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"Self-Feeding" Strain of *Salmonella typhimurium* with a Mutation in the *trpB* Gene and Nutritional Requirements of *trpA* Gene Mutants[1]

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An indole-requiring (Ind$^-$) mutant of *Salmonella typhimurium*, isolated from a culture of a leaky *trpA* mutant, was genetically analyzed by P22-mediated transduction. The mutation site giving the Ind$^-$ phenotype was shown to be in *trpB*, the second gene of the *trp* operon. A second mutation at this site resulted in change of nutritional requirement from indole to anthranilic acid (Anth$^-$). This phenotype is normally associated with mutations in the first *trp* gene, *trpA*. However, the Anth$^-$ mutant also excreted anthranilic acid and showed "self-feeding" on unsupplemented media. Of two possible explanations for this aberrant phenotype, the first, that the *trpB* mutations may be in the "unusual" region, was dismissed on genetic evidence and on the biochemical evidence that an active anthranilate synthetase (AS) is produced. The alternative explanation, that the affected enzymatic activity, phosphoribosyl transferase, is unstable in vivo, but its AS component 2 activity is stable, is considered more probable.

A cluster of five genes controlling the sequence of reactions from chorismic acid to tryptophan has been recognized in *Salmonella typhimurium* (2, 5). At one end of this cluster is the *trpA* gene, which controls the conversion of chorismic acid to anthranilic acid by the production of component 1 of anthranilate synthetase (AS). The adjacent *trpB* gene also controls the synthesis of anthranilic acid together with its conversion to *N*-5'-phosphoribosyl anthranilic acid (PRA) by the production of phosphoribosyl transferase (PRT), component 2 of AS (1). We have studied a mutant, an Ind$^-$ phenotype, that will grow on minimal medium with indole (MM + Ind) but not on MM with anthranilic acid (MM + Anth). Its mutation site is in the *trpB* gene. Further mutation at this site, or at a site very close to it and still within the *trpB* gene, changes the nutritional requirement from indole to anthranilic acid (Anth$^-$ phenotype), and the mutant will then grow on MM + Anth at 37 C (normally the phenotype of *trpA* mutants). However, this Anth$^-$ strain also excretes anthranilic acid and thus represents an unusual type of biochemical mutant. We shall consider two possible explanations representing mechanisms which may be operative in mutants with aberrant phenotypes. Both mechanisms invoke protein-protein interactions between the products of adjacent genes (1, 7) rather than the interference with transcription or translation found with antipolarity mutations (11).

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MATERIALS AND METHODS

Salmonella strains *trp-43*, *trp-107*, *trp-130*, *trp-BED 164*, and *supX33* are deletion mutants. They were kindly supplied by E. Balbinder.

Other mutants were obtained from the collection of the late M. Demerec. One of these is *trpA47*. Its slow-growing reversions (Anth-S phenotype) have been shown to be due to mutations that are inseparable from the *47* site, and their genotype was designated *trpA47s* (9). The Ind$^-$ mutant (TD3) which is described in this paper arose by a spontaneous mutation in one strain of *trpA47s*. A summary of the strains used is given in Table 1.

The method of making phage suspensions, the method of making transductions, and the composition of the media were similar to those described by P. F. Smith-Keary (10). In descriptions of crosses, the donor marker is said to be transduced to the recipient. The concentrations of anthranilic acid and indole in supplemented media were 5 and 20 μg/ml, respectively. Enzyme assays were performed at Syracuse and followed the procedure of Blume, Weber, and Balbinder (3).

TABLE 1. *Strains used*[a]

Designation	Genotype	Phenotype	Origin
	trpA47s	Anth-S	Partial revertant of *trpA47* (9)
TD3	*trpA47s B883*	Ind$^-$	Spontaneous mutation in a culture of *trpA47s*
TD10	*trpA47s B890*	Anth$^-$	Partial revertant of TD3
TD14	*trpB883*	Ind$^-$	TD3 by transduction: *trpE95* (x)TD3 on EMM + Ind
TD15	*trpB890*	"Leaky" Anth$^-$	TD10 transduction: *trpE95* (x) TD10 on EMM + Anth

[a] Anth-S, colonies growing more slowly than wild type (LT2) in the absence of anthranilic acid, indole, or tryptophan; no anthranilate excreted. Ind$^-$, colonies requiring indole or tryptophan for growth. Anth$^-$, colonies requiring anthranilic acid, indole, or tryptophan; no anthranilate excreted. "Leaky" Anth$^-$ colonies differ from Anth-S in their pattern of growth (dependent on cell density), and anthranilate is excreted.

RESULTS AND DISCUSSION

Whether the Ind$^-$ auxotroph that arose by mutation in *trpA47s* had retained the *A47s* site was investigated by two transductions. When *trpA47* was transduced to TD3, 26% of the transductants, selected on MM + Anth, had Anth-S phenotypes that were indistinguishable from that of *trpA47s*; 74% were small colonies of donor (*trpA47*) phenotype. No wild-type transductants were obtained. When *supX33* was used as donor, all of the 27 transductants that were tested had the Anth-S phenotype. We conclude that the Ind$^-$ auxotroph retained the *A47s* site in its *trpA* gene. The location of the additional mutation that was responsible for its requiring indole was investigated by further transductions. No Ind$^+$ transductants were found when *trp-43* and *trp-107* were transduced to the auxotroph. As these are deletion mutations that extend from *trpB* through *trpC*, we conclude that the additional mutation site is in this region (Fig. 1). That there were no Ind$^+$ transductants when *trp-130* was used as donor reduces the possible locations for the site to within the *trpB* gene and the adjacent part of the *trpE* gene. The results of reciprocal transductions between TD3 and point mutants (*trpE4*, *trpB121*, *trpB17*, and *trpB54*, the latter used as donor only), carried out on broth-enriched MM (Table 2), suggest that the Ind$^-$ mutation (designated *trp-883*) is located between *trpB17* and *trpB54* (Fig. 1).

Mutations to the right of *trp-883* (*trpE4* and *trpB54*) gave a majority of prototrophic recombinants in the samples of colonies tested when used either as donor or as recipient with TD3. Those to the left (*trpB17* and *trpB121*) gave a majority of Anth-S recombinants (*trpA47s* phenotype). Also, the activity of indoleglycerol phosphate synthetase (InGPS), the *trpE* gene product, was normal, although there was no detectable PRT activity even in fully derepressed cells (Table 3). It is concluded that the *883* mutation site is in the *trpB* gene. The genotype of the Ind$^-$ auxotroph TD3 can therefore be designated *trpA47s B883* (Table 1). The *A47s* site was removed by transduction to obtain *trpB883*. It is an Ind$^-$ auxotroph (strain TD 14) which, when used as a recipient on MM, gave minute colonies indicative of abortive transduction with *trpA*, *E*, *D*, and *C* mutants, but not with *trpB12*. This lack of complementation with a known *trpB* mutant is further evidence for the *trpB* location of *883*.

When TD3 was spread on media supplemented with anthranilic acid (EMM + Anth), the majority of reversions to Ind$^+$ were anthranilic acid-requiring. Four of these which had been independently isolated were crossed by transduction in all pair-wise combinations, and no prototrophic transductants were found. No prototrophic transductants were found when each was crossed with TD3. We conclude either that they retain the *883* site and a mutation elsewhere both suppresses the requirement for indole and creates a requirement for anthranilic acid, or that they arise by further mutation at the *883* site. If the *883* site is retained and the further mutation is to the left of it, we should find Ind$^-$ transductants when these reversions are transduced to *trpD6* on EMM + Ind. The recipient, *trpD6*, grows on EMM + tryptophan but not on EMM + Ind. In such transductions with each of the four reversions, no Ind$^-$ transductants were found in a total of 708 colonies tested. If the *883* site is retained and the further mutation is anywhere to the right of it, we should find Ind$^-$ transductants when the reversions are transduced to *cysB12* and Cys$^+$ transductants are selected on EMM + Ind. No Ind$^-$ transductants were re-

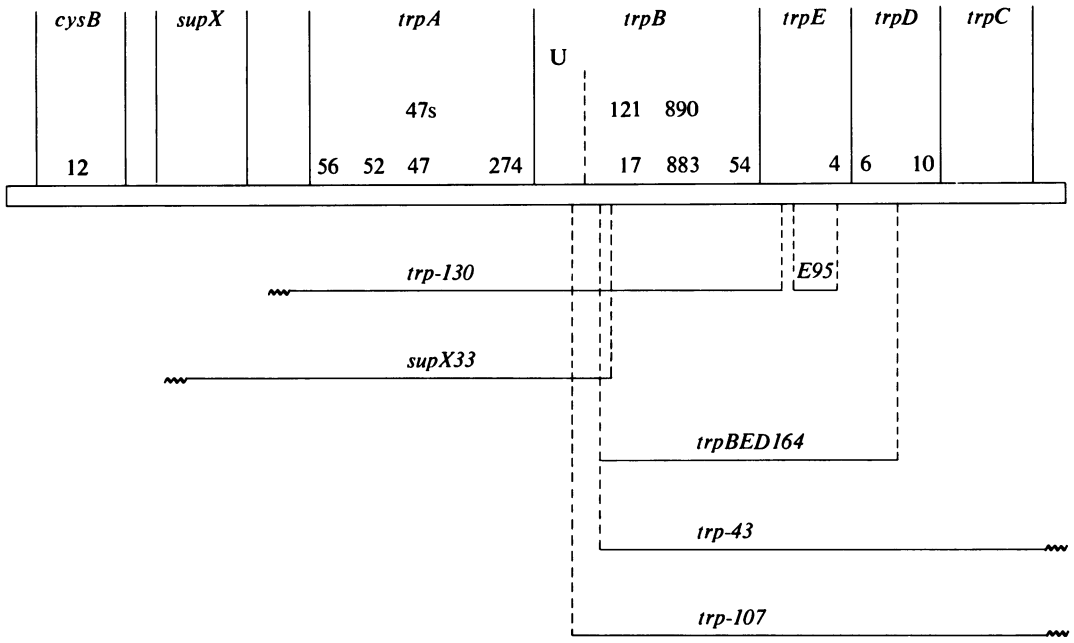

FIG. 1. *Map of the trp transducing fragment of S. typhimurium showing the relative locations of mutations used in this study. Thin bars represent the extents of known deletion mutations (2); U, region of "unusual" mutations (1).*

TABLE 2. *Transductions involving TD3 and Ind⁻ point mutants*[a]

Cross	Transductant phenotype	Point mutant						
		trpB121		*trpB17*		*trpB54* (D)	*trpE4*	
		D	R	D	R		D	R
Expt A, with TD3 (*trpA47s B883*)	Wild type	17	75	4	4	41	29	65
	Anth-S	37	108	14	14	13	7	9
Expt B, with *trpA47s E4*	Wild type	24	56					
	Anth-S	144	104					

[a] Figures refer to numbers of transductant colonies picked at random from EMM transduction plates and tested for growth phenotype by streaking for single colonies on MM and MM + Anth. Experiment B was a control; *trpE4* is known to be to the right of *trpB12* (Fig. 1). Anth-S, colonies growing more slowly than wild type in the absence of anthranilic acid. D, used as donor; R, used as recipient.

covered in 461 colonies tested from these transductions. We conclude that the mutation in these reversions is at, or very close to, the *883* site. That this mutation was not in the *trpA* gene was confirmed by finding Anth-S transductants indistinguishable from *trpA47s* when *supX33* was transduced to each of the four reversions. In addition, these *trpA47s* transductants, which also arose in crosses with *trpD6* and with *cysB12*, demonstrated the retention of the *47s* site in the *trpA* gene of the reversions. The genotype of these reversions can therefore be designated *trpA47s B890-3* (strains TD10 to 13). Further work (Table 1) has been confined to TD10 (*trpA47s B890*).

These experiments have demonstrated that, of two changes in the *trpB* gene that are inseparable by transduction, one is expressed as a requirement for indole and the other as a requirement for anthranilic acid or indole. This can be explained in two ways. There is evidence that the products of the *trpA* and *trpB* genes are normally associated in vivo and that PRT protein is required for AS activity, but not vice versa. However, some AS (component 1) activity can

TABLE 3. *Derepressed tryptophan biosynthetic enzymes in crude extracts*[a]

Mutant	AS	PRT	InGPS	TSα	TSβ2
trpA47s	0	+	+	+	+
trpA47s B883	0	0	+	+	+
trpA47s B890	0	?	+	+	+
trpB890	+	?	+	+	+

[a] Symbols: +, approximately nonpolar auxotrophic level (2); 0, no activity detected in vitro; ?, activity barely detectable at the level of resolution of the assay (0.02 enzyme unit per ml). Assays performed as described by Blume et al. (2). One enzyme unit causes the production or conversion of 0.1 μmole of product or substrate in 20 min at 37 C. Abbreviations: AS, anthranilate synthetase; PRT, phosphoribosyl transferase; InGPS, indoleglycerol phosphate synthetase; TSα, TSβ2, components of tryptophan synthetase.

be detected without the normal product of the *trpB* gene (6, 12), and anthranilic acid is accumulated by *trpBED164* (Fig. 1; 2). Also, we have observed feeding of *trpA* mutants by *trp-43* and *trp-107*. In addition, we can imagine changes in *trpB* which may not drastically affect the catalytic activity of the PRT protein, but which may cause this protein (AS component 2) to interfere with the AS activity of component 1, the *trpA* gene product ("pseudopolarity" mutants; 1, 7). However, such mutants would not accumulate anthranilic acid. Bauerle and Margolin (1) described Anth⁻ mutants bearing mutations assigned to the *trpB* gene ("unusual" mutants). These authors ascribe the Anth⁻ phenotype to the inability of the mutant component 2 to activate the AS (component 1), although retaining some PRT activity; however, this explanation is questionable (3). It should be noted that the *883* and *890* site(s) are not in the region of "unusual" mutants but are distal to them within the *trpB* gene.

Alternatively, the PRT produced in TD10 may be an inefficient or unstable enzyme which converts so little of the intracellular anthranilic acid to PRA that there is no detectable growth at 37 C on MM. Increasing the concentration of anthranilic acid by adding it to the medium may stabilize this PRT and enable it to convert a sufficient amount to promote growth and give TD10 its Anth⁻ phenotype. The amount of anthranilic acid formed by TD10 is likely to be small, since its mutant *trpA* probably produces an inefficient AS component 1; *trpA47s* grows slowly on MM at 37 C and has no AS detectable in vitro.

We have been able to show that TD10 will feed the Anth⁻ auxotrophs, *trpA52* and *trpA274*, on MM at 37 C. If an inefficient PRT in TD10 allows much of the small amount of presumptive anthranilic acid that is produced by these cells to accumulate, it could be excreted and could feed Anth⁻ strains having normal PRT. The *trpB* mutation (890) has been isolated from TD10 by transduction, and the resulting strain (*trpB890*) also requires anthranilic acid for optimal growth (see below). By means of paper chromatography (8), anthranilic acid was identified in ether extracts of liquid MM + 4 μg of L-tryptophan per ml from which cells of *trpB890* had been removed after growth for 21 hr at 37 C. Similar extracts of media in which *trpE4* (known to excrete anthranilic acid) and *trpA56* (unable to synthesize anthranilic acid) had been grown, as well as known samples of anthranilic acid and acetyl anthranilic acid, were used as controls on the same paper. Feeding of *trpA* mutants by *trpB890* was also observed. It is concluded that *trpB890* and hence probably its parent strain TD10 both excrete anthranilic acid. Although *trpB890* requires anthranilic acid for growth, in dense inocula on MM it appeared to be very leaky, and in streaks growth was always radial from the point of densest inoculation. This phenotype implies some form of "self-feeding," a phenomenon which may be accommodated in the explanation of the Anth⁻ phenotype on the assumption that the mutant PRT is enzymically unstable and may be rapidly inactivated within the growing cell. Very low PRT activity has been detected in in vitro assays of extracts of fully derepressed cells. Such catalytic inactivity would permit the accumulation of anthranilic acid in a manner similar to the accumulation of indole in *E. coli* by the leaky tryptophan synthetase mutant *trpB8* (note that *E. coli* nomenclature differs from that used for *S. typhimurium*) described by Crawford and Johnson (4), provided that the inactive PRT was still functional in the AS reaction. This activity has been demonstrated in extracts of *trpB890*. An elevated concentration of anthranilic acid, either by direct medium supplementation or by cellular excretion, may facilitate catalytic stability in growing cells by some mechanism, such as increased substrate binding, and thus produce the aberrant Anth⁻ phenotype of this *trpB* mutant concomitantly with the excretion of anthranilic acid. However, the precise biochemical interactions involved await further elucidation.

Our purpose in publishing this preliminary work on these strains is to report the isolation of this novel biochemical mutant and to re-emphasize some of the general problems in inter-

preting mutant phenotypes in biochemical terms (1).

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