletion events. We considered the simultaneous occurrence of independent mutations at two or more closely linked loci in the same cell as too rare an event to affect our analysis.

Transfer of the *E. coli* F'123 plasmid. The F'123 plasmid was introduced into *S. typhimurium* strains PM153 and PM236 by replicating single colonies of the *E. coli* K-12 plasmid-bearing strain KLF23/KL181 onto lawns of the recipient strains. The agar medium lacked the histidine and uracil required by the *E. coli* donor and the cysteine required by the recipients. The Cys^+ colonies which arose represented clones of recipient cells which had received the donor F'123 plasmid carrying *E. coli* genes equivalent to those removed by the deletions on the *S. typhimurium* chromosome. Introduction of the F'123 plasmid into PM153 resulted in a change in phenotype from Cys^- Leu^+ Trp^- Opp^- to Cys^+ Leu^- Trp^+ Opp^+, and the hybrid clone was designated PM767. Introduction of F'123 into PM236 changed the Cys^- Leu^+ Trp^- Chr^- ($TonB^-$) Opp^- phenotype to Cys^+ Leu^- Trp^+ Chr^+($TonB^+$) $Opp,^+$ and the hybrid clone was designated PM785. Transfer of mutated F'123 plasmids between *S. typhimurium* strains was carried out in a similar fashion.

Selection for *opp* and *supX* mutations. Selection for *opp* mutations was based upon resistance to growth inhibition by triornithine. The cells used had to be cultured in synthetic minimal medium before the selection since opp^+ cells grown in rich broth medium have significantly reduced sensitivity to triornithine (3). A 0.1-ml portion of cells grown in synthetic medium was spread onto appropriately supplemented minimal agar medium. After drying, a sterile filter paper disk (diameter, 6 mm) was placed on the spread area, and 30 μl of a 10-mg/ml solution of tri-L-ornithine was applied. After 48 to 72 h of incubation at 37°C triornithine-sensitive strains (opp^+) exhibited a clear zone of inhibited growth around the disk. Resistant clones, which appeared as colonies within the clear zone, were picked and taken through two single-colony reisolations. When retested for resistance with triornithine-impregnated disks, these clones showed no clear zone of growth inhibition.

Selection for *supX* mutations was carried out by omitting the leucine supplement and picking the smaller Leu^+ colonies which appeared on agar spread with cells harboring the *leu-500* mutation. This allowed us to select the colonies with Leu^+ phenotypes which resulted from suppression of the leucine auxotrophy by *supX* mutations. The relatively few true reversion mutations at the *leu-500* site gave rise to distinctly larger Leu^+ colonies (16).

Selection for simultaneous *opp supX* mutations was carried out by omitting leucine supplementation and applying triornithine-impregnated disks. Clear zones around the disks could be distinguished in the relatively light turbidity resulting from residual growth of the Leu^- cells. Colonies appearing in the clear zones were Leu^+ Opp^- and were retested for each phenotype after single-colony reisolation to confirm that they were *supX opp* clones.

RESULTS

Deletion mapping of the *opp* locus in *S. typhimurium*. The gene sequence *pyrF-cysB-supX-trpOEDCBA-chr(tonB)* has been demonstrated in *S. typhimurium* (10, 16, 17). A total of 16 strains previously selected (16) for deletions of the *supX* locus (each of independent origin) were characterized for the extents of their deletions, as described above. In eight of the strains the deletions extended from the *cysB* (six strains) or *supX* (two strains) locus into or through the *trp* operon but did not reach the *chr(tonB)* locus (previously termed *car* [7, 16]). These are illustrated in Fig. 1 as deletion types A and B. All eight strains were sensitive to triornithine. In growth tests on agar they produced clear or almost clear zones of inhibited growth around paper disks containing that tripeptide, thereby signaling the presence of an opp^+ allele. The other eight strains harbored deletions which extended from *cysB* (three strains) or *supX* (five strains) through the *trp* operon and into or through the *chr(tonB)* locus. These are illustrated in Fig. 1 as deletion types C and D. Seven of these *chr* strains were resistant to inhibition by triornithine, indicating an *opp* genotype. The remaining strain exhibited a very low level of sensitivity, which was observed as a barely discernable zone of slightly lessened growth around the disk containing triornithine. Two $supX^+$ strains with type E (Fig. 1) deletions which include all (*trp-101*) or part (*trp-107*) of the *trp* operon and extend into or through the *chr(tonB)* locus (6) were resistant to triornithine inhibition and therefore *opp*. The $supX^+$ strain *trp-167*, harboring type F (Fig. 1) deletion which removes all the *trp* genes but does not reach the *chr(tonB)* locus (6), was sensitive, indicating the presence of an opp^+ allele. These data from 19 deletion strains suggest that only deletions which reach the *chr(tonB)* locus cause an Opp^- condition. They imply that the *opp* gene of *S. typhimurium* lies on the *chr(tonB)* side of the *trp* operon and possibly beyond the *chr(tonB)* locus.

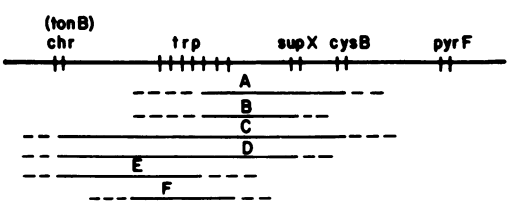

FIG. 1. *Classes of deletions in the chr-to-pyrF region of the S. typhimurium chromosome. Distances shown between genes are arbitrary. The solid lines below the chromosome line represent deletions and are the minimum extents of the deletions in each class, as determined by mapping. The dashed extensions represent varying possible additional lengths of the individual deletions in each class.*

Transduction mapping of the *opp* locus in *S. typhimurium*. Strain PM661 (*pyrF146 cysB529 trp-167 leu-500*) was used as a recipient in a phage P22 transduction-mediated cross with *opp* donor strain PM781 (*pyrF146 opp-102 leu-500*). The transduction mixture was spread onto minimal agar medium supplemented with uracil, tryptophan, and leucine to select for Cys^+ transductants. The transductant colonies were then replica-plated onto medium lacking tryptophan and onto tryptophan-supplemented medium containing triornithine to test for their Trp and Opp phenotypes. The results (Table 2) demonstrated that the *opp* gene does not lie between *cysB* and *trp*. If it did, the vast majority of the transductants which incorporated the $cysB^+$ and trp^+ genes of the donor would have also gotten the *opp* allele of the donor (double crossover required) rather than retaining the opp^+ allele of the recipient (quadruple crossover required). Table 2 shows just the reverse; 79.8% of the cys^+ trp^+ transductants retained the opp^+ allele of the recipient, indicating that *opp* is not located between *cysB* and the *trp* operon. Of the 22 cys^+ transductants which integrated the *opp* allele of the donor, 21 (95.5%) also integrated the trp^+ allele of the donor, suggesting that *trp* is located between *cysB* and *opp*. The resulting sequence, *pyrF-cysB-trp-opp*, is in agreement with the our deduction from the deletion mapping.

A second set of transductions utilized strain PM782 (*pyrF146 cysB529 trp-167 opp-103 leu-500*) as recipient and strain PM784 (*chr-51*) as donor. It should be noted that the *leu-500* allele is not linked by transduction to the region of the chromosome under study and therefore does not participate in the formation of recombinant classes among the selected transductants. Table 3 describes the four recombinant classes obtained by selecting for $pyrF^+$ transductants; the presence of the donor or recipient allele is indicated for each locus. It is quite clear that the degree of cotransduction linkage among the *pyrF-cysB-trp-chr(tonB)* markers is related to their relative positions; the closer the gene to the selected $pyrF^+$ marker, the greater the link-

TABLE 2. *Frequency of the four genotype classes resulting from selection of 219 cysB⁺ transductants in a cross between recipient strain PM661 (cysB529 trp-167 pyrF146 leu-500) and donor strain PM781 (opp-102 pyrF146 leu-500)*

Genotype[a]			Frequency (%)
cysB	*trp*	*opp*	
d	r	r	52.0 (114)[b]
d	d	r	38.0 (83)
d	d	d	9.6 (21)
d	r	d	0.4 (1)

[a] The genotype of each class is presented in terms of the donor (d) or recipient (r) origin of the alleles of each marker gene.

[b] Numbers in parentheses are total numbers of transductant colonies of each class of recombinants.

age. The absence of any detectable linkage with the *opp* marker suggests that it is beyond the *chr*(*tonB*) locus, in agreement with the deletion data.

Table 3 also describes the recombinant classes obtained when the *pyrF*⁺ and *trp*⁺ alleles of the transduction donor are selected simultaneously. The very small percentage (7.8%) which also incorporated the *opp*⁺ allele of the donor is further evidence that the *opp* locus does not lie between *pyrF* and *trp*. Table 3 shows the recombinant classes obtained by selecting for only the *trp*⁺ allele of the donor. If we examine the *trp*⁺ transductants which inherited the *chr*(*tonB*) and/or *opp*⁺ allele of the donor, we find strong evidence that the *chr*(*tonB*) locus lies between *trp* and *opp*; almost 94% of the transductants which received the *trp*⁺ and *opp*⁺ alleles of the donor also incorporated the *chr*(*tonB*) allele of the donor. Among the *trp*⁺ transductants which retained the *opp* allele of the recipient, just under 71% incorporated the *chr*(*tonB*) allele of the donor. This is also consistent with the deletion data. Another set of similar transductions used as the recipient a strain having the same set of marker alleles, except that the *trp* marker was *trpC171*, a small deletion within the *trpC* gene. The results (data not shown) were fully consistent with those just described. These data and our deletion data, combined with the previous evidence that *supX* lies between *cysB* and *trp* (10, 16), leads us to conclude that the gene order in *S. typhimurium* LT2 is *pyrF-cysB-supX-trp-chr*(*tonB*)*-opp*.

Deletion mapping of the *E. coli supX* and *opp* loci. The F'123 plasmid is known to bear the *pyrF-cysB-trp* region of the *E. coli* K-12 chromosome (14). Using conjugation, we introduced F'123 into an *S. typhimurium* strain (PM153) which harbored a deletion of the *cysB* gene, the *supX* gene, and the entire *trp* operon, as well as an *opp* mutation and the *leu-500* mutation. The resultant hybrid was designated PM767. As a consequence of acquiring the F'123 plasmid, the original Cys⁻ Leu⁺ Trp⁻ Opp⁻ phenotype changed to Cys⁺ Leu⁻ Trp⁺ Opp⁺. It should be noted that a *supX* mutation suppresses the leucine auxotrophy resulting from the *leu-500* promoter mutation. The change to a Leu⁻ phenotype as a result of the introduction of the plasmid indicates that F'123 carries a *supX*⁺ allele. Apparently the *E. coli* K-12 *supX* gene functions in *S. typhimurium*. The change from an Opp⁻ to an Opp⁺ phenotype also indicates the presence of an *opp*⁺ allele on the F'123 plasmid.

Four single-colony clones of the hybrid PM767 strain were grown in liquid culture. The cells were spread onto minimal agar plates supplemented with tryptophan, and a filter paper disk containing triornithine was placed on the agar surface of each plate. The absence of leucine supplement selected for cells with *supX* muta-

TABLE 3. *Recombinant classes obtained in transductions by using strain PM782 (pyrF146 cysB529 trp-167 opp-103 leu-500) as recipient and strain PM784(chr-51) as donor*

Selected donor marker(s)	Genotype[a]					Frequency (%)
	pyrF	*cysB*	*trp*	(*tonB*) *chr*	*opp*	
pyrF⁺(332)[b]	d	r	r	r	r	53.6 (178)[b]
	d	d	r	r	r	37.7 (125)
	d	d	d	r	r	5.4 (18)
	d	d	d	d	r	3.3 (11)
pyrF⁺*trp*⁺(332)	d	d	d	d	r	45.5 (151)
	d	d	d	r	r	42.5 (141)
	d	d	d	d	d	6.0 (20)
	d	r	d	r	r	2.4 (8)
	d	r	d	d	r	1.8 (6)
	d	d	d	r	d	0.9 (3)
	d	r	d	d	d	0.6 (2)
	d	r	d	r	d	0.3 (1)
trp⁺(491)	r	r	d	d	r	28.1 (138)
	r	d	d	d	r	26.5 (130)
	r	r	d	r	r	11.8 (58)
	r	d	d	r	r	9.8 (48)
	r	r	d	d	d	7.1 (35)
	d	d	d	d	r	6.3 (31)
	r	d	d	d	d	5.1 (25)
	d	d	d	r	r	3.5 (17)
	r	r	d	r	d	0.4 (2)
	d	r	d	d	r	0.4 (2)
	d	r	d	r	r	0.4 (2)
	r	d	d	r	d	0.4 (2)
	d	d	d	d	d	0.2 (1)

[a] The genotype of each class is presented in terms of the donor (d) or recipient (r) origin of the alleles of each marker gene.

[b] Numbers in parentheses are total numbers of transductant colonies.

tions on the F'123 plasmid, which, combined with the preexisting chromosomal deletion of *supX*, produced the Leu$^+$ phenotype needed to form colonies. Within the clear zones surrounding each disk, any colony which appeared indicated that the cells were *opp* (triornithine resistant) as well as *supX*. The lack of cysteine supplementation served to indicate that cells in growing colonies retained the F'123 plasmid carrying the *cysB*$^+$ allele. A total of 22 Leu$^+$ Opp$^-$ colonies from the four PM767 clones were picked and purified by single-colony streaking and reisolation. As noted above, the occurrence of simultaneous mutations to *opp* and *supX* on the F'123 plasmid is most readily explained by a deletion involving both genes. The 22 *opp supX* clones all required tryptophan for growth, indicating that they had also become *trp*. To examine the state of the *tonB*(*chr*) gene the F'123 plasmids from the 22 clones were transferred to a *chr*(*tonB*) strain (PM454). In the absence of citrate, the growth of *S. typhimurium chr* mutants and *E. coli tonB* mutants is inhibited by the level of chromium ion normally present in agar (7). Tests on citrate-free agar revealed that in all 22 cases the F'123 plasmid had also become *tonB*(*chr*). These results suggest that *tonB*(*chr*) and *trp* lie between *opp* and *supX* since in all 22 clones, single deletions which eliminated *opp* and *supX* also eliminated *tonB*(*chr*) and *trp*.

To further test this interpretation, we once again selected in strain PM767 for simultaneous mutation to *opp* and *supX*, but this time omitted tryptophan as well as leucine from the medium and supplemented the medium with cysteine. The absence of tryptophan supplementation made the ability of each cell to grow dependent upon the retention of an F'123 plasmid which remained *trp*$^+$. No colonies arose in the clear zones of triornithine inhibition, indicating that no *opp supX* clones had appeared. However, many *supX* colonies appeared outside the triornithine inhibition zones. Of 30 *supX* clones examined, 25 proved to be mutant at the *supX* locus only, and 5 were *supX cysB*. These results also suggest that *trp* lies between *opp* and *supX* since the requirement that *trp*$^+$ be retained prevents the appearance of clones with deletions which extend from *opp* to *supX*.

The appearance of five clones which became simultaneously *supX* and *cysB* but remained *trp*$^+$ suggests that the F'123 plasmid can sustain deletions which eliminate the *supX* and *cysB* genes but do not affect the *trp* genes. In a previous selection experiment the deletions which eliminated the *opp*, *tonB*(*chr*), *trp*, and *supX* genes did not affect *cysB*. The simplest interpretation of these results is that the *supX* locus lies between *trp* and *cysB* on the segment of the *E. coli* K-12 chromosome carried by F'123.

Our interpretation that *tonB*(*chr*) and *trp* lie between *opp* and *supX* because all deletions which eliminate the latter two genes also eliminate the former two genes is weakened by the existence of an alternative explanation. If there are highly preferred deletion endpoints in this region of the *E. coli* K-12 chromosome, then the joint elimination of *tonB*(*chr*) and *trp* with *opp* and *supX* may simply result from the fact that these genes all lie between preferred deletion endpoints. Evidence for such preferred classes of deletions has been reported previously (20, 21). To resolve this question, we again resorted to conjugation and introduced the F'123 plasmid into an *S. typhimurium* strain (PM236) harboring the *leu-500* mutation, as well as a deletion which eliminated the *cysB*, *supX*, *trp*, *chr*(*tonB*), and *opp* genes. This permitted us to determine the state of all of the pertinent F'123 genes directly, without the need to transfer the mutated plasmids in order to examine *tonB*(*chr*) function. The resultant hybrid strain, designated PM785, was then subjected to selection for *opp* mutants on medium supplemented with tryptophan and leucine but lacking cysteine so as to require retention of the cysB$^+$ F'123 plasmid. A total of 50 triornithine-resistant (*opp*) colonies were selected from the clear zones around the disks, and the state of each of the other F'123 genes in question was determined; 28 of these clones were *opp tonB*$^+$ *trp*$^+$ *supX*$^+$ *cysB*$^+$, 6 were *opp tonB trp*$^+$ *supX*$^+$ *cysB*$^+$, and 16 were *opp tonB trp supX*$^+$ *cysB*$^+$. It is evident from the six clones in which single deletions eliminated the *opp* and *tonB* genes that these two loci must both be on the same side of the *trp* operon since the latter was not affected. The fact that all 16 clones in which a deletion removed the *opp* and *trp* genes simultaneously became *tonB* strongly suggests that *tonB* lies between *opp* and *trp*. This is supported by the report that a strain with a deletion extending from *tonB* through the *trp* genes remained *opp*$^+$ (3). We conclude that the gene sequence in *E. coli* is identical to that in *S. typhimurium* [*opp-tonB*(*chr*)-*trp-supX-cysB*], although inverted with respect to orientation on the chromosome.

DISCUSSION

The data presented here which place the *E. coli tonB* locus between the *opp* locus and the *trp* genes (*opp-tonB-trp-supX-crysB*) contradict the findings of other investigators (3, 8) that the *opp* gene is located on the *cysB* side of the *trp* operon. One group based its conclusion on the very high cotransduction linkage between the

opp and *trp* genes but did not take into consideration the fact that although the *trp* marker used was actually a *tonB*-to-*trp* deletion, it would act like a point mutation in recombination. The other group based its conclusion on the data from a three-point conjugation mating involving *pro* as the selected marker and unselected *trp* and *opp* markers. Their interpretation assumed that these three loci provided the necessary linkage relationships to provide a valid three-point test of sequence. In fact, the *pro* marker is quite distant from *trp* and *opp*. Recombination events at the closely linked *trp* and *opp* genes probably occur independently of those at the *pro* marker.

We provided B. A. D. Stocker with four *S. typhimurium* strains, each of which has a deletion of independent origin which eliminates the *trp*, *chr*(*tonB*), and *opp* genes. He has informed us that, based upon their patterns of sensitivity to phages, these strains remain *galU*$^+$. This indicates that *opp* resides between *chr*(*tonB*) and *galU*, so that the gene sequence in this organism can be extended to *pyrF-cysB-supX-trp-chr*(*tonB*)*-opp-galU*.

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