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# Tryptophan Biosynthesis in *Salmonella typhimurium*: Location in *trpB* of a Genetic Difference Between Strains LT2 and LT7

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*Salmonella typhimurium* prototrophs carrying a *trpR* mutation synthesize tryptophan biosynthetic enzymes constitutively. When feedback inhibition of anthranilate synthetase but not 5′-phosphoribosylpyrophosphate phosphoribosyltransferase activity was by-passed by growing cells on media supplemented with anthranilic acid, all *trpR* prototrophs overproduced and excreted tryptophan. However, the rate of tryptophan production depended on both the ancestry of the *trpR* strain and the integrity of its *trpA* gene. Prototrophs with *trp* genes derived from *S. typhimurium* strain LT2 produced tryptophan more efficiently than those with *trp* genes derived from strain LT7. This strain difference was cryptic insofar as it did not affect the growth rate; it was revealed only as a rate-limiting step in the constitutive biosynthesis of tryptophan in the presence of anthranilic acid, and was due to a lesion in the LT7-derived *trpB* gene. Strains with LT7-derived *trp* genes bearing a deletion in *trpA* produced tryptophan as readily as LT2 *trpR* prototrophs. This indicated that LT7-specific 5-phosphoribosylpyrophosphate phosphoribosyltransferase must be aggregated with the *trpA* gene product to give an observable reduction of constitutive tryptophan production. The discovery of this strain difference has particular implications for studies involving the activities of *trpA* and *B* genes and their products in *S. typhimurium* and may have general significance for other studies involving different strains of *Salmonella*.

Since the advent of *Salmonella* genetics (34) the possibility of cryptic genetic differences between strains LT2 and LT7 of *S. typhimurium* (18) seems to have been minimized or largely ignored (1). In the literature the origins of particular mutants are often unspecified (3, 4, 11), and occasionally LT7 strains have been inadvertently designated as LT2 derivatives (29, 30, 32). In the course of experiments to map threonine (*thr*) mutations using a mutation in the tryptophan regulatory gene, *trpR*, as an outside donor marker, I found differences in the levels of tryptophan excreted by *trpR* prototrophs. These were determined in a feeding test with auxotrophic strain *trpE95* (5). The degree of feeding depended on the recipient strain into which the *trpR* allele had been transferred (30).

In *Escherichia coli* and presumably in *Salmonella*, the $trpR^+$ product is a protein (aporepressor) which becomes an active repressor of the *trp* operon when combined with at least one type of corepressor molecule. The biosynthetic end product, tryptophan, and its analogue, 5-methyltryptophan (5MT), apparently act as corepressors (2, 21, 24, 26). Mutations in *trpR* allow constitutive *trp* enzyme synthesis and concomitant 5MT resistance. In addition to repression control of enzyme synthesis, tryptophan also controls its own biosynthetic pathway by feedback inhibition of the activities of the enzyme complex anthranilate synthetase (AS component I)-anthranilate 5-phosphoribosylpyrophosphate phosphoribosyltransferase (PRT) (13, 14, 23). The AS-CoI-PRT complex catalyzes the first two steps in the synthesis of tryptophan from chorismic acid and is specified by genes *trpA* and *trpB* in *Salmonella* (15) (Fig. 1). The amino-terminal one-third of the PRT polypeptide is specified by region I of *trpB* and functions as a glutamine amidotransferase (GAT or AS-CoII) in the AS reaction: chorismic acid + glutamine $\xrightarrow{Mg^{2+}}$ anthranilate + pyruvate + glutamate. It is not required for the PRT reaction: anthranilate + PP-ribose-P $\xrightarrow{Mg^{2+}}$ *N*-(5′-phosphoribosyl) anthranilate + inorganic pyrophosphate (12, 15). Results from several laboratories indicate that there is definitely a

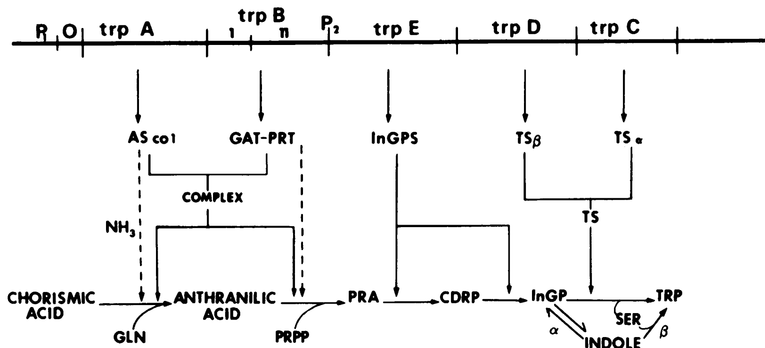


FIG. 1. *Tryptophan operon and biosynthetic pathway in S. typhimurium. Broken lines indicate reactions carried out by unaggregated products of trpA and trpB genes. P1 and P2 are primary and secondary trp promoter regions, 0 is trp operator. Abbreviations: InGPS, indoleglycerol phosphate synthetase; TSα and TSβ, subunits of tryptophan synthetase; GLN, L-glutamine; PRPP, 5-phosphorylribose 1-pyrophosphate; PRA, N-(5'-phosphoribosyl) anthranilate; CdRP, 1-(o-carboxyphenylamino)-1-deoxyribulose 5-phosphate; InGP, indole-3-glycerolphosphate; ser, L-serine; trp, L-tryptophan.*

tryptophan binding site on the *trpA* product, AS-CoI, in both *E. coli* and *S. typhimurium* LT2 (3, 14, 33), and that activities of both AS-CoI and PRT are more sensitive to tryptophan (or 5MT) inhibition when aggregated (as in prototrophs) than when unaggregated. However, the existence of a tryptophan binding site on the bifunctional GAT-PRT polypeptide, and the susceptibility of unaggregated PRT to feedback inhibition seem less certain (3, 14, 33). Feedback (5MT)-resistant mutants have lesions in *trpA* but apparently not in *trpB* (2, 23). The AS-CoI-PRT complex of *trpR* prototrophs should retain feedback sensitivity, although inhibition of AS-CoI but not PRT activity can be passed-by by growing cells in the presence of anthranilic acid. Under these conditions, as shown in the present paper, feedback inhibition of aggregated PRT activity in vivo appears sufficient to prevent excess production of tryptophan in *trpR* prototrophs if the gene encoding PRT (*trpB*) is derived from an LT7 strain of *S. typhimurium*, but not when the *trpB* gene is from an LT2 strain.

## MATERIALS AND METHODS

**Media.** Descriptions of most media used have been given previously (29, 30). Final concentrations of supplements added to minimal agar (M agar) for growth of *trp* auxotrophs were: anthranilic acid (MAnt), 5 μg/ml; indole (MInd) and tryptophan, 20 μg/ml. Other abbreviations used include: nutrient (N) agar; EM agar, 0.01% broth-enriched minimal agar.

**Bacterial strains.** The list of representative substrains of *S. typhimurium* LT2 and LT7 used in this study is given in Table 1; nonlysogenic prototrophs isolated from transduction crosses and used to define phenotypic differences between strains LT2 and LT7, and *trp* auxotrophs used in mapping experiments, are listed in Tables 2 and 3, respectively. The *mut* (mutator) genotype of strains with LT7 parentage (presence, $mut^-$, or absence, $mut^+$, of a lesion causing high frequencies of mutation) was determined by testing the frequency of mutation from streptomycin sensitivity to resistance in cultures streaked on N agar plus 100 μg of streptomycin/ml (20). Genetic symbols follow the recommendations of Demerec et al. (8) and have been defined by Sanderson (25).

**Transduction procedures.** Routine methods of transduction using the integration-deficient mutant, *int7*, of phage P22 (previously designated P22L7) (16, 28) have been described (29, 30). In later experiments the direct plating procedure without preincubation (10) was used, usually with recipient cells resuspended in single strength minimal salts solution (29). Transductant clones were characterized after one or, in later experiments, after two single-colony isolations on M agar; nearly all isolates were then phage free. All experiments were conducted at 37 C.

**Phenotypic characterization.** Responses to 5MT of phage-free prototrophic transductants were tested by a filter-paper disk method: about $10^7$ stationary-phase cells were spread onto M agar and allow to dry; a sterile 12-mm diameter filter-paper disk was then placed on the inoculated plate, and one drop (ca. 0.05 ml) of 0.2% 5MT solution was added. Plates were incubated overnight after which any zone of growth inhibition around the disk was noted. A disk containing sterile water was included as a control on each plate. The stab feeding test (29, 30) was used to detect excretion of tryptophan by *trpR* prototrophs: a diffuse halo of growth of the tester auxotroph, *trpE95*, around the stab, distinct from surface growth of the tested bacteria, indicated feeding. Strain *trpE95* accumulates and excretes anthranilic acid and no other supply of this compound was normally required to by-pass the feedback inhibition of AS-CoI activity by tryptophan which would have prevented

TABLE 1. *Substrains of Salmonella typhimurium LT2 and LT7*

| Strain[a] | Genotype | Origin of *trp* region | Source and/or reference[b] |
|---|---|---|---|
| | *argA85* | LT2 | A |
| SO144 | *cysB403 trpR531* | LT2 (*cysB403* transduced into LT7 recipient strain *trpR531 trpA47*) | P. P. McCann, thesis, Syracuse Univ., Syracuse, 1970. |
| SO595 | *cysB403 trpR1281* | LT2 (*cysB403*) | 29 |
| mtr-1 | *cysB517 trpR1352* | LT2 | 4 |
| | *metA22 trpE2* | LT2 | A, 27 |
| | *metB23* | LT2 | D. A. Smith, 27 |
| SU47 | *serB80 thrC59* | LT2 | A, 29, 30 |
| | *serA94* | LT2 | A |
| | *thrA5* | LT2 | A |
| | *thrA16* | LT2 (*pro* region from LT7) | A, 30 |
| | *thrB2 cysA21* | LT2 (?) probably strain *cysA21* | 27, 30 |
| | *thrB9* | LT2 (*pro* region from LT7) | A, 30 |
| SD90 | *thrB10* | LT2 (SU47) | 30 |
| SD30 | *thrC59 trpR582* | LT2 (SU47) | 30 |
| | *trpA8* | LT2 (*cysB12*) | 8 |
| | *trpA703* | LT2 | P. Margolin |
| | *trpB12* | LT2 | 5 |
| | *trpB258* | LT2 | B |
| | *trpE95* | LT2 | 5 |
| SO243[c] | *trpABE130* | LT2 | 5 |
| | *trpBED164* | LT2 | 5 |
| SD158 | *supX33 leu*$^+$ | LT2 | *supX33 leu500* (4) |
| Wt | + | LT2 | C |
| | *metA124 mut*$^-$ | LT7 | A |
| | *proAB47* | LT7 | A, 21 |
| | *serA103* | LT7 | A |
| | *thrA11* | LT7 (*pro* region from LT2) | A, 30 |
| | *thrA20* | LT7 | 11 |
| | *thrA23* | LT7 | A, 30 |
| | *thrA26* | LT7 | 11 |
| | *thrB8* | LT7 | 30 |
| | *thrB10* | LT7 (*pro* region from LT2) | 30 |
| SO396 | *thrBC12 trpR1329* | LT7 (*pro* region from LT2) | 29 |
| | *thrC29 mut*$^-$ | LT7 | A, 30 |
| | *trpA28* | LT7 | 5, 15 |
| SO641 | *trpAB684* | LT7 (*trpA28*) | 15 |
| | *trpE45* | LT7 | 5 |
| SO167 | *trpA47 trpR533* | LT7 (*trpA47*) | 2, 5, 29 |
| | *trpA49* | LT7 | 5 |
| | *trpB54* | LT7 | 5 |
| SO501 | *trpA512* | LT7 (*trpA49*) | 1, 31 |
| SO115 | *trpAB514* | LT7 LT7 (*trpB46*) | 15 |
| | *trpB883* | LT7 (*trpA47*) | 31 |
| SO136 | *trpA278B883* | LT7 (*trpB883 cysB12*) | by transduction, Stuttard, unpublished. |

[a] All mutants without strain designations were originally isolated at the Cold Spring Harbor Laboratory under the direction of the late M. Demerec. Records of their origins were kindly supplied by K. E. Sanderson.

[b] A, K. E. Sanderson, Salmonella Genetics Stock Centre (SGSC), Calgary University, Calgary, Alberta, Canada. B, Elias Balbinder, Biology Department, Syracuse University, Syracuse. C, Dublin University collection, originally from M. Demerec. Wt, Wild type.

[c] Strain SO243 is an Ind$^-$ strain isolated from the Trp$^-$ polar deletion mutant, *trpABE130*, and has either a small extension of the original mutation to end in-phase or a compensating phase-shift mutation very close to one end of *trpABE130*.

excess tryptophan production in the tested strains. However, to reduce possible variations in the levels of anthranilic acid present in different test plates, later tests were made by inoculating transductants on a small area of 4-ml overylays of 0.7% agar containing about 2 × $10^8$ *trpE95* cells on MAnt

plates. The overlay method was also used to compare feeding of the Trp⁻ deletion strain, *trpABEDC167*, on M, MAnt, and MInd agar.

## RESULTS

**Initial observations.** A differential effect of genetic background on the expression of *trpR* mutations was first noticed in crosses made to map different *thr* mutations with respect to *trpR* (29). When strain SD30 (carrying *trpR582 thrC59*) was used as donor in transductions with different *thr* recipients, two classes of 5MT-resistant (*trpR*) prototrophs were obtained (see below) depending on which *thr* recipient was used: (i) those which, when stabbed into M agar seeded with the tester auxotroph *trpE95*, promoted rapid growth of *trpE95* cells and formation of a dense halo around the stabs (rapid-feeding [RF] prototrophs); (ii) those which promoted much slower growth of strain *trpE95* in similar tests (slow-feeding [SF] prototrophs). Nonfeeding (NF), 5MT-sensitive ($trpR^+$) prototrophs were also produced in each cross. The feeding responses on different plates were scored with reference to control stabs in each plate: control strains were SD101 (NF), SD139 (RF), SD140 (SF) (Table 2, Fig. 2). In the reciprocal crosses all 5MT-resistant (*trpR*) recombinants were RF prototrophs. Thus an apparent modifier of the *trpR* phenotype (slow-feeding determinant, *sfd*) was present in substrains of *S. typhimurium* LT7 including strains *thrA11*, *B8*, *B10*, *C29*, and *C1001* (the latter derived from LT7 strain *serA103*), but not in LT2 derivatives, and was not genetically linked to the *trpR-thr* region. That *sfd* was nonspecific in its effect on *trpR* expression was shown when one of the *sfd*-carrying strains, *thrB10*, was used as the recipient in crosses with 17 different *trpR* donors selecting for $thr^+$ recombinants on EM agar. All *trpR* recombinants from each cross were SF prototrophs. Control crosses involving 4 of the 17 *trpR* donors and recipient strain SD90 (*thrB10* in LT2 genetic background) all gave 5MT-resistant prototrophs which were RF, none was SF.

Phenotypes of *trpR* strains with and without *sfd*: phage-free isolates of recombinants carry-

TABLE 2. *Phenotypes of prototrophs carrying different trpB genes*

| Strain | Derivative[a] | *trpR* allele | Source of *trpB* | *trpE95* feeding response[b] | Sensitivity to 5MT[c] |
|---|---|---|---|---|---|
| SD139 | mtr-1 × *thrC29* | 1352 | LT2 (mtr-1) | RF | 0 |
| SD140 | SD129 × *thrC29* | 1352 | LT7 (*trpA512*) | SF | 0 |
| SD142 | *thrC29 mut⁻* × wt | + | LT7 (*thrC29*) | NF | 37 |
| SD101 | SD90 × SO167 | + | LT2 (SU47) | NF | 30 |
| Wt LT2 | | + | LT2 | NF | 30 |

[a] By transduction: recipient × donor, see Tables 1 and 3.
[b] See Fig. 2. RF, Rapid feeding, large halos in stab or spot tests after 24 h; SF, slow feeding, very small halos around stabs after 24 h not visible in spot tests; NF, nonfeeding, no halos produced.
[c] Average inhibition zone size (millimeters, diameter), range of variation in repeat tests was less than 2 mm.

TABLE 3. *Derivation of trp auxotrophs carrying trpR1352*

| Strain[a] | Phenotype | Genotype[b] | Derived by transduction[c] |
|---|---|---|---|
| SD129 | Ant⁻ | *trpA512 sfd* | mtr-1 × SO501 |
| SD130 | Ind⁻ | *trpE95* | mtr-1 × *trpE95* |
| SD145 | Trp⁻ | *trpBED164* | mtr-1 × *trpBED164* |
| SD147 | Ant⁻ | *trpA47 sfd* | SD145 × SO167 |
| SD149 | Ant⁻ | *trpAB514 sfd* | SD145 × SO115 |
| SD150 | Ind⁻ | *trpABE130*[d] | mtr-1 × SO243 |
| SD151 | Ind⁻ | *trpB258* | mtr-1 × *trpB258* |
| SD152 | Ind⁻ | *trpA278B883 sfd* | mtr-1 × SO136 |
| SD155 | Ind⁻ | *trpB12* | mtr-1 × *trpB12* |
| SD157 | Ant⁻ | *trpA91* | mtr-1 × *trpA91* |
| SD159 | Ind⁻ | *supX33* | mtr-1 × SD158 |
| SD162 | Ant⁻ | *trpAB684* | SD155 × SO641 |

[a] Transductants were selected on tryptophan enriched media.
[b] Markers in addition to *trpR1352; sfd*, LT7 slow-feeding determinant.
[c] Recipients are in the left-hand column.
[d] Deletion modified as in strain SO243.

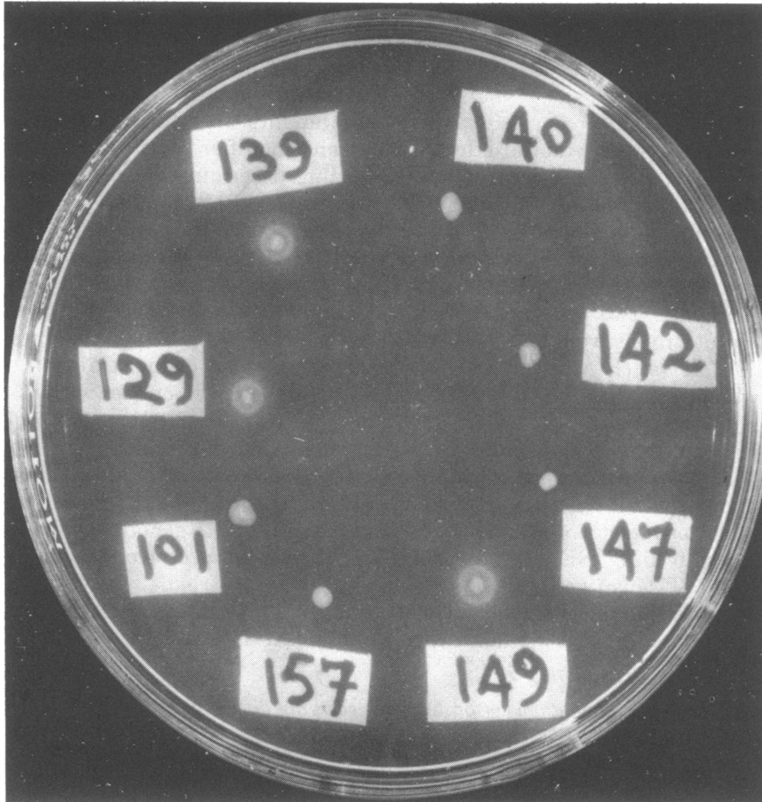


FIG. 2. *Feeding test with tester strain trpE95 in soft agar overlay on MAnt agar after 24 h at 37 C. Test inocula were transferred to the overlay by means of sterile toothpicks. Halos of tester growth around RF strains, SD129 (trpR1352 trpA512 sfd), SD139 (trpR1352, LT2 prototroph), SD149 (trpR1352 trpAB514 sfd) are clearly distinguishable from surface growth of NF strains, SD101 (LT2 prototroph) and SD142 (LT7* $mut^-$ *sfd prototroph); the SF strains, SD140 (trpR1352 LT7 sfd prototroph), SD147 (trpR1352 trpA47 sfd) and SD157 (trpR1352 trpA91) at this stage are indistinguishable from NF strains.*

ing various combinations of *trpR1352* and *trp* operon alleles were obtained from transduction crosses, and their phenotypes were compared (Table 2, and Fig. 2). The prototrophic *trpR* strain SD139, carrying LT2-derived *trp* genes, was RF, whereas strain SD140, carrying LT7-derived *trp* genes, was SF. The $trpR^+$ control strains SD101 (LT2) and SD142 (LT7 $mut^-$) were both NF. In the same tests the auxotrophic *trpR* strains, SD129 and SD149, carrying LT7-derived deletions *trpA512* and *trpAB514*, respectively, were RF. This suggested that an intact aggregate of AS-CoI and PRT (LT7) might be necessary to give an SF phenotype. Strains SD147 (LT7) and SD157 (LT2) also had SF phenotypes, presumably because of the polarity of mutations *trpA47* and *trpA91* respectively (3) caused reduced levels of PRT synthesis in these strains. No feeding by any *trpR* strains of deletion strain *trpABEDC167* in soft agar overlays was detected on M agar; on MAnt agar strain SD139 gave much larger halos than strain SD140, and both gave about the same halos on MInd agar. Strains SD142 and WtLT2 gave no halos except on MInd agar where very slight feeding was detected. The difference in feeding by strain SD139 compared with strain SD140 was more apparent when *trpE95* was the tester strain on MAnt agar.

The growth rates of all prototrophs and the $Ant^-$ strain SD149 were about the same in liquid minimal medium whether supplemented with 50 $\mu$g of tryptophan or 5 $\mu$g of anthranilic acid per ml.

In tests for 5MT sensitivity (see Materials and Methods), the WtLT2 strain showed an inhibition zone of 30 mm average diameter, strain SD142 had an average inhibition zone size of 37 mm with sharper edges and less background growth than with WtLT2. This suggested that the LT7 genes in strain SD142 may make the strain slightly more sensitive to

5MT than is the WtLT2 strain, although the presence of *mut*⁻ in strain SD142 might have some effect. None of the *trpR* prototrophs had inhibition zones in these tests. So *sfd* apparently had no effect on 5MT resistance of *trpR* prototrophs, but caused only a reduction in their capacity to overproduce tryptophan.

**Location of sfd. (i) Linkage with trp operon.** Various recipient strains carrying *trpR1352* and markers in or closely linked to the *trp* operon (Table 3) were used in mapping experiments. In preliminary two-point co-transduction tests selecting prototrophic recombinants from recipient strains mtr-1 (*trpR1352 cysB517*) or SD130 (*trpR1352 trpE95*) *sfd* was identified as a genetic lesion in or very near the *trp* operon of LT7 *trp*⁺ donors, including eight *thr* strains (Table 1). This was confirmed in crosses between strains carrying *sfd* and mutants with extensive deletions of the *trp* operon. The results (Tables 4, 5, 6) indicated that *sfd* was located outside of deletion *supX33* in strain SD159, in the region of overlap between deletions *trpBED164* and *trpABE130* (Fig. 3). This region includes virtually all of *trpB* region II which specifies that portion of the PRT polypeptide required for the PRT reaction, but none of *trpB* region I (the "unusual" region [3]) which specifies component II of anthranilate synthetase (the glutamine amidotransferase [GAT] portion of PRT). Absence of recombination between overlapping deletions *supX33* and *trpBED164* was confirmed in control crosses. These data did not entirely exclude the possibility that *sfd* lay within the *trpE* gene between deletion *trpE95* and the operator-distal end of modified deletion *trpABE130*. However, the recovery of RF prototrophs from crosses with strain SD159 showed that *sfd* was not a primary *trp* promoter (P1) or *trp* operator mutation nor was it in the *trpA* gene or in *trpB* region I since these elements are entirely deleted by the *supX33* mutation (15, 19). Thus *sfd* was present in all 21 strains with LT7-derived *trpB* genes (Table 1) but not in any of 20 strains with LT2-derived *trpB* genes. In subsequent mapping it was assumed that *sfd* occupied only one site regardless of recent parentage of each LT7 strain; all results were compatible with this assumption. In the cross between recipient strain SD159 and *trpB883* (Table 6) the absence of SF transductants among the sample tested suggested that *sfd* might be located distal to the *B883* site within *trpB* region II and would be included in prototrophic recombinants only by rare quadruple crossovers. Results of three-point tests to further define the location of *sfd* within *trpB* (Table 7) supported this possibility.

TABLE 4. *Location of sfd in region covered by deletion trpBED164: crosses with recipient strain SD145 (trpR1352 trpBED164) selecting prototrophs on M agar*

| Donor *trp* marker | Source of donor *trp* genes | Transductants | | |
|---|---|---|---|---|
| | | No. tested | Phenotype[a] (%) | |
| | | | RF | SF |
| *trpA8* | LT2 | 8 | 100 | 0 |
| *trpA91* | LT2 | 10 | 100 | 0 |
| *trpA703* | LT2 | 11 | 100 | 0 |
| *trpA28* | LT7 | 16 | 0 | 100 |
| *trpA47* | LT7 | 137 | 0 | 100 |
| *trpA49* | LT7 | 33 | 0 | 100 |
| *trpA512* | LT7 | 40 | 0 | 100 |
| *trpAB514* | LT7 | 38 | 0 | 100 |

[a] See Table 2.

TABLE 5. *Location of sfd in region covered by deletion trpABE130[a]: crosses with recipient strain SD150 (trpR1352 trpABE130[a]) selecting prototrophs on EM agar*

| Donor strain | Source of donor *trp* genes | Transductants | | |
|---|---|---|---|---|
| | | No. tested | Phenotype[b] (%) | |
| | | | RF | SF |
| *argA85* | LT2 | 6 | 100 | 0 |
| *cysB403* | LT2 | 6 | 100 | 0 |
| *metB23* | LT2 | 15 | 100 | 0 |
| *metA22 trpE2* | LT2 | 16 | 100 | 0 |
| *trpE95* | LT2 | 22 | 100 | 0 |
| *Wt* | LT2 | 6 | 100 | 0 |
| *serA103* | LT7 | 59 | 0 | 100 |
| *thrC29* | LT7 | 64 | 0 | 100 |
| *trpE45* | LT7 | 12 | 0 | 100 |

[a] Modified as in strain SO243 (Table 1.)
[b] See Table 2.

TABLE 6. *Location of sfd outside region covered by deletion supX33: crosses with recipient strain SD159 (trpR1352supX33)[a] selecting prototrophs on EM agar*

| Donor strain | Source of donor *trp* genes | Transductants | | |
|---|---|---|---|---|
| | | No. tested | Phenotype[b] (%) | |
| | | | RF | SF |
| *trpB12* | LT2 | 16 | 100 | 0 |
| *trpB258* | LT2 | 16 | 100 | 0 |
| *trpE45* | LT7 | 61 | 34 | 66 |
| *trpB883* | LT7 | 16 | 100 | 0 |
| *proAB47* | LT7 | 22 | 4.5 | 95.5 |

[a] See Table 3.
[b] See Table 2.

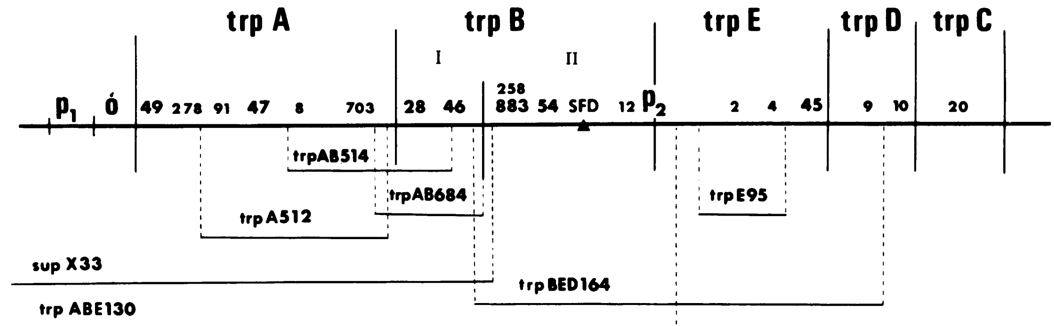


FIG. 3. *Partial map of the trp operon in S. typhimurium, (3, 5, 15, 31, 32). The bacterial chromosome is represented by the wider horizontal line; solid vertical lines represent gene boundaries (spacing is arbitrary). Larger numbers above the "chromosome" line indicate LT7 mutation sites, smaller numbers indicate LT2 mutation sites; sfd, relative location of LT7 lesion giving the SF phenotype in trpR prototrophs. Narrow horizontal lines represent the extents of deletion mutations as designated. P1, Principal trp promoter; P2, secondary trp promoter; 0, trp operator.*

TABLE 7. *Location of sfd within trpB region II*[a]

| Cross[b] | | Transductants | | | Probable site order[d] |
|---|---|---|---|---|---|
| Recipient | Donor | No. tested | Phenotype[c] (%) RF | SF | |
| 1. *trpA47 sfd* | *trpB12* | 48 | 23 | 77 | *A47-sfd-B12* |
| 2. *trpA47 sfd* | *trpB258* | 48 | 0 | 100 | *A47-B258-sfd* |
| 3. *trpB258* | *trpA49 sfd* | 31 | 7 | 93 | *A49-B258-sfd* |
| 4. *trpE95* | *trpA49 sfd* | 16 | 31 | 69 | *A49-sfd-E95* |
| 5. *trpA512 sfd* | *trpB258* | 74 | 5 | 95 | *A512-B58-sfd* |
| 6. *trpA512 sfd* | *trpE95* | 48 | 23 | 77 | *A512-sfd-E95* |
| 7. *trpE95* | *trpA512 sfd* | 10 | 30 | 70 | *A512-sfd-E95* |
| 8. *trpA512 sfd* | *trpBED164* | 112 | 0 | 100 | *sfd* covered by *BED164* |
| 9. *trpAB514 sfd* | *trpBED164* | 240 | 0[e] | 100 | *sfd* covered by *BED164* |
| 10. *trpAB514 sfd* | *trpB258* | 64 | 3 | 97 | *AB514-B258-sfd* |
| 11. *trpAB514 sfd* | *trpB12* | 52 | 25 | 75 | *AB514-sfd-B12* |
| 12. *trpAB514 sfd* | *trpE95* | 48 | 30 | 70 | *AB514-sfd-E95* |
| 13. *trpAB684 sfd* | *trpB12* | 32 | 23 | 77 | *AB684-sfd-B12* |
| 14. *trpAB684 sfd* | *trpB258* | 46 | 11 | 89 | *AB684-258-sfd* |
| 15. *trpA278 B883 sfd* | *trpB12* | 80 | 66 | 34 | *A278-B883-sfd-B12* |
| 16. *trpB12* | trpB883 sfd | 48 | 52 | 48 | B883-sfd-B12 |
| 17. *trpB12* | *trpB54 sfd* | 52 | 75 | 25 | *B54-sfd-B12* |
| 18. *trpB258* | *trpB54 sfd* | 73 | 86 | 14 | *B258-B54-sfd* |
| 19. *trpB258* | *trpE45 sfd* | 63 | 49 | 51 | *B258-sfd-E45* |
| 20. *trpB12* | *trpE45 sfd* | 48 | 12 | 88 | *sfd-B12-E45* |
| 21. *trpE95* | *trpE45 sfd* | 24 | 8 | 92 | *sfd-E95-E45* |

[a] Prototrophs selected on M or EM agar.
[b] For convenience, only counterselective *trp* markers and the LT7 marker *sfd* are indicated; all recipients carried *trpR1352* (see Table 3).
[c] See Table 2.
[d] On the assumption that progeny requiring most crossovers have the lowest frequencies.
[e] One RF colony was recovered but was apparently a relatively stable merodiploid.

The order of markers, *trpB883*, *B258*, *B54*, and *B12*, used to locate *sfd* within *trpB* region II, was checked by control crosses: all gave recombinants with *supX33*; *trpB883* and *trpB258* (a very stable mutation, possibly small deletion) did not recombine with each other but both gave prototrophic recombinants with *trpB54* and *trpB12*; all gave abortive transductants with *trpA* and *trpE* mutants but not with each other; in the cross SO136 (*trpA278 trpB883*) × *trpB12* (donor) on anthranilate-supplemented media less than 10% of recombinants

were Ant$^-$ (*trpA278*) indicating the site order: *trpA278—B883—B12*. The relative order *trpA—B883—B54—B12* had been determined previously (3, 5, 31). This knowledge of relative order of markers in *trpB* region II was then used to locate *sfd*: in Table 7, crosses 1 and 2 indicated that *sfd* present in LT7 strain *trpA47* was located between *trpB258* and *trpB12*; crosses 3 to 8 showed a similar location for *sfd* in strain *trpA49* and its derivative strain SO501 (*trpA512*); likewise for strain SO115 (*trpAB514*) derived from LT7 strain *trpB46* (crosses 9 to 12), and strain SD162 (*trpAB684 trpR1352*) derived from LT7 strain *trpA28* (crosses 13 and 14). Excretion of anthranilic acid by *trpB* recipient strains, including strain SD145 (carrying *trpBED164*) prevented adequate counterselection of donor-type transductants on M agar in crosses with Ant$^-$ donor strains (e.g., SO501 and SO115). Consequently such crosses were not analyzed. Results of crosses 15 and 16 were in agreement with the result given in Table 6 which indicated *sfd* was indeed linked to *trpB883* and lay between *trpB883* and *trpB12*. The alternative order A278—*sfd—B883—B12* would necessitate rare quadruple exchanges to give SF recombinants in both crosses, whereas the observed SF frequencies were much more compatible with the given order. Similarly, analysis of strain *trpB54* (crosses 17 and 18) showed the presence of *sfd* between *trpB54* and *trpB12*. Finally, in analyzing the location of *sfd* linked to *trpE45* (crosses 19 to 21), it was concluded that *trpE45* was actually situated at the operator-distal end of *trpE* rather than at the operator-proximal end as given previously (5). This was confirmed in control crosses with recipient strain *trpA47sE4* (Stuttard, unpublished data; see Fig. 3). Unpublished data of Balbinder and coworkers (personal communication) also supports the conclusion that the relative order of several *trpE* markers with respect to *trpB* is the reverse of that given earlier by Blume and Balbinder (5).

## DISCUSSION

In the present studies I have found that the wild-type *trpB* gene originating in *S. typhimurium* strain LT7 is genetically different from the homologous wild-type gene of strain LT2. A genetic lesion, *sfd*, has been identified as the cause of reduced tryptophan biosynthesis in *trpR* prototrophs carrying a *trpB* gene originating in strain LT7 compared with those carrying an LT2-derived *trpB* gene. Since *sfd* is located in *trpB* region II, the affected gene product should be PRT. This inference was supported by preliminary results of assays for PRT activity in crude extracts of strains SD139 and SD140 grown in the absence of tryptophan, which indicated that the specific activity of LT2 PRT in strain SD139 was considerably higher and had a lower $K_m$ for anthranilic acid than the LT7 PRT found in strain SD140 (M. Dooley, personal communication, see also Table 3 in reference 15). Besides a reduction in catalytic activity of LT7 PRT compared with LT2 PRT the LT7 *trpB* product may (i) be made in lesser amounts, or (ii) have enhanced end-product inhibition, or (iii) have enhanced substrate inhibition in the presence of anthranilic acid and an intact *trpA* product, or both, when compared with PRT specified by LT2 *trpB* gene under the same conditions.

When *trpR* auxotrophs carrying LT7 deletion mutations *trpA512* or *trpAB514* were also tested for feeding of *trpE95* on MAnt agar no differences from LT2 *trpR* prototrophic phenotype were detected (Fig. 2). Strain SO501 carrying deletion *trpA512* produces an unaggregated GAT-PRT polypeptide (AS-CoII) able to complement the free AS-CoI of strain *trpBEDC43* (3, 15) to give glutamine-dependent AS activity; strain SO115 (*trpAB514*) produces a PRT polypeptide with no AS-CoI complementing (GAT) activity, and in which the enzymically active carboxy-terminal segment is probably fused to an amino-terminal segment specified by the operator-proximal region of *trpA* (Stuttard, unpublished observations). So it is likely that PRT activity in *trpR* strains carrying either of these deletions would not be subject to the same degree of feedback inhibition, or any other effect of AS-CoI when aggregated with PRT, as would the activity of aggregated PRT in *trpR* prototrophs. This would explain why LT2 *trpR* prototrophs and LT7 *trpR trpA* deletion strains are both RF, whereas LT7 *trpR* prototrophs are SF. However, considerably more data on the effects of *sfd* on the various parameters of LT7 PRT compared with LT2 PRT activity are required before more definite conclusions can be drawn.

The discovery of this difference between apparently wild-type *trpB* genes of strains LT2 and LT7 of *S. typhimurium* is a little disconcerting in view of the prevalent practice of considering them to be entirely equivalent and interchangeable according to their convenience for genetic or biochemical analysis. The presumption of equivalence may be valid in many instances. For example, there appear to be no differences between LT2 and LT7 strains in their restriction and modification of foreign

(phage or bacterial) deoxyribonucleic acid (7). However, the present evidence shows that subtle differences can exist and may be important in particular studies. The differential effects on PRT activity ascribed to some polarity mutations in *trpA* (3, 32) may actually be a consequence of differing parentages of *trpB* genes in the strains used. Also since some of the LT7 strains carry the *mut*⁻ allele (20) the ancestral presence of the mutator gene in these strains might be expected to have caused any number of genetic alterations which remain to be revealed. However, unless all present LT7 substrains are derived from one ancestral *mut*⁻ strain, it seems that the *trpB* gene difference from the LT2 wild-type is indeed characteristic of the LT7 wild-type strain rather than of LT7 *mut*⁻-derived strains alone.

I have also found that nearly all strains with a basically LT7 parentage, but none of the LT2 strains, are lysogenic for a phage as yet unidentified. It forms very small, almost clear plaques on LT2 strains. Supernatant fluid taken from an overnight culture of any LT7 strain, except *thrC29*, grown in L broth contain about $10^5$ plaque-forming units per ml. Strains carrying a *cysB-trp* region of LT2 origin in an otherwise LT7 genome, such as strain SO144, are also lysogenic for this phage. So this particular lysogeny has no role in the production of an SF phenotype, nor does it affect sensitivity to phages P22 and KB1 (6). No further analysis of this LT7 phage has yet been made, although it may be one of the factors by which wild-type strains LT2 and LT7 were originally differentiated (18).

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