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Restoration of Phosphoribosyl Transferase Activity by Partially Deleting the *trpB* Gene in the Tryptophan Operon of *Salmonella typhimurium* [1]

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The amber mutant *trpA28*, which contains a mutation mapping within the so-called "unusual" region of the tryptophan (*trp*) operon of *Salmonella typhimurium* (between the genes *trpA* and *trpB*), lacks both components of the anthranilate synthetase (AS)-phosphoribosyl transferase (PRT) enzyme complex, the products of the genes *trpA* and *trpB*, respectively. Twenty-six revertants of this mutant selected on minimal medium supplemented with anthranilic acid, a substrate of PRT, contain deletions of various segments of the "unusual" region and make a species of PRT different in every respect from the wild-type, dissociated form of this enzyme. The results indicate that the unusual region corresponds to the operator proximal end of the *trpB* gene. Mutants in the unusual region, however, show unexpectedly low levels of AS activity and in two cases (*trpA515* and *trpA28*) no detectable activity of this enzyme component.

The first two reactions of tryptophan synthesis in the *Enterobacteriaceae* are catalyzed by a bifunctional enzyme aggregate composed of anthranilate synthetase component I (AS-CoI or AS) and anthranilate-5-phosphoribosyl pyrophosphate phosphoribosyl transferase (AS-CoII or PRT) (4, 19; Fig. 1). Both in *Escherichia coli* (18) and in *Salmonella typhimurium* (4, 6) AS has been shown to be the product of the first, and PRT of the second, structural genes of the tryptophan (*trp*) operon. These are designated *trpA* and *trpB* in *S. typhimurium* (6). AS (AS-CoI) catalyzes the reaction: chorismate + NH_3 $\xrightarrow{Mg^{2+}}$ anthranilate + pyruvate.

Aggregation of AS-CoII allows glutamine to be utilized as a substrate in the above reaction: chorismate + glutamine $\xrightarrow{Mg^{2+}}$ anthranilate + pyruvate + glutamate.

The aggregate or unaggregated PRT (AS-CoII) catalyzes the second reaction of tryptophan synthesis: anthranilate + PP-ribose-P $\xrightarrow{Mg^{2+}}$ *N*-(5'-phosphoribosyl) anthranilate (PRA) + inorganic pyrophosphate (PPi).

As a rule, mutants for *trpA* lack AS but not PRT activity and can utilize anthranilic acid as a growth factor. Mutants in *trpB*, on the other hand, are deficient in PRT but have AS activity and were defined by their inability to utilize anthranilic acid as a growth factor while accumulating it into the culture medium (6). A group of mutations mapping in the region of the boundary between *trpA* and *trpB* proved to be exceptional in that they were simultaneously deficient for both activities of the complex. These were called "unusual" mutations by Bauerle and Margolin (4) who suggested that they defined the operator proximal portion of *trpB*, since the addition of saturating amounts of wild-type, free PRT to extracts of these mutants restored glutamine-dependent AS activity. This interpretation was questioned (2, 7) on the basis of the following observations: (i) All unusual mutations show polarity effects (reduced derepression levels of the last three gene products of the operon), suggesting that they may be nonsense (chain terminating) mutations. Since most of the unusual mutants are leaky and possess low PRT activity, it would be difficult to explain how a chain-terminating mutation in the early part of *trpB* could still allow synthesis of the

[1] Portions of this report have been abstracted from a Master's thesis of L. J. La Scolea.

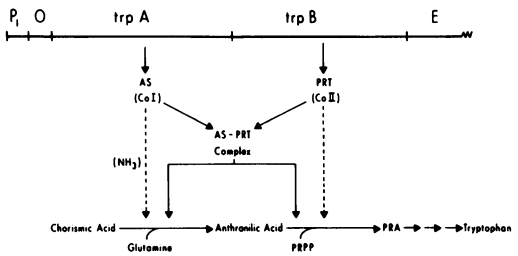

FIG. 1. *AS-PRT complex of the trp operon of Salmonella typhimurium. Broken lines, reactions which can be carried out by each component of the complex in the dissociated state; solid lines, reactions carried out by each component as part of the complex.*

enzyme. (ii) When the AS of two leaky unusual mutants was assayed in the glutamine-dependent reaction in the presence of saturating amounts of PRT, the specific activities found were lower than normal. (iii) A strain with an amber mutation mapping in the unusual region, *trpA28*, has no detectable activity for either AS or PRT under all assay conditions (2, 7). On the basis of these observations, an unambiguous assignment of the unusual region to either *trpA* or *trpB* could not be made at the time (2, 7).

The *trpA28* strain differs from the majority of the unusual mutants in its total lack of activity for both components of the AS-PRT complex and, consequently, is not leaky. On the other hand, it exhibits the same kind of polarity effect on the levels of the last three gene products as do the leaky unusual mutants (2, 7). This combination of properties make *trpA28* unique. Another exceptional mutation mapping in the unusual region is *trpA515* (3, 8). Strains carrying *trpA515* can utilize anthranilic acid as a growth factor when the analogue 5-methyltryptophan (MT) is present, but not when it is absent. This peculiar phenotype stems from both the nature of the *trpA515* mutation as well as its location. Under repressing conditions (excess tryptophan or MT), *trpA515* can be recognized as a "promoter" (transcription start signal), and an active species of PRT is produced. However, under derepressing conditions (growth on limiting tryptophan) it is probably read as a structural mutation in the unusual region, and only residual PRT activity is produced (8). No AS activity is detected under either of these growth conditions (8). In this last property *trpA515* resembles *trpA28*.

Recent developments have shed new light on the role and meaning of the unusual region. Several reports (12, 17; Bauerle et al., Fed. Proc. **30**:1058, 1971) indicate that PRT is a bifunctional enzyme composed of two parts: the amino terminal portion (about 40% of the PRT polypeptide) which possesses glutamine amidotransferase activity and complements AS in the glutamine-dependent anthranilate synthetase reaction, and the carboxy-terminal portion (the remaining 60% of the enzyme) which is responsible for the PRT reaction. These two portions can be physically separated in the proper genetic mutants, or by mild proteolytic digestion of PRT. Grieshaber and Bauerle (12) have evidence which indicates that the unusual region corresponds to the operator proximal segment of *trpB* and codes for the glutamine amidotransferase portion of PRT. In agreement with this, we have observed that auxotrophic revertants of unusual mutants selected for their ability to grow on anthranilic acid supplement contain deletions which enter the unusual region, indicating that this region is not essential for PRT activity (2). The present report represents a study of a group of such revertants obtained from the unusual amber mutant (*trpA28*). Whereas our results indicate that most, if not all, of the unusual region corresponds indeed to the operator-proximal end of the *trpB* gene, they also show that mutations in this region affect the level of AS activity (the *trpA* gene product) present in the cell.

Many of the deletions obtained by reverting *trpA28* on anthranilate-supplemented medium extend into *trpA*, and one of them deletes most of this gene. We have taken advantage of this material to look for a possible role of *trpA* or its polypeptide product in the regulation of the *trp* operon. We will also present some observations on the effect of deletions on recombination.

MATERIALS AND METHODS

Nomenclature. The system proposed by Demerec et al. (11) has been used to designate the bacterial mutants employed in this work.

Bacterial strains. With the exception of the revertants of *trpA28* which are the subject of this report, all mutant strains employed have been previously described (2, 4, 6). The *supX* deletions employed in mapping were a gift of P. Margolin and have also been described (24). The mutants *trpE95* and *trpE2*, altered in the third (middle) gene of the *trp* operon (6), were used as sources of wild-type AS-PRT complex. Free PRT was obtained from the mutant *trpA703*, which carries a chain-terminating mutation in *trpA* (15).

Media. The defined minimal medium routinely used was that of Vogel and Bonner (30) supple-

mented with 0.005% tryptophan-free acid-hydrolyzed casein (28) and 0.2% glucose. Enriched minimal medium was prepared by omitting the casein and substituting 1.25 (v/v) nutrient broth (Difco) in minimal medium. For solid media 1.5% (w/v) Difco agar was added to the liquid media. Nutrient broth and agar (Difco) were used as complex growth media. Routinely, supplements were added to the minimal medium for a final concentration of 20 μg/ml except for limiting anthranilic acid (LAA) which was used at a concentration of 2 μg/ml, and the tryptophan analogues 5-methyltryptophan (MT, K & K Chemicals, Plainview, N.Y.) and 6-fluorotryptophan (FT, Sigma Chemical Co.) which were employed at final concentrations of 100 μg/ml each.

Nitrous acid mutagenesis and selection of revertants. Revertants of *trpA28* capable of normal growth on anthranilic acid-supplemented minimal medium were recovered following treatment with nitrous acid according to a procedure suggested by J. Calvo (*personal communication*). A 1.0-ml sample of an overnight broth culture of the mutant *trpA28* was inoculated into 200 ml of prewarmed (37 C) nutrient broth. This culture was incubated on a rotatory shaker at 37 C with a constant aeration for 3 hr, and the bacteria were then centrifuged in a Sorvall RC-2 refrigerated centrifuge at 7,000 rev/min for 15 min. The pellet was washed twice in 100 ml of sterile saline and resuspended in 9.8 ml of sterile, prewarmed acetate buffer (0.1 M, *p*H 4.5). To this was added 0.2 ml of a sterile solution of sodium nitrite (0.5 M). After incubation for 15 min at 37 C, the reaction was stopped by the addition of 90 ml of cold, sterile 0.1 M phosphate buffer at *p*H 7.5. The cells were centrifuged, the supernatant was decanted, and the pellet was resuspended in 200 ml of sterile nutrient broth and incubated for 2 hr at 37 C. This culture was also centrifuged, the supernatant was decanted, and the pellet was resuspended in 10 ml of sterile saline. Samples of 0.2 ml of the resuspended cells were plated on LAA, and the plates were incubated at 37 C and scored after 48 hr. Single colonies were isolated and streaked out on minimal and LAA media, after suspending in 1.0 ml of saline. The anthranilic acid requirers were kept for further study, and single-colony isolates were obtained after streaking for three successive times on LAA.

Reversion studies. Strains recovered following mutagenesis of *trpA28* with nitrous acid were tested for the ability to revert to prototrophy, both spontaneously and following induction with chemical mutagens by the spot-test method (1). The mutagens employed were *N*-methyl-*N*-nitroso-*N'*-nitroguanidine (NG, K & K Chemicals, Plainview, N.Y.), diethylsulfate (DES, Eastman Organic Chemicals, Rochester, N.Y.), and ICR-191A (acridine half-mustard, generously donated by H. J. Creech of the Institute of Cancer Research, Philadelphia, Pa.).

Transduction experiments. A nonlysogenizing mutant of the phage P22,L7 (27) was employed in all genetic crosses. Lysates were prepared and stored, and transduction experiments were carried out according to the procedures described by Blume and Balbinder (6) with minor modifications. The full-plate test (6) was used in all cases. Crosses were routinely repeated two or three times except for those which tried to define the termini of a deletion. These were repeated from a minimum of 5 to a maximum of 24 times. An average of three plates was used per cross. Reciprocal crosses were performed in a number of cases. As controls, platings of uninfected bacteria, phage lysates, and bacteria infected with phage grown on the same strain were performed routinely.

Extract preparation and enzyme assays. Cultures were grown in 2,800-ml Fernbach flasks in 1-liter volumes. Repressed cultures were grown to late log phase in minimal medium supplemented with 50 μg of L-tryptophan per ml. Derepressed cultures were grown to stationary phase in the same medium supplemented with only 2 μg of L-tryptophan/ml. All incubations were carried out on a rotary shaker at 37 C. The procedures for harvesting the cells and obtaining crude extracts are those described by Balbinder et al. (2) with the following modifications: (i) cells were washed with 0.05 M potassium phosphate buffer (*p*H 7.4) containing 1 mM 2-mercaptoethanol, instead of tris(hydroxymethyl)aminomethane (Tris)-hydrochloride; (ii) cells used to assay the various activities of the AS-PRT complex were resuspended in the same buffer used for washing, whereas those used to assay for tryptophan synthetase, component β (TS-β) were suspended in 0.1 M potassium phosphate buffer (*p*H 7.8) containing 1 mM 2-mercaptoethanol.

The activities of the AS-PRT aggregate (i.e., AS in the glutamine-dependent reaction, and PRT) were assayed by measuring the formation and disappearance, respectively, of anthranilate according to the procedures of Henderson et al. (14, 15) with the following modifications. (i) The assays were carried out in a final volume of 3.0 ml. (iii) 2-Mercaptoethanol was substituted for dithiothreitol. The NH_3-dependent reaction of AS-CoI was assayed by the procedure of Zalkin and Kling (34). Complementation assays of AS, i.e., activation by PRT both in the NH_3- and glutamine-dependent reactions were carried out by the procedure of Yanofsky et al. (33). A 10- to 30-fold excess of PRT obtained from the chain-terminating mutant *trpA703* (15) was used in these assays. A Farrand spectrophotofluorometer set for an activation wavelength of 325 nm and an emission wavelength of 400 nm was employed in all these assays. One unit of enzyme activity represents the production or the disappearance of 1 nmole of anthranilate per min per ml.

The β_2 subunit of tryptophan synthetase (TS-β_2) was assayed in the conversion of indole to tryptophan as described by Smith and Yanofsky (28). One unit of TS-β_2 activity is defined by the utilization of 0.1 μmole of indole in 20 min. Protein concentration was determined by the method of Lowry et al. (22). Specific activity is defined as units per milligram of protein.

RESULTS

Stable anthranilic acid-utilizing revertants of trpA28. The mutant *trpA28* maps in the unusual region and has been tentatively

characterized as an amber mutation (2). It has no detectable AS or PRT activities and is therefore incapable of growth on either minimal or minimal medium supplemented with anthranilic acid (references cited above). Like the other mutations mapping in the unusual region, it produces revertants of two distinct phenotypes: prototrophs and anthranilic acid-utilizing auxotrophs (2). Of 110 revertants obtained following mutagenization with nitrous acid (*see above*), 83 were found to be prototrophs and 27 were anthranilic acid requirers. None of the latter could revert to prototrophy either spontaneously or after treatment with several mutagens (NG, DES, ICR-191). A detailed genetic analysis of 26 of these revertants showed that they contained multisite mutations, probably deletions of various sizes covering the site of the *trpA28* mutation and extending both to its right and to its left (Fig. 2). Many of these extended into *trpA*, and in one case (*trpAB688*) actually deleted all, or most, of this gene. Other deletions (i.e., *trpAB684*) had most, or all, of the unusual region missing.

In the process of mapping these *trpAB* deletions by crossing them against *trpA* point mutations and *supX* deletions, we observed that the recombination frequencies were much lower in crosses between two deletions than between a deletion and a point mutation, although the position of the point mutation was closer to the operator-proximal terminus of the *trpAB* deletion than the operator-distal end of the *supX* deletion. Some representative results are given in Table 1 (see also Fig. 2). Reciprocal tests gave the same results. Similar observations have been reported for the histidine (*his*) operon (23). The most probable explanation for these observations is that two large non-overlapping deletions in two homologous deoxyribonucleic acid molecules will distort synapsis and interfere with the recombination process. From these results it should be obvious that, in mapping the termini of genetic

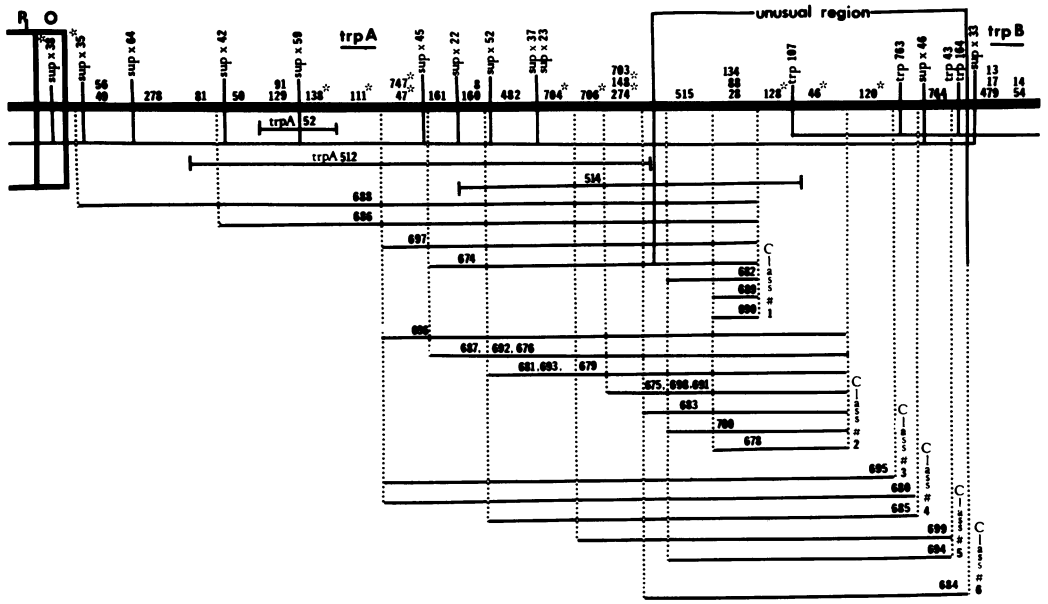

FIG. 2. *Multisite revertants of trpA28 and the revised fine-structure map of trpA. The bacterial chromosome is represented by the wide horizontal line. The numbers above the "chromosome" line indicate the approximate sites of trp point mutations. Where the sequence of the genetic markers is not known, the numbers are arranged in a column. The spacing between these sites is arbitrary and does not reflect physical distances. The narrow lines underneath the bacterial chromosome represent the approximate regions of the genome covered by the various deletions, each one designated by its corresponding number. Except for the deletions trpA52 and trpA512, which do not extend past the intercistronic trpA-trpB boundary, all the others shown in this map seem to span this boundary and are considered to be trpAB deletions. The termini of the supX deletions (24) and the multisite mutations trpBEDC43, trpBED164, trpBED763, and trpBEDC107 (4, 6) are indicated above the "chromosome" line by vertical bars. Other designations are: O = operator; P1 = promoter. The deletions trpA52, trpA512, and trpAB514 have been described (2, 7, 31). All other deletions were isolated as revertants of trpA28. The asterisks (*) indicate a change in the map positions of the mutations so designated as a result of this investigation.*

deletions, crosses to point mutations are more reliable than crosses to other deletions. The map as presented in Fig. 2 is the one most compatible with the data, and it updates the map of *trpA* and the unusual region. The *trpAB* multisite mutations have been classified into six different groups by the position of the right-hand terminus within the unusual region.

All twenty-six revertants were sensitive to the growth-inhibiting effects of the tryptophan analogues 5-methyltryptophan (MT) and 6-fluorotryptophan (FT) which can act as false co-repressors of the *trp* operon (3). This includes the multisite mutation *trp-AB688*, whose operator-proximal end seems to lie between *supX38* and *supX35* (Fig. 2). From the recombination data we cannot exclude the possibility that it penetrates slightly into the *trp* operator (*trpO*). The fact that the tryptophan biosynthetic enzymes of this mutant can be normally repressed by tryptophan (Table 2) would suggest, however, that this is not the case, or that the penetration is so slight as to produce no detectable damage to the integrity of the operator. Strains containing deletions extending into the operator (*trpO*) and lacking the entire *trpA* gene have been obtained by selecting anthranilic acid-utilizing revertants of unusual mutations in the presence of MT or FT (9). The synthesis of the tryptophan biosynthetic enzymes of these mutants is no longer repressed by tryptophan (9).

TABLE 1. *Recombination frequencies (per 10^{-9} bacteria) in crosses between non-overlapping deletions as compared to crosses between deletions and point mutations*[a]

Cross (recipient × donor)	Recombination frequencies
trpAB675 × *ΔsupX-37*	0.28
trpAB675 × *ΔsupX-23*	1.5
trpAB675 × *trpA706*	11.7
trpAB685 × *ΔsupX22*	0.6
trpAB685 × *trpA8*	7.5
trpAB691 × *ΔsupX37*	0.75
trpAB691 × *Δsup23*	0.3
trpAB691 × *trpA706*	10.8
trpAB695 × *ΔsupX-59*	1.8
trpAB695 × *trpA138*	4.2
trpAB697 × *ΔsupX59*	0.5
trpAB697 × *trpA138*	3.2
trpAB698 × *ΔsupX23*	0.58
trpAB698 × *trpA706*	10.9
trpAB699 × *ΔsupX37*	<0.05
trpAB699 × *ΔsupX23*	<0.05
trpAB699 × *trpA704*	3.2

[a] The deletion strains obtained as revertants of *trpA28* were used as recipients in transduction crosses with phage grown on a selected group of *supX* deletions and *trpA* point mutants. The actual location of these mutations within *trpA* is shown in Fig. 2. The procedure for the full plate test including controls is given in Materials and Methods. The number of infected bacteria per plate was always approximately 5×10^8.

Regulation of the trp operon in mutants carrying trpAB multisite mutations. There have been recent suggestions (13, 20, 21) that in operons controlling amino acid biosynthetic pathways, the feedback-sensitive enzyme (generally the product of the first structural gene) plays a role in the repression of the operon. This idea can be readily tested for the *trp* operon by measuring the levels of one of the tryptophan biosynthetic enzymes under conditions of repression and derepression in strains carrying deletions of all, or part, of the *trpA* gene and not extending into the operator. Several of our anthranilic acid-utilizing revertants have these characteristics, and were therefore tested in this manner. The results of these experiments are given in Table 2, and show conclusively that neither the first structural gene of the *trp* operon (*trpA*) nor its product (AS-CoI) plays a major role in the control by repression of the *trp* operon. The same conclusion has been reached by Hiraga and Yanofsky (16) from studies of deletions entering the first structural gene of the *trp* operon of *E. coli*. These results agree with, and further extend, the observations of Cordaro and Balbinder (10) who showed that the operator and the first structural gene of the *trp* operon are two separable genetic entities.

Effect of point mutations and deletions in the unusual region upon the activities of the

TABLE 2. *Repression and derepression of tryptophan synthetase β_2 in deletion-carrying mutants derived from trpA28*

Strain	Genotype	TS-β_2 specific activity[a]	
		Rep	Derep
	trpA8	0.56	47
SO-645	*trpAB688*	0.29	30
SO-637	*trpAB680*	0.32	38
SO-652	*trpAB695*	0.36	24

[a] Abbreviations: TS-β_2, tryptophan synthetase, component β_2; Rep, repressing growth conditions (50 μg of tryptophan per ml); Derep, derepressing growth conditions (2 μg of tryptophan per ml).

AS-PRT enzyme aggregate. Deletion of the amber mutation *trpA28* seems to be sufficient to restore PRT activity in the revertant strains (i.e., Fig. 2: *trpAB689*, *trpAB690*), even if the entire unusual region is deleted (Fig. 2, *trpAB684*) indicating that this region is indeed dispensable for PRT activity. This is consistent with the idea that the unusual region codes for the glutamine amidotransferase portion of PRT, which is needed for activating AS in the glutamine-dependent anthranilate synthetase reaction but is dispensable for the PRT reaction (12). If this were the case, we may expect that the enzyme responsible for PRT activity in the deletion-carrying revertants will be different from wild-type uncomplexed PRT. This is indeed what we find. Although the revertants must have PRT activity to utilize anthranilic acid for growth, extracts of some of these revertants showed no detectable PRT activity under standard assay conditions (Table 3). One anthranilate-utilizing revertant derived from the unusual mutation *trpB46* carries the deletion *trpAB514* which covers a large portion of the unusual region (Fig. 2, reference 2). This strain makes a species of PRT which shows activity only under modified assay conditions (Table 3). In preliminary experiments with the revertant strain *SO*-646 (*trpAB689*, Table 3), it has been possible to detect PRT activity under modified assay conditions when the cells were suspended in 0.1 M phosphate buffer (*p*H 7.8) in 40% glycerol and 1mM β-mercaptoethanol. The PRT of this strain has been examined for its heat stability, *p*H optimum, and the K_m values for both substrates, anthranilate and 5-phosphorylribose-1-pyrophosphate (PRPP). In all these parameters the revertant enzyme differed sharply from free wild-type PRT obtained from *trpA703* (*unpublished data*). A detailed characterization of these altered enzymes is in progress.

None of our revertants showed glutamine-dependent AS activity, an observation consistent with the conclusion that the unusual region (or a major portion of it) is the segment of the *trpB* gene coding for the glutamine amidotransferase activity of PRT. Surprisingly, however, none of them showed any detectable NH_3-dependent AS activity (Table 4). This reaction can be carried out by dissociated AS and requires no complementation by PRT. This was true for deletions extending past the mutation *trpA515* into *trpA* as well as for small deletions like *trpAB689* and *trpAB690*, which do not cover the site of *trpA515* (Fig. 2). It could be argued that all these deletions, including the short ones, span the intercistronic *trpA-trpB* boundary and therefore inactivate both glutamine amidotransferase and AS. Certain properties of *trpA515*, however, suggest that the intercistronic boundary may be located near the site of this mutation. We will discuss this problem below.

Table 4 shows the results of NH_3-dependent AS assays for all available unusual mutants, the deletions *trpBED763* and *trpBEDC107*, which penetrate the unusual region but do not extend into *trpA*, and some of the *trpA28* revertants bearing small deletions in this region. In a selected sample of these strains, AS activity was assayed in all the reactions carried out by this enzyme component. The results are given in Table 5. The data presented in these tables show that: (i) neither *trpA515*, *trpA28*, nor any deletions covering the *trpA28* site has any detectable AS activity; (ii) the remaining unusual mutants and the deletion *trpBEDC107* have detectable AS activities, but the levels are lower than those shown by either wild type or *trpBEDC43*, which carries a deletion not extending significantly into the unusual region and is our standard source of dissociated AS. Interestingly, the deletion *trpBED763*, extending a short distance into the unusual region, shows a normal level of AS activity (Table 4). On the basis of these observations, we can tentatively conclude that there are two kinds of unusual mutants: one group represented by *trpA515* and *trpA28*, both of which map in the operator proximal portion of the unusual region, and a second group represented by the remaining mutations. The last two columns on Table 5 indicate that the ratios of the various activities of AS are the same for the unusual mutants as for the wild-type complex, thus suggesting that the decreased levels of activity we observe in the mutants do not result from alterations of AS structure but rather from decreased amounts of this enzyme component. This will be considered further below.

DISCUSSION

Our results show that PRT activity can be restored in a mutant carrying an amber mutation in the unusual region by deleting the site of this mutation and part, or all, of this region. The species of PRT recovered in these cases are different in every respect from uncomplexed, wild-type PRT. These observations tell us that (i) the unusual region, or most of it, corresponds to the operator-proximal end of the *trpB* gene and (ii) this portion of the gene carries no information which is essential for PRT activity and thus its most likely role is to

code for that portion of the PRT polypeptide responsible for glutamine amidotransferase activity. This interpretation is consistent with the results mentioned earlier of Grieshaber and Bauerle (12). To follow the terminology proposed by Grieshaber and Bauerle (12), we shall refer henceforth to the unusual region as *trpB*-region 1, and the portion containing the PRT activity as *trpB*-region 2.

Two of our observations do not entirely fit this picture and indicate that some questions about the status and role of the genetically defined unusual region still remain. The first of these observations is that there are two distinct types of unusual mutants: one type represented by the exceptional mutants *trpA28* and *trpA515*, and the second by all the rest.

TABLE 3. *PRT activities of some deletion strains derived from trpA28*

Strains	Genotype	PRT specific activity	
		Standard assay[a]	Modified assay[b]
	trpE95	22.0	31.7
	trpA703	27.0	35.0
SO-646	*trpAB689*	ND[c]	ND
SO-657	*trpAB700*	ND	ND
SO-635	*trpAB678*	ND	ND
SO-639	*trpAB682*	ND	ND
SO-641	*trpAB684*	ND	ND
SO-645	*trpAB688*	ND	ND
SO-115	*trpAB514*[d]	ND	0.6

[a] pH = 7.4, PRPP = 20 μm (final concentration).
[b] pH = 8.2, PRPP = 1 mM.
[c] Not detectable, less than 7×10^{-3}.
[d] S. Marcus, *unpublished data*.

One major difference between these two groups is clear: *trpA28* and *trpA515* have no detectable AS activity, whereas all the other mutants do. The second observation is that AS activity is distinctly lower than expected in all the unusual mutants that have it.

The two exceptional mutations *trpA28* and

TABLE 4. *NH_3-dependent AS activity in "unusual" region mutants*

Strain	Genotype	Specific activity
	trp E2[a]	100.5
	ΔtrpBEDC43	16.2
	ΔtrpBED763	14.5
	ΔtrpBEDC107	1.0
	trpB744	5.1
	trpB120	4.2
	trpB46	5.8
	trpA28	ND[b]
	trpB128	4.5
	trpB88	6.2
	trpB134	3.75
	trpA515	ND
SO-657	*trpAB700*	ND
SO-651	*trpAB694*	ND
SO-647	*trpAB690*	ND
SO-646	*trpAB689*	ND
SO-641	*trpAB684*	ND
SO-639	*trpAB682*	ND
SO-635	*trpAB678*	ND
SO-637	*trpAB680*	ND
SO-631	*trpAB674*	ND

[a] *trpE2* carries the wild-type AS-PRT complex. The ratio of NH_3-dependent to glutamine-dependent AS activities for this complex is about 2 (see Table 5). All other strains in the table had no detectable glutamine-dependent AS activity.
[b] Not detectable, less than 8×10^{-4}.

TABLE 5. *Specific activities in the various reactions catalyzed by AS-CoI in some "unusual" mutants and deletions derived from trpA28*

Strain genotype	AS-NH_3 (uncomp)[a]	AS-NH_3[b] (comp)	AS-glutamine[b] (comp)	NH_3 (comp)/ NH_3 (uncomp)	NH_3 (comp)/ glutamine (comp)
trpE2 (w.t.)		100.5	51.8		1.95
ΔtrpBEDC43	13	132	76	10.2	1.7
ΔtrpBEDC107	2.8	36	22.8	12.9	1.6
trpB46	5.5	67.4	40	12.3	1.7
trpB128	6.7	73.5	39.7	11	1.9
trpB88	4.8	50	30.7	10.4	1.7
trpB134	7.4	77.8	48	10.5	1.6
trpA28	ND	ND	ND		
trpA515	ND	ND	ND		
trpAB678	ND	ND	ND		
trpAB689	ND	ND	ND		
trpAB690	ND	ND	ND		

[a] Abbreviations: uncomp, uncomplexed; comp, complex; ND, not detectable.
[b] These activities were assayed in the presence of saturating amounts of free PRT (AS-CoII) obtained from mutant *trpA703*.

trpA515 map at the operator-proximal end of the unusual region, and the possibility that they define a genetic region different in its function from *trpB*-region 1 cannot be entirely eliminated at this time. The crucial question, and one which this investigation is trying to answer, is: where is the *trpA-trpB* intercistronic boundary located? The fact that small deletions like *trpAB689* and *trpAB690* show no AS activity, in addition to an altered species of PRT, may indicate that they extend past this boundary into *trpA*. On the other hand, there are some indications that the intercistronic *trpA-trpB* boundary may be close to the site of the *trpA515* mutation, which is not covered by the deletions *trpAB689* and *trpAB690* (Fig. 2). Recent results (*unpublished data*) suggest that *trpA515* may be an insertion in the region of the *trpA-trpB* boundary which has two effects. It erases the intercistronic boundary, and, at the same time, introduces a new "promoter" into this region. Thus, under repressing conditions, transcription (and translation) would begin at, or near, the *trpA515* mutation and an active form of PRT would be produced. Under derepressing conditions, transcription and translation would start normally near the operator and, in the absence of a translation, stop between *trpA* and *trpB* and a fused CoI-CoII peptide would result, thus accounting for the lack of AS and the residual PRT activity shown by *trpA515* under these growth conditions (8). This hypothesis is being tested at present. We also hope that the study of the PRT species produced by deletions like *trpAB689* and others shown in Fig. 2 may help us in determining the exact location of the *trpA-trpB* boundary. This information should help us to understand the peculiar properties of *trpA515* and *trpA28*. For the time being we are tentatively considering that *trpA28* and *trpA515* correspond to the operator-distal portion of *trpA*, and this is reflected in our nomenclature.

The observation that deletions and mutations in what is now *trpB*-region 1 have decreased levels of AS activity is in agreement with an earlier report (2). By definition, this could be considered a case of antipolarity, that is, the reduction of the relative level of a polypeptide specified by the gene of an operon immediately preceding a gene with a chain-terminating mutation (32). We had previously reported (2) that several of the unusual mutations were polar although they were leaky (4), i.e., they made some active PRT as well as AS. Recently, Yanofsky et al. (33) have characterized mutants of *E. coli* which are equivalent to our unusual mutants as possessing chain-terminating mutations. These mutants are leaky, and also polar. Since the distal portion of *trpB* is sufficient to code for an active PRT moiety, leakiness of the unusual mutants can be due to reinitiation of polypeptide synthesis near the termination codon (25, 33). Antipolarity was reported for the last gene of the *trp* operon in both *E. coli* (32) and *S. typhimurium* (2). However, Yanofsky et al. (33) failed to detect any antipolar effects in PRT mutants of *E. coli* which were the counterparts of our unusual mutants. Their observations in this respect seem to be at variance with ours for reasons which are not obvious at the present time. One possibility that should be considered is that degradation of early *trp* messenger ribonucleic acid or early *trp* mRNA fragments may be faster in *S. typhimurium* than in *E. coli*.

The selection of anthranilic acid-utilizing revertants of the unusual mutation *trpA28* after nitrous acid treatment has proved to be an extremely powerful technique for selecting multisite mutations ending at different points within *trpA* and *trpB*-region 1. This approach should provide excellent material for studies of the AS-PRT complex in *S. typhimurium*. Similar deletion-carrying strains have been obtained by Tanemura and Bauerle (*personal communication*). All anthranilic acid-utilizing revertants of *trpA28* we recovered were multisite mutations. This may indicate that missense mutations at the *trpA28* site which can restore PRT activity without simultaneously restoring AS activity cannot be obtained, a possibility which is consistent with the notion that *trpA28* may be located outside the *trpB*-region 1. Prototrophic revertants of *trpA28* (restoring both activities) were recovered, but were not analyzed in this study.

There have been several reports of preferential sites for deletion end points ("hot-spots") in bacteria and bacteriophage following nitrous acid mutagenesis (5, 26, 29). We have found two presumptive hot spots in *trpB*-region 1: 50% of our deletions end between *trpB46* and *trpB120* in this region (class 2, Fig. 2), and another 25% between the group of mutations *trpA28*, *trpB88*, and *trpB134* (which have not yet been resolved) and the site of mutation *trpB128* (class 1, Fig. 2). However, we have not excluded the possibility that the regions between these sites are large, and deletion termini occur at random within them. More information is needed before we can decide whether we have hot spots or not.

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