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# Polar and Antipolar Mutants in the Tryptophan Operon of *Salmonella typhimurium*

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Polar mutations in *trpA*, the first structural gene of the tryptophan operon of *Salmonella typhimurium*, have an uncoordinate effect on the expression of the distal genes, with *trpB*, the second gene, being more drastically affected than the last three. A number of these polar mutant strains grow very poorly on anthranilic acid-supplemented minimal medium. By selecting for more rapid growth in the presence of anthranilic acid, secondary mutant clones showing a correction of the polar effect were isolated. A few of these were analyzed and shown to contain deletions of various segments of the *trpA* gene. Ten randomly isolated deletion mutants missing various segments of the *trp* operon were analyzed for possible pleiotropic effects. Five of them showed a pleiotropic effect of some sort and five did not. Of those showing pleiotropic effects, one had lost the promotor-like elements necessary to initiate expression of the operon, three showed possible antipolar effects, and one showed both polar and antipolar effects simultaneously.

The tryptophan (*trp*) operon of *Salmonella typhimurium* consists of five structural genes whose protein products catalyze the conversion of chorismic acid, the branch point compound in the pathway of aromatic amino acid biosynthesis, to L-tryptophan (9). The order of the five structural genes and the enzymes and reactions involved are shown in Fig. 1. The biosynthetic reactions involved and the order of the genes have been shown to be identical in *Escherichia coli* (16, 22, 29).

In both organisms, the tryptophan cluster has been shown to constitute an operon, the expression of the five genes being under the control of tryptophan, the end product of the pathway. The formation of the tryptophan biosynthetic enzymes in *E. coli* has been found to be coordinate under a variety of conditions (16, 22). In *S. typhimurium*, Bauerle and Margolin (4, 6, 19) reported that, although the entire *trp* cluster functions as a single unit in its response to tryptophan, possessing a single operator region at the *trpA* end (Fig. 1), the expression of the genes in this operon is semicoordinate, the first two genes (*trpA* and *trpB*) constituting one unit of coordinate expression and the last three genes (*trpE*, *trpD*, and *trpC*) a second coordinate unit. From studies of deletion mutations entering the operon from the operator end, and polar mutations in *trpA*, these workers concluded that this operon contains two initiators of gene expression or "promotor-like elements": one (P1) located at the beginning of *trpA* and a second (P2) between *trpB* and *trpE* (*see* Fig. 2). They ascribed the apparent semicoordinate derepression of this operon to the existence of these two elements.

The occurrence of structural mutations with pleiotropic effects has been reported in several systems, including the *trp* operon. Mutations with polar effects, i.e., affecting the expression of genes located on the operator distal side of the mutation, have been reported for the tryptophan operons of both *S. typhimurium* and *E. coli* (4, 5, 16, 30). In addition, mutations with an antipolar effect, i.e., affecting the expression of genes located on the operator proximal side of the mutation, have been described in the tryptophan operon of *E. coli* (16, 31). The present report and a companion paper (10) are concerned with the characteristics and behavior of various pleiotropic mutations in the *trp* operon of *S. typhimurium*. A number of these were uncovered because they deviated from the criteria established to classify mutations for each structural gene of

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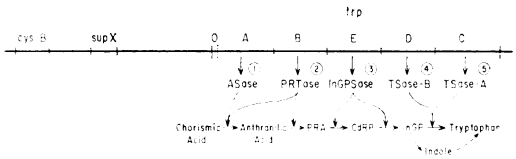


FIG. 1. *Organization of the tryptophan operon of Salmonella typhimurium. The circled numbers refer to the sequence in which the products of the five tryptophan (trp) genes participate in the biosynthesis of L-tryptophan; the capital letters denote the various trp genes. The trpA gene codes for anthranilate synthetase (ASase), and trpB codes for phosphoribosyl transferase (PRTase). PRTase and ASase are required in a complex for the conversion of chorismic acid to anthranilic acid (AA) but PRTase, complexed or free, converts AA to N-5'-phosphoribosyl anthranilate (PRA; 5, 17). Indoleglycerol phosphate synthetase (InGPSase), the trpE gene product, catalyzes two sequential reactions: PRA to 1-(O-carboxyphenylamino)-1-deoxyribulose-5-phosphate (CdRP), and this intermediate to indole-3-glycerol phosphate (InGP). Tryptophan synthetase (TSase) components A and B, the trpD and trpC gene products, respectively, are required for the conversion of InGP to tryptophan. The O indicates the general location of the trp operator region.*

the operon. These criteria correlated such properties of each mutant strain as (i) the ability to utilize intermediates of the pathway (anthranilic acid and indole) as growth factors instead of tryptophan, (ii) the accumulation of intermediates in culture filtrates, (iii) missing enzymatic activities, and (iv) map position (9). As we will show, some of these strains contain polar mutations, mainly in *trpA*, the first structural gene of the operon. Others have deletions showing polar or antipolar effects, and in one instance probably both of these simultaneously.

Of particular interest are some of the strains carrying *trpA* polar mutations. These did not grow or grew slowly when anthranilic acid was substituted for tryptophan as the growth factor, but grew normally in the presence of indole and accumulated no pathway intermediates (9). This property has allowed us to select from these strains secondary mutant clones which grow normally on anthranilic acid as a result of the elimination or diminution of the polar effects. We have analyzed a few of these secondary mutant strains in which polarity has been corrected by deletions of various segments of the *trpA* gene.

In this paper we will describe some of the characteristics of strains carrying polar and antipolar mutations for this operon and also those of three secondary "polarity correction" strains. A second paper (10) deals with the kinetics of derepression of several of these strains.

## MATERIALS AND METHODS

*Nomenclature.* The system of nomenclature used for the bacterial mutant strains is the one proposed by Demerec et al. (11) and will supersede that used in earlier publications.

*Bacterial strains.* The *trp* mutants employed in this work (Table 1) are from the collection of M. Demerec. They have been previously described (9). In addition to these, strains *supX38*, *supX42*, and *supX45*, obtained from P. Margolin, were used in deletion mapping. Strains SB-391 (formerly 644 FLA), SB-392, and SB-393, used for the identification of nonsense mutations, were generously provided by David Berkowitz (*manuscript in preparation*; *see* 28). Strain SB-391 contains a histidine deletion (GDCBHAF 644) and the episome obtained from *E. coli* strain F'*lac*$^-$ amber X82; SB-392 is the same as SB-391 but with the episome of U-281 (23). SB-393 has an F'*lac* episome carrying an ochre mutation in the *lacZ* gene (Newton's YA-596) and on the chromosome a *his* ochre mutation (*hisC117*), an ochre suppressor, and mutations causing requirements for arginine and isoleucine. All bacterial stocks as well as working strains were prepared and kept according to the techniques of Hartman, Loper, and Serman (15).

*Media.* Nutrient Broth (Difco) served as a routine complex medium. The defined minimal medium routinely used (MG) was that of Vogel and Bonner (27) supplemented with 0.005% acid-hydrolyzed casein (25) and 0.2% glucose. For genetic experiments in which a solid medium was needed, the above was solidified by the addition of 1.5% (w/v) agar (Difco). Enriched minimal medium (EMG) consisted of the MG medium plus 1.25% (v/v) liquid Difco Nutrient Broth. Additional growth factors were added as supplements as indicated. Some derepression experiments were carried out in MG medium devoid of glucose (UMM medium). This contained 0.2% citrate as the sole source of carbon. A modified minimal medium (ML), made up by eliminating the citrate in the Vogel medium, altering the ratio of monobasic to dibasic phosphate to bring the *p*H to 7, and adding lactose (1%) instead of glucose, was used in suppression tests (Berkowitz, *private communication*).

*Transduction experiments.* Mapping was performed by phage P22-mediated transduction. The procedure used in deletion mapping by spot tests has been previously described (9). For full plate tests, recipient bacteria were grown to saturation in aerated broth culture and infected with transducing phage at a multiplicity of 10. The transduction mixture was plated in 0.5-ml samples (approximately $10^9$ infected bacteria) per plate on solid MG medium. All crosses were carried out in duplicate and repeated at least once. Reciprocal crosses were performed in a number of cases. Control platings of uninfected bacteria as well as of phage lysates (for sterility) were performed routinely.

*Reversion and phenotypic curing experiments.* The ability of different mutagens to induce revertants was determined by the spot test method (3). The mutagens employed and the concentrations are given in the legend to Table 1. Phenotypic curing tests with kanamycin, neomycin, and streptomycin were performed

by spot test technique of Whitfield, Martin, and Ames (28). (For further details, *see* legend to Table 1.) The sources of the mutagens were as follows: diethyl sulfate (DES) was purchased from Eastman Organic Chemicals, Rochester, N.Y.; *N*-methyl-*N*-nitroso-*N'*-nitroguanidine (NG) from K and K Chemicals, Plainview, N.Y.; acridine-half mustard (ICR-191A) was generously donated by H. J. Creech of the Institute for Cancer Research, Philadelphia, Pa. The sources of the antibiotics were as follows: kanamycin sulfate was purchased from Bristol Laboratories, Syracuse, N.Y.; neomycin sulfate, The Upjohn Co., Kalamazoo, Mich.; streptomycin sulfate, from E. R. Squibb & Sons, New Brunswick, N.J.

*Identification of nonsense mutations.* Nonsense mutations were tentatively identified as amber [uridine (U), adenosine (A), guanosine (G)] or ochre (UAA) by the procedure of Berkowitz et al. (*private communication*) as described by Whitfield, Martin, and Ames (28). The test is based on the inability of *S. typhimurium* to ferment lactose. This enables one to identify amber and ochre suppressors among externally suppressed revertants. Identification of these suppressors was done by mating the revertants with strains SB-391, 392, and 393 which contain an F'*lac* episome with either an amber or ochre mutation of the β-galactosidase gene. The *trp* mutants to be tested were induced to revert to growth on minimal medium (MG) by NG or ICR-191 (as the case warranted), and the prototrophic revertants were replicated onto a lawn of a tester strain (e.g., SB-391) on solid ML medium. The SB strain by itself is unable to grow because of its *lac* mutation and amino acid requirements, and the revertants are unable to grow on lactose because *Salmonella* is lactose-negative. However, if a revertant receives the F'*lac*$^-$ episome and it has a suppressor for the particular *lac*$^-$ amber or ochre mutation, it will grow on ML. The tests were found to be highly reproducible. Each of the *trp* mutants to be tested was grown overnight in nutrient broth, and 0.1 ml of each culture was plated by spreading on solid MG medium containing tryptophan (0.5 μg/ml). A crystal of NG or a drop of a solution of ICR-191 (*see* legend to Table 1) was placed in the center of each plate, and the plates were incubated at 37 C for 2 to 3 days. The colonies obtained were replica-plated on the lawns of tester SB strains as described above, incubated for 6 days, and scored after 2, 4, and 6 days. Those showing good growth on SB-391 or SB-392 were classified as amber. Revertants which gave rise to restricted areas of growth after 4 to 6 days on these two tester strains were tentatively classified as ochres (Berkowitz, *private communication*). Only one mutant, *trpA284*, showed a good response when revertants were replicated on a lawn of SB-393, together with a weak response on SB-391. This mutant has been classified as ochre.

*Extract preparation and enzyme assays.* Cells were harvested by centrifugation, washed twice in cold tris-(hydroxymethyl)aminomethane-chloride buffer (*p*H 7.8), resuspended in a small volume of the same buffer, and disrupted with a Bronwill Biosonik probe sonic oscillator. The cell debris was removed by centrifugation at 40,000 × *g* in a Sorvall RC-2 refrigerated centrifuge for 30 min. Anthranilate synthetase (ASase) and phosphoribosyl anthranilate transferase (PRTase) were assayed as described by Ito and Crawford (16), with the difference that the increase or decrease in the fluorescence of anthranilic acid was measured in a Turner fluorometer. In some mutants which contain uncomplexed ASase this enzyme was assayed in the presence of saturating amounts of free PRTase obtained from crude extracts of *trpA148* or *trpA8*, the latter after freezing and thawing to dissociate the ASase-PRTase complex (5). After incubation of the reaction mixture at 37 C for 20 min, the reaction was stopped by addition of 0.1 ml of 1 N HCl, and anthranilic acid was extracted into 4 ml of ethyl acetate. Indole glycerol phosphate synthetase (InGPase) was measured according to the method of Smith and Yanofsky (25) as modified by Ito and Crawford (16). Tryptophan synthetase (TSase) A and B components were assayed as described by Smith and Yanofsky (25). Protein was estimated by the method of Lowry et al. (18). One unit of specific activity is defined as the production or conversion of 0.1 μmole of product or substrate in 20 min at 37 C per mg of protein.

*Determination of repressed levels of the tryptophan enzymes.* Cells were grown at 37 C in MG medium supplemented with L-tryptophan (50 μg/ml). Growth flasks were inoculated from overnight broth cultures, and the cells were harvested after approximately 4 to 5 hr on a rotary shaker (final concentration, $10^9$ cells/ml). Growth was estimated by turbidimetric measurements in a Klett-Summerson colorimeter. After harvesting the cells, the presence of unused tryptophan in the culture medium was determined by the tryptophanase assay of DeMoss (12).

*Determination of derepressed levels.* To measure maximal derepressed levels of the tryptophan enzymes, growth was carried out in MG medium supplemented with only 5 μg (per ml) of tryptophan at 37 C on a rotary shaker for 18 to 20 hr. When a more accurate comparison of derepressed enzyme levels of various strains was desired, a repressed culture was washed twice in 0.9% saline, suspended in in prewarmed (37 C) UMM medium (with citrate as the only source of carbon), and allowed to derepress at 37 C on a rotary shaker for specified time periods.

## RESULTS

*Description of polar mutants.* The mutations studied and the tests used to classify them as nonsense (amber or ochre), frameshift, or missense are given in Table 1. Mutations were classified as presumed frameshifts, nonsense, or missense by the criteria of Whitfield, Martin, and Ames (28). These correlate the response of each mutant to chemical mutagens, phenotypic curing by several antibiotics, and suppression tests. The mutagens DES and NG act as alkylating agents and are believed to cause base pair substitutions by transition and transversion. ICR-191 was found to be an effective mutagen of *S. typhimurium* and is believed to induce, primarily,

TABLE 1. *Criteria for the classification of polar mutants*[a]

| Mutant | Origin | Mutagenesis (reversion to prototrophy)[b] | | | | Phenotypic curing[b] | | | Type of mutant |
|---|---|---|---|---|---|---|---|---|---|
| | | Sp | DES | NG | ICR-191 | N | K | S | |
| *trpA56* | Sp | + | − | − | + | − | − | − | FS |
| *trpA49* | SP | + | + | + | −(?)[c] | − | + | + | AM |
| *trpA81* | NA | + | − | − | + | − | − | − | FS |
| *trpA82* | NA | + | − | − | + | − | − | − | FS |
| *trpA111* | X ray | + | + | + | + | + | + | − | OC (?) |
| *trpA278* | UV | + | + | + | − | + | + | + | AM |
| *trpA28*[d] | Sp (*mut*⁻)[d] | + | + | + | − | − | + | − | AM |
| *trpA120* | Fast neutrons | + | ? | ? | + | + | + | + | NS (?) |
| *trpA46* | Sp | + | + | + | + | + | ? | ? | NS (?) |
| *trpA284* | UV | + | + | + | − | − | + | + | OC |
| *trpA(?)194* | NA | − | − | − | − | − | − | − | Deletion (?) |
| *trpE286* | UV | + | + | + | − | + | + | + | AM |
| *trpA8* | UV | + | + | + | − | − | + | + | MS |

[a] Abbreviations: Sp = spontaneous; NA = nitrous acid; UV = ultraviolet light; DES = 0.1 ml of an undiluted diethyl-sulfate solution; NG = 0.1 ml of a 300 μg/ml solution of *N*-methyl-*N*-nitroso-*N'*-nitroguanidine; ICR-191 = 0.1 ml of a 1.0 mg/ml solution; N = 0.1 ml of a 20 mg/ml solution of neomycin sulfate; K = 0.1 ml of a 3.3 mg/ml solution of kanamycin sulfate; S = 0.1 ml of a 15 mg/ml solution of streptomycin sulfate; FS = frameshift; AM = amber; OC = ochre; MS = missense; NS = nonsense.

[b] Lawns of each mutant were prepared by spreading 0.1 ml of an overnight broth culture on the surface of a minimal glucose (MG) plate for "leaky" mutants or an enriched minimal (EMG) plate for "nonleaky" mutants. For reversion studies, 0.1 ml of a solution of the mutagen was applied on a sterile filter paper disc in the center of each plate. In control plates this was replaced with sterile water. ICR-191 was applied in subdued light and plates were incubated in the dark. For phenotypic curing, four sterile filter paper discs were placed at four corners of the plate: one, the control, was spotted with 0.1 ml of sterile water; the remaining three, with 0.1 ml of antibiotic solutions. Plates for mutagenesis and phenotypic curing were scored after 48 hr of incubation at 37 C, and were reincubated for an additional 2 days. Generally, no further changes were observed after additional incubation.

[c] Question marks indicate that tests could not be scored unambiguously, or that classification is uncertain.

[d] Mutant *trpA28* arose spontaneously in strain LT7 carrying a mutator gene (*mut*⁻). Since it has a high spontaneous reversion frequency in this genetic background, it was transduced into an LT7 *mut*⁺ strain for study.

frameshift mutations (2, 20). The antibiotics streptomycin, kanamycin, and neomycin lead to phenotypic curing by causing misreading of messenger ribonucleic acid (mRNA). Phenotypic curing is manifested in spot tests by the appearance of a solid ring of growth peripheral to the zone of inhibition caused by the antibiotic, and was found to be quite specific in tests with *hisC* mutants: all amber and ochre mutants showed phenotypic curing, while frameshift mutants gave negative results and only 33% of the missense mutants tested responded to at least one of the antibiotics (28). The suppression tests further distinguished the nonsense from the frameshift and missense classes and allowed for a preliminary identification of various nonsense mutations as ambers or ochres. Our results are in good agreement with those of Whitfield et al. (28). Mutant strains which were reverted by NG and DES, with only three exceptions which will be mentioned later, did not respond to ICR-191. They also showed phenotypic curing and gave positive results in suppression tests. These were classified as containing nonsense mutations. Strains classified as containing frameshift mutations were reverted only by ICR-191, were not susceptible to phenotypic curing, and gave negative results in suppression tests. Mutant strains reverted by DES and NG but not ICR-191, and which gave negative results in suppression tests, were considered to contain missense mutations.

By these criteria, three of the *trp* mutations listed in Table 1 (*A56*, *A81*, and *A82*) can be definitely classified as frameshifts. One, *A8*, is probably a missense. It resembles those *hisC* missense mutations which were susceptible to phenotypic curing (28). The mutation *trp-194* has been tentatively classified as a deletion. This mutation never reverted to prototrophy either spontaneously or after induction with various

mutagens, and the strain was not susceptible to phenotypic curing. Of the remaining eight mutations listed in Table 1, one, *E286*, is an amber mutation in *trpE* and the other seven are mutations in *trpA*. Of these, four have been tentatively identified as ambers, two as ochres, and two, *A46* and *A120*, remain unclassified. Berkowitz et al. (*private communication*), working with histidine (*his*) mutants, found that not all *hisC* ochre mutants tested responded to the suppression test. Mutations *A46* and *A120* could consequently be ochres, represent a third nonsense triplet (UGA; 24), or belong to the missense class. As we shall see later, they constitute a special case. Both of these mutations, as well as *A111*, showed some of the characteristics of nonsense mutations: they were subject to phenotypic curing and were induced to revert by DES and NG, but they also responded to ICR-191 in reversion tests. Similar cases have been reported among several *hisC* mutations (28). It is possible that ICR-191 is inducing suppressor mutations in these mutants. However, suppression tests of ICR-191-induced revertants against our tester strains were negative.

Table 2 gives some additional characteristics of the mutants analyzed in Table 1, including their growth rates on anthranilic acid-supplemented minimal (MG) medium, their accumulation products, and their ability to give rise to prototrophic revertants as well as to secondary mutants which grow well on anthranilic acid supplement. As one would expect, *trpA* mutants accumulate no intermediates for the tryptophan pathway, since the first enzymatic activity in the sequence is missing. Growth rates on anthranilic acid-supplemented MG medium varied from no detectable growth over a period of 12 hr, the duration of one experiment, to normal growth (45 to 55 min for one doubling of the bacterial population). Table 2 also shows that those mutants which have low growth rates on anthranilic acid supplement can give rise to two types of revertants under appropriate selection conditions: (i) complete prototrophs and (ii) auxotrophs which in the presence of anthranilic acid grow better than the original mutant strain. Some of these secondary mutants will be discussed later. One mutation, *trp-194*, can give rise only to the second type of revertant in very low frequency, and only after ICR-191 induction. For technical reasons (9), it has not been possible to map this mutation nor *trpA284*. From the available data, we tentatively consider that *trp-194* is an "out of phase" deletion with a polar effect which ICR-191 corrects by restoring the proper phase of reading.

The levels of the tryptophan biosynthetic enzymes shown by various nonpolar mutant strains and wild-type LT2 under repressed and derepressed conditions are shown in Table 3. With the exception of derepressed wild type, these levels are approximately equal to the repressed and derepressed standards within the limits of accuracy of the assays and, for the derepressed group, the limitations inherent in the derepression procedure which will be mentioned later. In the wild type, the tryptophan enzymes are only partially derepressed (reaching only about 4% of the levels of the fully derepressed auxotrophs), owing to repression by endogenously synthesized tryptophan. The repressed and derepressed levels of these same enzymes in the strains described in Tables 1 and 2 are given in Table 4. These levels are uniformly lower than those found in nonpolar mutants (Table 3). Polar mutants exhibit the loss

TABLE 2. *Some characteristics of trpA polar mutants*

| Mutant | Growth rate in AA[a] | Accumulation products[b] | Recoverable revertant types | |
|---|---|---|---|---|
| | | | Prototrophs | Normal AA utilizers[c] |
| *trpA56* | 180 | None | + | + |
| *trpA49* | 240 | None | + | + |
| *trpA81* | 66 | None | + | + |
| *trpA82* | 70 | None | + | + |
| *trpA111* | 50 | None | + | CNT |
| *trpA278* | 48 | None | + | CNT |
| *trpA28* | No growth | None | + | + |
| *trpA120*[d] | 132 | None | + | NT |
| *trpA46*[d] | Slow growth (leaky) | None | + | + |
| *trpA284* | 228 | None | + | + |
| *trpA194* | No growth | None | − | + |
| *trpA8* (nonpolar) | 48 | None | + | CNT |
| *trpE268* (nonpolar) | No growth | AA + CdR | + | − |
| *trpE286* (polar) | No growth | AA (CdR not determined) | + | − |

[a] Growth rate is the time interval in minutes needed for a doubling of the average number of cells in MG medium supplemented with 10 μg of anthranilic acid (AA) per ml. All mutants listed showed normal generation times when indole or tryptophan was used as supplement (45 to 55 min). The bacteria were cultured in growth flasks on rotary shakers at 37 C. Growth rate was determined turbidimetrically. Readings were taken in a Klett-Summerson colorimeter, equipped with a red filter, at regular intervals during the experiment. Klett units were correlated to cell numbers by a standard curve.

[b] The methods for the determination of intermediates accumulated in culture filtrates have been described (8). CdR = 1-(*O*-carboxyphenylamino)-1-deoxyribulose.

[c] The general procedure to induce revertants was as described in Table 1, but selection was performed on MG medium supplemented with anthranilic acid (2 μg/ml in some experiments, 10 μg/ml in others). CNT= could not be tested; NT= not tested.

[d] Leaky on minimal medium.

of the activity corresponding to the enzyme coded by the mutated gene with a concomitant reduction of the activities of the enzymes coded by the genes located on the operator distal side of the mutation. It is immediately apparent (Table 4) that those mutations classified as nonsense or frameshift (Table 1) are polar, whereas *A8*, which was classified as a missense mutation, is not polar (Table 3). This agrees with all available reports (13, 21, 30) which show that only nonsense or frameshift mutations have polar effects but missense mutations do not.

The intensity of a polar effect can vary from one mutation to another and has been shown to depend on the distance between the polar mutation and the operator end of the mutated gene. Gradients of polarity have been demonstrated for genes in various operons, the polar effects being more severe for mutations near the operator proximal end of a gene and decreasing in intensity for mutations located closer to its operator distal end (4, 5, 21, 23, 30). Structural genes adjacent to the operator have very steep polarity gradients (4, 14, 23, 30); the internal genes of an

TABLE 3. *Relative levels of the tryptophan biosynthetic enzymes in nonpolar trp mutants under conditions of repression and derepression*[a]

| Mutants | Repressed (% standard basal level) | | | | | Derepressed (% standard maximal level) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | ASase | PRTase | InGPSase | TSase B | TSase A | ASase | PRTase | InGPSase | TSase B | TSase A |
| *trpA8* | <25 | 175 | 93 | 98 | 89 | <0.1 | 112 | — | 74 | 107 |
| *trpE268* | 150 | — | <5 | 100 | 70 | 125 | 90 | <0.5 | 82 | 103 |
| *trpC3* | 100 | 100 | 100 | 100 | 95 | — | — | 100 | — | — |
| *trpD1* | 100 | 100 | 95 | — | — | — | — | — | — | — |
| *trpE201* | 200 | 100 | <5 | 98 | 75 | 80 | 100 | <0.5 | 98 | 87 |
| *trpB223* | — | <25 | — | 150 | 95 | — | <0.1 | 100 | 120 | 112 |
| LT2 | 100 | 100 | 100 | 100 | 100 | 3 | 4 | 3.3 | 4.4 | 6 |
| SSA | 0.02 | 0.02 | 0.16 | 0.33 | 0.36 | 4 | 3 | 9.0 | 27.6 | 28.6 |

[a] Repressed and derepressed extracts were prepared. Repressed enzyme levels are given as relative to those found in fully repressed LT2 (wild type) cells (taken as the standard and equal to 100%). Derepressed levels are given as relative to those found in fully derepressed nonpolar auxotrophs (taken as 100%). The standard maximal levels are the averages of those found in fully derepressed nonpolar auxotrophs. The standard specific activities (SSA) for both repressed and derepressed conditions are given. Dashes indicate that a particular assay was not performed. Abbreviations used for designating the different enzymes are the same as in Fig. 1.

TABLE 4. *Relative levels of the tryptophan biosynthetic enzymes in various polar trp mutants under conditions of repression and derepression*[a]

| Polar mutant | Repressed (% standard basal level) | | | | | Derepressed (% standard maximum level) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | ASase | PRTase | InGPSase | TSase B | TSase A | ASase[b] | | PRTase | InGPSase | TSase B | TSase A |
| | | | | | | −PRTase | +PRTase | | | | |
| *trpA56* | <25 | <25 | 57 | 61 | 48 | <0.1 | — | <0.1 | 13 | 15 | 13 |
| *trpA49* | <25 | <25 | 36 | 54 | 45 | <0.1 | — | <0.1 | 8 | 8 | 5 |
| *trpA81* | <25 | <25 | 30 | 60 | 54 | <0.1 | — | 8 | 14 | 11 | 25 |
| *trpA82* | <25 | <25 | 35 | 45 | 40 | <0.1 | — | 10 | 17 | 16 | 14 |
| *trpA111* | <25 | <25 | 21 | 40 | 60 | <0.1 | — | 8 | 15 | 26 | 35 |
| *trpA278* | <25 | <25 | 57 | 67 | 38 | <0.1 | — | <0.1 | 5 | 7 | 6 |
| *trpA28* | <25 | <25 | 30 | 60 | 42 | <0.1 | <0.1 | <0.1 | 8 | 19 | 12 |
| *trpA120* | <25 | <25 | 30 | 54 | 42 | <0.1 | 4.2 | <0.1 | 37 | 36 | 37 |
| *trpA46* | <25 | <25 | 50 | 60 | 37 | <0.1 | 4.2 | <0.1 | 38 | 23 | 18 |
| *trpA284* | — | — | — | — | — | <0.1 | — | 1.5 | 6 | 7 | 7 |
| *trpA194* | — | — | — | — | — | <0.1 | — | <0.1 | — | 4 | 4 |
| *trpE286* | 100 | — | <5 | 12 | 6 | 75 | — | 83 | <0.5 | 18 | 16 |

[a] Enzyme levels are expressed as relative to the standard (taken as 100%), as described in Table 3. Abbreviations as in table 3.

[b] ASase was assayed in the absence of excess PRTase (− PRTase) as well as in its presence (+PRTase).

operon show much shallower gradients (14, 30) or no polarity gradient at all (14).

While the results in Table 4 are adequate as a qualitative test to establish the difference between polar and nonpolar mutations, they are not sufficiently accurate to firmly establish quantitative differences in the intensities of the polar effects of the various polar mutations. In the first place, the assays for ASase and PRTase which we employed were not sufficiently sensitive to measure with accuracy very low levels of activity. Second, we observed, as did Yanofsky and Ito (30), that the synthesis of the tryptophan enzymes is not coordinate in derepressed cultures, and that the relative enzyme levels observed could vary with the time at which the cells were harvested. For these reasons we feel that no firm conclusions regarding the coordinate or semicoordinate expression of the *trp* genes, or the existence of a polarity gradient for *trpA*, can be based on these data. These questions are fully dealt with in a companion report (10). Keeping in mind the limitations just stated, certain conclusions can be drawn from Table 4.

(i) In agreement with the observations of Bauerle and Margolin (4, 19), it appears that polar *trpA* mutants show a basal level of expression for the genes *trpE*, *trpD*, and *trpC* which cannot be reduced by even the most severe polar mutations of *trpA*. Our data show that among the *trpA* mutants all measurable enzyme levels observed under repression (PRTase was too low to measure) were about 30 to 70% of the levels of fully repressed wild type; no differences could be discerned among individual mutants. In contrast, the repressed levels of the gene products affected by the polar effect of *E286* (last two genes) were significantly lower than those shown by any of the *trpA* polar mutants.

(ii) Differences in the intensities of the polarity effects of the various *trpA* polar mutations, not evident under repressed conditions, are evident under derepression. Furthermore, if one compares the derepressed levels of the tryptophan enzymes shown by these mutants with their position on a map of the *trpA* gene (Fig. 3), a rough gradient of polarity can be discerned. For example, the polar effect of *A49* upon the expression of *trpE*, *trpD*, and *trpC* is stronger than that of *A111*, which is in turn stronger than *A46*. This correlation, however, does not hold throughout (e.g., *A28*). More accurate measurements of polarity are presented in the accompanying paper (10).

(iii) The polar effects of the *trpA* polar mutations seem to be more stringent on the level of PRTase activity, the *trpB* gene product, than on the remaining enzymes: PRTase is consistently lower than the other gene products under conditions of derepression. Under conditions of repression, this could not be clearly demonstrated because of the insensitivity of the assay, as mentioned above. As Table 4 shows, the activity of PRTase in repressed cultures was too low to be detected by our assay procedure. However, a level of PRTase similar to those of the other enzymes (30 to 70% of fully repressed wild type) could have been detected. The fact that no activity could be found suggests that the levels of this enzyme were more drastically affected than the rest under repression as well as derepression. This effect on the level of expression of the adjacent operator distal gene seems to be specific for *trpA* polar mutants: *E286* affects the expression of both genes distal to it equally.

Two mutants, *A46* and *A120*, in addition to polar levels for the distal enzymes, showed low levels of ASase activity when assayed in the presence of saturating amounts of PRTase (Table 4). These mutants are leaky on minimal medium (5). A third mutant, *A28*, mapping in the same region (*see* Fig. 3) shows no detectable activity under the same assay conditions. *A46*, *A120*, and *A28* belong to the group of "unusual," mutants which Bauerle and Margolin (5) placed at the operator proximal end of the *trpB* gene.

A correlation between the low growth rates on anthranilic acid shown by some of our polar mutants and limiting amounts of one of the tryptophan enzymes could be expected. Since all strains grow normally on indole, TSase (both components A and B) does not appear to be the limiting enzyme. Somerville and Yanofsky (26) interpreted the slow growth on anthranilic acid supplement of certain polar mutants in the ASase gene of *E. coli* as resulting from the inhibition of InGPSase by anthranilic acid in strains having decreased levels of this enzyme. While we have indications that InGPSase in *S. typhimurium* may be subject to inhibition by anthranilic acid (*unpublished data*), a comparison of Tables 2 and 4 shows no correlation between InGPSase levels and growth rate on anthranilic acid supplement. The last possibility is PRTase, which decreases more drastically in the polar mutants than the last three enzymes. However, a correlation between growth rate on anthranilic acid and PRTase levels is not obvious from a comparison of Tables 2 and 4.

Our inability to detect a correlation between enzyme levels and slow growth on anthranilic acid supplement stems from the limitations of the derepression data which were mentioned earlier. In the accompanying paper (10) we show a correlation between growth on anthranilic acid and the rate of synthesis of PRTase.

*Pleiotropic effects of deletion mutations.* The maximal levels of the tryptophan biosynthetic enzymes under derepression in mutant strains

carrying deletions of various sizes and terminating at various points within the *trp* operon are given in Table 5. The extent of the different deletions is shown in Fig. 2.

Of our sample of 10 randomly isolated deletions, 5 showed a pleiotropic effect of some sort. Deletion *trpABE 130*, which deletes the first two genes from the operator end and extends into the third one, shuts off the expression of the remaining two intact genes, *trpD* and *trpC*. This extreme pleiotropic effect of the *ABE 130* deletion has been interpreted by Bauerle and Margolin (6, 19) as resulting from the removal of both "initiators" P1 and P2 (Fig. 2). Secondary mutants in which expression of the last two genes has been restored have been isolated by selection on indole-supplemented minimal medium (6, 9, 19). A short deletion in the last gene of the cluster, *trpC109*, shows an antipolar effect on the expression of the adjacent genes *trpD* and *trpE* located on the operator proximal side of the mutation. The intensity of the effect is strongest on the expression of the immediately adjacent gene (*trpD*) than on the next one (*trpE*). The strain carrying the deletion *trpBED 164*, which has lost the three middle genes of the operon (retaining only the first and the last), has decreased levels of both TSase A and ASase, even when the activity of the latter is assayed in the presence of excess PRTase. Low levels of ASase are also shown by deletion mutants *BEDC 43* and *107*. Both of these strains have lost the entire distal end of the operon and have retained only *trpA*, or most of it. In a previous report (9), *BEDC 43* and *107* were considered antipolar on the basis of no detectable ASase activity. It has since been shown that PRTase and ASase have to form a complex for ASase activity to appear (5, 17). When assays were performed in the presence of excess PRTase, ASase activity was detected in derepressed cells, but its level was only a fraction (about 7%) of the maximal derepressed level. Very little, if any, activity was detected in the absence of external PRTase. It is likely that the low levels of ASase activity found in the presence of the deletions *43*, *107*, and *164* were the result of antipolar effects of the deletions. However, the possibility that the efficiency of the "in vitro" complementation between ASase and PRTase was not very high has not been excluded. For this reason, any antipolar effect for these three deletions, while still possible, is not absolutely certain.

The deletions *CD 196*, *E230*, *E141*, *E95*, and *E171* show no detectable polar or antipolar effects.

*Revertants of polar trpA mutations selected for efficient utilization of anthranilic acid as a growth factor.* As indicated earlier (Table 2), those polar *trpA* mutants which grow subnormally on anthranilic acid supplement give rise to two types of revertants: full prototrophs and more efficient anthranilic acid-utilizing auxotrophs. We considered the possibility that some revertants of the second type represented instances of full or partial polarity correction. The selection of these

TABLE 5. *Levels of the tryptophan biosynthetic enzymes in several trp deletion mutants grown under conditions of derepression*[a]

| Mutant | ASase | | PRTase | InGPSase | TSaseB | TSaseA |
|---|---|---|---|---|---|---|
| | Noncomplemented | Complemented with PRTase | | | | |
| *trpC109* | 88 | — | — | 63 | 26 | * |
| *trpBEDC43* | 0.5 | 9.2 | * | * | * | * |
| *trpBEDC107* | <0.1 | 7.5 | * | * | * | * |
| *trpBED164* | <0.1 | 7.5 | * | * | * | 0.1 |
| *trpDC196* | 85 | — | — | 87 | * | * |
| *trpE141* | 110 | — | 80 | * | 100 | 123 |
| *trpE230* | — | — | 118 | * | — | 82 |
| *trpE95* | 232 | — | 109 | * | — | 78 |
| *trpE171* | 105 | — | 125 | * | — | 100 |
| *trpABE130* | * | * | * | * | <0.03 | <0.03 |

[a] The enzyme levels are expressed as relative to the standard maximal levels of fully derepressed nonpolar auxotrophs (taken as 100%), as in Table 3. The asterisks indicate the enzymes missing as a result of each deletion.

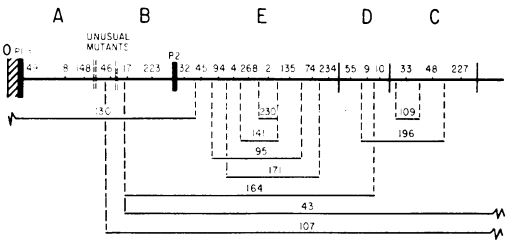


FIG. 2. *Map of the trp operon of Salmonella typhimurium, showing the extent and location of the deletions in the mutant strains described in Table 4; this map is based on previously published data (9). The bacterial chromosome is represented by the dark horizontal line. The numbered bars beneath the chromosome represent the deletion mutants, and their length indicates the physical segment of chromosome missing in each. The numbers above the chromosome refer to individual point mutants and are given as points of reference. The large capital letters refer to the trp genes. The symbol O indicates the operator, and P1 and P2 mark the proposed locations of the two promotor-like elements within the operon (6, 19).*

secondary mutants on anthranilic acid is analogous to Beckwith's (7) selection of revertants of 0° mutations in the lactose operon and to the recovery by Ames, Hartman, and Jacob (1) of secondary mutants from a strain carrying a small deletion of the terminal part of the first gene in the histidine (*his*) cluster which rendered the undamaged genes nonfunctional. Ames, Hartman, and Jacob (1), by selecting secondary mutants of their deletion-carrying strain in the presence of histidinol, an intermediate in the histidine pathway, recovered strains in which the activities of the undamaged genes had been restored. Similarly, by using melibiose, an $\alpha$-galactoside which requires only the permease of the lactose operon for its metabolism, Beckwith (7) was able to select for phenotypic $z^-$ $y^+$ revertants in which the pleiotropic effect of the 0° mutations was removed. The rationale in our case is identical: we select for phenotypic *trpA* *trpB*$^+$ revertants of extreme *trpA* polar mutations. Beckwith (7, 8) showed that the correction of the polar effect could be achieved by suppressors or deletions. A preliminary analysis of the *trpA* revertants selected for improved utilization of anthranilic acid indicates that the same mechanisms operate in our system. Some anthranilic acid-utilizing revertants are capable of further reversion to prototrophy, whereas others have lost this ability. We have not analyzed extensively revertants of the first type and cannot therefore ascertain whether their properties are due to the presence of suppressors or of secondary mutations at the original *trpA* site. On the other hand, the stable anthranilic acid utilizers, those which do not revert to prototrophy, have been studied in some detail and we will discuss a few of them.

As we have indicated, these stable secondary mutants can no longer revert to prototrophy even after treatment with a wide spectrum of mutagenic agents (i.e., NG, DES, and ICR-191). Three of these have been mapped (Fig. 3). The levels of the tryptophan enzymes for these secondary mutants and their strains of origin under conditions of repression and derepression are given in Table 6. Note that the derepression procedure followed in these experiments is different from that used for the determinations given in Table 4. The mutants were allowed to derepress for given periods of time in the absence of tryptophan or any other supplement and with citrate as the sole source of carbon. Under these conditions, derepression takes place virtually in the absence of growth (10).

Polarity can be corrected by a deletion which excises the site of the polar mutation if the deletion itself ends "in phase." This condition seems to be represented by the small deletion *A513* in strain SO-82. As Table 6 shows, the levels of the last three enzymes under repression are equal to the normal levels of repressed wild-type LT2 (about twice the levels of *trpA49*). PRTase could not be measured accurately in the repressed samples; however, after 300 min of derepression, PRTase as well as the last three enzymes was at the same level shown by nonpolar strains which had been allowed to derepress for the same periods of time. Table 6 shows that under derepression PRTase increases in nonpolar mutants about twice as much as the remaining enzymes. Although these results resemble those of Bauerle and Margolin (4) in showing a higher multiplicity of derepression for *trpB* as compared to the last three genes and could be taken as supporting evidence for their conclusion that derepression of the *trp* operon is semicoordinate, they should be interpreted with caution. The levels given in Table 6 are relative to those shown by fully repressed wild-type cells. Because of the insensitivity of our assay for the first two enzymes at low levels of activity, which we have mentioned earlier, these data do not allow us to make a clear-cut distinction between coordinate or semicoordinate derepression. Our assay procedures for these enzymes differed in some respects from those used by Bauerle and Margolin (4).

Polarity can also be corrected by a deletion distal to but not covering the site of a polar mutation. This is based on the fact that the extent of polarity of a chain-terminating mutation in the first gene of an operon (14) is determined, at least in part, by the distance between it and the end of the gene. The deletion in effect moves the polar

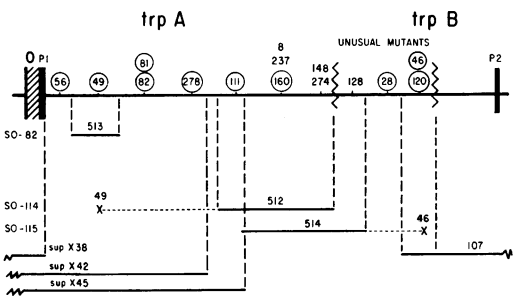


FIG. 3. *Partial map of the trpA gene, indicating the location and extent of the deletion in some secondary mutants selected for improved growth on anthranilic acid (see Table 6). The bacterial chromosome is represented by the wide horizontal line. The narrower lines underneath represent the location and extent of the deletions in the secondary mutants. The deletions supX 38, 42, 45, and trpBEDC 107 are given as reference points. The numbers above the chromosome represent various point mutants arranged in their map order. Polar mutants are indicated by a circle.*

TABLE 6. *Relative levels of the tryptophan biosynthetic enzymes in two polar mutants (A49 and A46) and in secondary mutants derived from them by selecting revertants on anthranilic acid (2 μg/ml)-supplemented MG medium*[a]

| Strain designation | Genotype | Derived from | Derepression time | Relative enzyme level | | | |
|---|---|---|---|---|---|---|---|
| | | | | PRTase | InGPSase | TSaseB | TSaseA |
| | | | *min* | | | | |
| — | *trpA49* | LT7 (*mut*$^+$) | 0 | <25 | 40 | 60 | 60 |
| | | (wild tpye) | 200 | <25 | 50 | 100 | 100 |
| SO-82 | *trpA513* | *trpA49* | 0 | — | 90 | 100 | 100 |
| | | | 300 | 2,000 | 1,700 | 2,000 | 2,900 |
| SO-114 | *trpA49* | *trpA49* | 0 | <25 | 60 | 60 | 40 |
| | *trpA512* | | 300 | 1,200 | 2,000 | 3,000 | 3,000 |
| — | *trpA46* | LT7 (*mut*$^+$) | 0 | <25 | 40 | 80 | 50 |
| | | (wild type) | 200 | <25 | 400 | 1,030 | 610 |
| SO-115 | *trpA46*(?) | *trpA46* | 0 | <25 | 90 | 110 | 100 |
| | *trpA514* | | 300 | 350 | 2,500 | 4,000 | 2,300 |
| Relative levels shown by nonpolar mutants | | | 0 | 100 | 100 | 100 | 100 |
| | | | 200 | 3,500 | 1,300 | 2,180 | 1,900 |
| | | | 300 | 5,000 | 2,000 | 3,200 | 2,800 |
| | | | 400 | 7,000 | 2,800 | 4,300 | 3,800 |

[a] The secondary mutants carry deletions of various sizes (*see* Fig. 3). The nomenclature for the derived strains follows the recommendations of Demerec et al. (11). The enzyme levels are given as relative to the standard fully repressed LT2 wild type, taken as 100% (Table 3). The zero-time sample represents fully repressed conditions. Cells were allowed to derepress in UMM medium in the absence of tryptophan with citrate as the sole source of carbon for the times indicated. ASase activity was absent in all of these strains.

mutation closer to the end of the gene. Cases of this type have been reported for the *lac* operon of *E. coli*(32) and are represented in our system by the strain SO-114. As shown in Fig. 3, this strain contains the original polar mutation *A49* in addition to the deletion *A152* which covers the distal part of the *trpA* gene. This fact has been clearly established, since SO-114 yields prototrophic recombinants when crossed with *A278*, and the *A49* mutation has been separated from *A512* in crosses between SO-114 and *cysB403* with a recombination frequency of about 0.3% (Stuttard, *unpublished data*; Weber, *unpublished data*). Bauerle (*private communication*) confirmed these results by separating *A512* from *A49*. Strain SO-114 shows the enzyme levels typical of *trpA* polar mutants under conditions of repression (Tables 4 and 6). However, the levels observed under derepression are similar to those shown by nonpolar mutants under identical conditions. It seems, then, that in this strain the presence of the deletion has not eliminated polarity, as is the case with *A513*, but it has diminished its intensity.

Table 6 also contains information about a deletion, *trpA514*, which corrects the polar effect of *A46*. The correction of polarity seems complete for the last three enzymes but only partial for PRTase. As shown in Fig. 3, *A46* is located in the "unusual" region, and the deletion *A514* extends in a proximal direction into *trpA* but does not seem to have excised the *A46* site. This conclusion is based on crosses of SO-115 to *A28*, which yielded prototrophic recombinants. The data available at this time indicate that *A28* is closer to the operator than *A46*, but this should be confirmed further. Attempts to separate *A46* from *A514* in strain SO-115 have been unsuccessful thus far.

## DISCUSSION

Polar mutants in *trpA* show an uncoordinate effect on the expression of the distal genes of the *trp* operon, with *trpB* being more drastically affected than the last three. These observations agree with similar ones reported by Bauerle and Margolin (4) and are consistent with their idea that a second "initiator," P2, located between *trpB* and *trpE* is present in the *trp* operon. An alternative interpretation of the uncoordinate

effect of *trpA* polar mutations on PRTase levels would postulate a differential stability between the free and complexed forms of this enzymatic component, with the free form being the less stable one. Although we have observed that the dissociated form of PRTase is less stable in crude extracts than the complexed form, the magnitude of the difference in stability does not appear to be sufficiently large to account for the extremely low levels of PRTase activity that we find in *trpA* polar mutants. While it is possible that a lower stability of dissociated PRTase may be a contributing factor to the low activities which we observed, it would not account for all the results.

Bauerle and Margolin also reported (4, 6) that polar mutants in *trpA* showed, under repressed conditions, a basal level of expression for the genes *trpE*, *trpD*, and *trpC*. This basal level was independent of the intensity of the polar effect each mutation showed under derepressed conditions. They interpreted this independence as an indication that the expression of the last three genes was largely initiated by P2. We have made essentially the same observations. Under repression, all of our polar *trp* mutants showed the same basal level of expression, and this was approximately the same as that reported by Bauerle and Margolin. Under derepression, some showed more severe polar effects than others. The fact that this basal level of expression is independent of the severity of the polar effect which a given polar mutation shows under derepression is clearly illustrated by a comparison between the strain carrying the mutation *A49* and the double mutant *A49 A512* (SO-114) which was derived from it. Both of these strains have practically the same levels of tryptophan biosynthetic enzymes under repressed conditions, but under derepression the double mutant is capable of making higher levels of these enzymes than is the single mutant. Both strains carry the *A49* polar mutation, but strain SO-114 carries in addition the deletion *A512*, which decreases the intensity of the polar effect of *A49* by shortening the distance between it and the operator distal end of the gene. This effect of *A512* is apparent only under derepression; under repression, both strains show the same enzyme levels. The mutation *E286* leads to lower levels of expression of the genes *trpD* and *trpC* under repressed conditions than any of the *trpA* polar mutations. This observation is also consistent with the postulate that an initiator (or "promoter-like" element) exists between *trpB* and *trpE*. The polar mutation *E286* would be distal to P2 and therefore should be expected to show lower basal levels of expression for the last two genes than those shown by polar mutations proximal to P2.

One of the implications of the existence of a second initiator is that extreme polar mutations in the first structural gene (0° type), which completely eliminate the expression of the entire operon will not be found in the *trp* cluster. Thus far, in a sample of 190 randomly isolated *trp* mutants, we have failed to observe a single mutation of the 0° type.

In conclusion, our data are consistent with the postulate of Bauerle and Margolin (4, 6, 19) that a second "promoter-like" element exists in the *trp* operon of *S. typhimurium*. We have no data which are inconsistent with this idea.

Some of our deletion-carrying strains show probable antipolar effects. This is clear in the case of *trpC109*, which has decreased levels of TSase B, the product of the adjacent operator proximal gene *trpD*. It is possible, although not certain for reasons given earlier, that the decreased levels of ASase found in the presence of deletions *BED 164*, *BEDC 43*, and *BEDC 107*, when the activity of this enzyme component was assayed in the presence of saturating amounts of PRTase, are due to an antipolar effect of these deletions. At any rate, the case of *trpC109* shows that antipolarity exists in the *trp* operon of *S. typhimurium*, as well as in the one of *E. coli* (16, 30, 31). Fink and Martin (14), after an extensive search, found no indications that antipolar effects occur in the *his* operon of *S. typhimurium*. Thus far, then, antipolar effects have been reported only for the *trp* operon of *E. coli*, and our results show that they also exist in the *trp* operon of *S. typhimurium*. This suggests that antipolarity may be exclusive to the *trp* operon and may constitute a type of control perhaps related to the existence of enzyme complexes in this system. More information is needed about this phenomenon before its significance can be understood.

Revertants (or secondary mutants) in which polarity can be corrected or diminished by selecting for strains with increased activity for the second enzyme in the operon have been reported by Beckwith for the lactose operon of *E. coli* (7). It was shown that β-galactoside permease, the product of the second gene in the lactose operon, is restored in a revertant of a strongly polar nonsense mutant by the deletion of a large segment of the first gene (*z*). Zipser and Newton (32) extended these investigations on the effect of deletions on polarity and concluded that the degree of polarity created by a nonsense codon in *z* is in part a function of its distance from the *z-y* punctuation, and that the distance between the nonsense mutation and the operator end of the gene does not influence per se the rate of expression of distal genes. (Addition of an operator proximal deletion to a nonsense mutation does not in-

fluence the degree of polarity.) Among our secondary mutants showing a correction of polarity by deletions in *trpA*, we can distinguish three different situations. The first is illustrated by strain SO-82 and deletion *A513*. In this case a small "in phase" deletion seems to have excised the site of the polar mutation. The situation with strain SO-114, which contains the original *A49* polar mutation and the distal deletion *A512*, has already been mentioned. This case is identical to that described by Zipser and Newton (32), i.e., polarity has been diminished by shortening the distance between the site of the mutation and the end of the gene. As mentioned above, Zipser and Newton (32) have shown in the *lac* operon that deletions which correct polarity are always located in an operator distal position with respect to the polar mutation being corrected; those located in an operator proximal direction do not correct polarity. Thus, although deletion *A512* in strain SO-114 agrees with their observations, deletion *A514* in strain SO-115 does not. It is possible that the deletion *A514* does not end in phase and generates a frameshift which makes the polar mutation at *A46* (presumably a nonsense) vanish. If *A46* is near the end of the gene, the chance that a second nonsense codon will appear at a position where it could show a detectable polar effect could be very low. As we have indicated, however, we are not completely certain of the genetic characterization of strain SO-115 and will defer further discussion of this problem until this question has been settled.

Mutants *A28*, *A46*, and *A120* present a problem in interpretation at this time. They map at the extreme distal end of *trpA*, they are clearly polar, and one of them, *A28*, has been characterized as an amber mutation. However, they show less PRTase activity than polar mutants located proximally to them and having a much stronger overall polar effect. Bauerle and Margolin (5) have called these mutants and others like them "unusual." Based on the fact that some of these strains possess uncomplexed ASase capable of activity when normal PRTase is added to assay mixtures, in addition to low levels of PRTase, they concluded that these mutations correspond to the operator proximal end of *trpB* and the low PRTase activity corresponds to a mutationally altered enzyme. We have reported here some observations which are difficult to reconcile with this interpretation. The first observation is that these mutations are polar. If the low levels of PRTase found in *A46* and *A120* correspond to an enzyme altered by a missense mutation, we will have to assume that some missense mutations can be polar. This assumption is unattractive since all the evidence thus far is consistent with the idea that polarity effects result from the presence of chain-terminating triplets within a gene coded as part of a polycistronic mRNA (5, 13, 21, 23, 30). The second observation has to do with the characteristics of *A28*. Unlike *A46* or *A120*, this strain has no detectable ASase or PRtase activities. We feel that, based on the information available at this time, an unambiguous assignment of the "unusual" mutants to *trpA* or *trpB* cannot be made. This problem is discussed in more detail in the companion report (10).

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