THE FUNCTIONAL ORGANIZATION OF THE TRYPTOPHAN GENE CLUSTER IN *SALMONELLA TYPHIMURIUM**

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Information for the synthesis of the enzymes which catalyze the terminal steps of tryptophan biosynthesis in *Salmonella typhimurium* is encoded in a cluster of five genes located near *cysB* on the chromosome (Fig. 1).¹ In *Escherichia coli* the gross structure of the *try* region is identical with that of *S. typhimurium*, and it has been concluded that the genes constitute a classical operon since the major tenets of the Jacob-Monod model² have been satisfied. The data obtained with *E. coli* include (a) demonstration of an operator region at the anthranilate synthetase end (the *tryA* end in *Salmonella* nomenclature) of the cluster,³ (b) detection of a polycistronic messenger RNA corresponding to the *try* genes,⁴ (c) isolation of mutations in the anthranilate synthetase gene which have pleiotropic effects on all the *try* genes,⁵ and (d) demonstration of apparent coordinate expression of the genes.⁶

FIG. 1.—Order and the enzyme products of the tryptophan genes in *S. typhimurium*. In earlier reports,^{8, 11, 26} the *supX* locus was referred to as *su leu500*.

In this report it is shown that in *S. typhimurium* the first two genes of the *try* cluster (*tryA* and *tryB*) form one coordinate unit of expression, while the last three genes (*tryE*, *tryD*, and *tryC*) comprise a second coordinate unit. It is unlikely that this is due to the existence of two independent but adjacently linked *try* operons, since we find that polarity mutations in *tryA* affect not only the function of *tryB* but also of *tryE*, *tryD*, and *tryC*. In agreement with the results on the kinetics of repression, the polar effects are not coordinate for all four genes; rather *tryB* expression is more severely limited than that of the other genes. These data are consistent with the results obtained in a study of the effects of deletion mutations into the *tryA* (operator) end of the gene cluster.⁷⁻⁹ It has been concluded from this work that the *try* gene cluster in *S. typhimurium* functions as a unit with respect to regulation by tryptophan, possessing a single operator region at the *tryA* end, but is composed of two independent units (*tryA-tryB* and *tryE-tryD-tryC*) with respect to initiation of the expression of gene activity.

It has also been possible to dissect the *tryA* gene into seven distinct regions by deletion mapping. Twenty-two *tryA* point mutations have been mapped, and the mutant strains were characterized biochemically. Of these, 16 are of the polarity type. By comparing the position of each mutant on the linear map to the degree of its polarity effect, it has been found that those mutations most proximal to the operator end of the gene exert the most severe pleiotropic effect. Mutations more distal to the operator are proportionately less polar. This demonstration of a "polarity gradient" in the *tryA* gene is similar to that reported from a study of polarity mutations in the lactose operon of *E. coli*.¹⁰

Materials and Methods.—*Bacterial strains:* The tryptophan auxotrophs of *S. typhimurium* were derived from strains LT2 and LT7. Many strains were kindly supplied by M. Demerec. The

remaining mutants were isolated in this laboratory. The general procedure used in selecting the strains which possess deletions into the operator (*tryA*) end of the *try* gene cluster has already been described.¹¹ The 5-methyl tryptophan-resistant (*mt^r 1*) mutant was isolated from strain *cysB 517* by direct selection, using minimal agar¹² supplemented with cysteine (20 μ g/ml) and 5-MT (100 μ g/ml). Stocks were maintained on Dorset Egg Agar slants (Difco) at 2°C.

Transduction and recombination: The general procedures for transduction using phage PLT22 have been described previously.¹³ Recipient cells were grown overnight at 37°C in a broth medium consisting of 1.0% Difco tryptone and 0.5% Difco yeast extract. Approximately 2×10^9 cells were infected at a multiplicity of 10. In experiments involving deletion mapping of the *try* point mutations, 0.1-ml aliquots of the transduction mixtures were spread onto four plates of minimal agar¹² supplemented with 0.1% Difco casamino acids. In many cases, reciprocal crosses were performed, but usually the point mutants were used as recipients, since the deletion strains are in general very poor recipients in transduction tests.

Culture methods and preparation of extracts: The minimal medium of Davis and Mingioli¹² modified by the exclusion of citrate was used routinely. Cultures in which repressed levels of the *try* enzymes were desired were grown at 37°C with forced aeration to late log phase in medium containing 25 μ g/ml tryptophan and 0.25% glucose. For preparing cultures of *try* mutants with derepressed enzyme levels, growth under conditions of limiting tryptophan was carried out in a bacterial chemostat. The rate of tryptophan supplementation was adjusted so as to maintain the cell population at approximately 10^9 /ml with a generation time of about 3 hr. The cells were collected by centrifugation, washed once with cold 0.9% NaCl, resuspended in cold 0.1 M Tris-HCl buffer (pH 7.8) containing 10^{-4} M EDTA, and broken at 5–10°C by ultrasonic disintegration. Debris was removed by centrifugation at $25,000 \times g$ for 30 min, and the protein extracts containing 6–8 mg protein/ml (method of Lowry *et al.*¹⁴) were separated from low-molecular-weight materials by gel filtration at 2°C using a 25-ml G-25 Sephadex column. The same Tris buffer was used for column equilibration and elution. Extracts were assayed immediately, since several of the enzyme activities were found to decay upon standing or freezing and thawing.

Enzyme assays: Anthranilate synthetase (ASase) was assayed by measuring fluorometrically the formation of anthranilic acid from chorismic acid. The reaction mixture contained 0.5 μ mole chorismic acid, 10 μ moles $MgCl_2$, 20 μ moles L-glutamine, 100 μ moles KPO_4 (pH 7.0), and enzyme extract in a final volume of 1.0 ml. After incubation at 37°C (usually for 10 min) the reaction was stopped by the addition of 0.1 ml of 1 N HCl. The anthranilic acid formed was extracted into 4 ml of ethyl acetate (EtOAc) and was quantitated using a Turner fluorometer. When necessary, dilution of the EtOAc extract was made with water-saturated EtOAc. Chorismic acid was isolated according to the method of Gibson¹⁵ and then crystallized from ether.

Phosphoribosyl transferase (PRTase) was assayed by measuring fluorometrically the disappearance of anthranilic acid. The reaction mixture contained 25 μ moles anthranilic acid, 0.6 μ mole 5-phosphoribosyl pyrophosphate (Sigma Chemical Co.), 5 μ moles $MgCl_2$, 50 μ moles KPO_4 (pH 8.2), 2 units of indoleglycerol phosphate synthetase (this enzyme aids in pulling the PRTase reaction by removing phosphoribosyl anthranilate, the product of PRTase, and was supplied by the addition of a Sephadexed crude extract of mutant *tryB 13* which lacks PRTase), and enzyme extract in a final volume of 0.5 ml. After incubation at 37°C the reaction was stopped by the addition of 0.1 ml of 1 N HCl. The unconverted anthranilic acid was extracted into 4 ml EtOAc and quantitated using a Turner fluorometer. The amount of anthranilic acid removed was calculated as the difference between the treated reaction mixtures and an untreated substrate blank. In this assay as well as in the ASase assay, the enzyme extract did not interfere with or contribute to the fluorescence determination.

Indoleglycerol phosphate synthetase (IGPSase) was assayed essentially according to the method of Smith and Yanofsky.¹⁶ The reaction mixture contained 0.7 μ mole of anthranilic deoxyribulotide (synthesized chemically¹⁶), 50 μ moles Tris-HCl (pH 7.8), and enzyme extract in a final volume of 0.5 ml. Incubation was carried out at 37°C for 20 min.

The A and B subunits of tryptophan synthetase (TSase) were assayed individually by measuring the removal of indole in the presence of a threefold excess of the purified complementary subunit.¹⁶ TSase-A protein was purified according to Smith and Yanofsky¹⁶ from derepressed cultures of mutant *tryA 148*, a strain lacking ASase; TSase-B protein was purified from derepressed cultures of mutant *tryC 71*, a strain lacking the TSase-A protein.

In all assays one unit of enzyme activity is defined as 0.1 μ mole of product formed, or substrate used, in 20 min at 37°C.

Results.—Kinetics of expression of try genes: A comparison of the levels of each of the tryptophan biosynthetic enzymes of *S. typhimurium* grown under conditions of repression and derepression indicate that the five *try* genes are not expressed as a coordinate unit. It was found that ASase and PRTase (the products of *tryA* and *tryB*, respectively) are derepressed coordinately with respect to one another, but not with respect to the remaining three enzyme proteins of the pathway. On the other hand, these latter proteins, IGPSase and the A and B proteins of TSase (the products of *tryE*, *tryC*, and *tryD*, respectively), apparently do constitute a coordinate unit within themselves. Table 1 contains data obtained from a study of 5-MT-resistant derivative (*mtr 1*) of *S. typhimurium* which, when grown in minimal medium, possesses elevated levels of all five enzyme proteins. This overproduction of the tryptophan enzymes is constitutive, since inclusion of high levels of tryptophan in the growth medium has no repressive effect (Table 1). By comparing the enzyme levels of *mtr 1* with the activities of fully repressed control cells, i.e., the 5-MT-sensitive parental strain, it is seen that in *mtr 1* the ASase and PRTase are present at about 40 times the control level while IGPSase, TSase-A, and TSase-B are derepressed only about 25 times (Table 1).

This lack of total coordination in *mtr 1* is not due to some aberrant pleiotropy caused by the mutational event leading to 5-MT resistance, for example by an alteration of the *try* operator or of the *tryA* gene which codes for the important allosteric protein of the pathway (ASase). The ASase of *mtr 1* appears to possess normal kinetics and is as sensitive to end product inhibition by tryptophan as the ASase of wild-type cells. But, more significantly, the *mtr 1* mutation is not linked in transduction tests to the *try-cysB* region of the chromosome (Table 2). Strain *mtr 1* is a derivative of strain *cysB 517* and thus possesses a cysteine auxotrophy. Normal genetic linkage between *cysB 517* and *try* auxotrophic markers, for example, in a cross with *tryE 200*, is about 15 per cent (Table 2). In a similar cross *mtr 1* was used as a recipient in a transduction with wild-type phage, and selection was made for incorporation of the *cysB*⁺ marker. If the *mtr 1* locus were located somewhere in the *try* gene cluster, about 15 per cent of the *cysB*⁺ recombinants would also have received all or part of the wild-type *try* region and thus become 5-MT-sensitive. However, all *cysB*⁺ recombinants remained 5-MT-resistant (Table 2).

TABLE 1
CONSTITUTIVE LEVELS OF TRYPTOPHAN BIOSYNTHETIC ENZYMES IN STRAIN *mtr 1*

Enzyme	Gene locus	Relative Specific Activity	
		Minimal medium	Minimal medium + tryptophan (25 μ g/ml)
ASase	<i>tryA</i>	42	38
PRTase	<i>tryB</i>	44	43
IGPSase	<i>tryE</i>	24	22
TSase-B	<i>tryD</i>	28	22
TSase-A	<i>tryC</i>	26	25

Cells were grown to late log stage with forced aeration at 37°C. The medium was also supplemented with L-cysteine (40 μ g/ml), since *mtr 1* was isolated as a derivative of *cysB 517*. The enzymatic activities were determined as described in *Materials and Methods*. The relative specific activities were calculated by designating as unity the specific activities of the control extract prepared from *cysB 517* grown under conditions of maximal repression (25 μ g/ml tryptophan). Thus the relative specific activity is a direct measure of the multiplicity of enzyme derepression over the fully repressed, control level. The actual specific activities (0.1 μ mole of substrate converted or of product formed/mg protein/20 min) corresponding to unity were: ASase, 0.18; PRTase, 0.27; IGPSase, 0.44; TSase-B, 0.86; TSase-A, 0.65.

TABLE 2
ANALYSIS OF LINKAGE OF *mtr 1* TO *try-cysB*

Recipient	Donor phage	Selected markers	Total transductants	Number incorporating the indicated unselected markers
<i>cysB 517</i>	<i>try 200</i>	<i>cysB</i> ⁺	1663	231 (<i>tryE 200</i>)
<i>mtr 1, cysB 517</i>	Wild-type	<i>cysB</i> ⁺	866	0 (<i>mtr</i> ⁺)
<i>tryABEDC 101</i>	<i>mtr 1, cysB 517</i>	<i>try</i> ⁺	398	84 (<i>cysB 517</i>), 0 (<i>mtr 1</i>)
<i>tryABEDC 101</i>	<i>mtr 1, cysB 517</i>	<i>try</i> ⁺ + <i>mtr 1</i>	0	—

Transduction tests were performed as described in *Materials and Methods*. Determination of the incorporation of the unselected marker was accomplished by replica-plating the transductant colonies onto the appropriate media. The concentration of 5-MT was 100 µg/ml.

Also when strain *tryABEDC 101*, an auxotroph harboring a deletion of the entire *try* region, is transduced to prototrophy with *mtr 1* phage, no incorporation of the *mtr* trait was found (Table 2). Normal *cysB* linkage was observed in this cross, and all recombinants which received the *cysB 517* marker in addition to the entire *try* region of *mtr 1* also remained 5-MT-sensitive. Furthermore, when in this same cross simultaneous selection for *try*⁺ and *mtr* was attempted, no recombinant colonies appeared. Thus, it is concluded that the *mtr 1* mutation is not an alteration of any of the *try* genes and is not carried in the same transducing particle as the *cysB-try* region. The obvious alternative is that *mtr 1* is a mutation in a *try* regulator gene analogous to that reported in *E. coli*.¹⁷

The data obtained with strain *mtr 1* (Table 1) indicate that the tryptophan genes of *S. typhimurium* constitute not one but two units of coordinate expression. This conclusion is strongly supported by the results of experiments carried out with various *try* mutant strains (Table 3). Derepression of the remaining, unaltered enzymes of these auxotrophs was effected by growth in a chemostat under conditions of tryptophan limitation. The extent of derepression obtained in chemostat-grown cells was 3–4 times greater than the constitutive levels present in strain *mtr 1*, and the disparity between the multiplicity of derepression of the *tryA-tryB* unit and of the *tryE-tryD-tryC* unit was always found. In most cases this disparity was greater than that seen in *mtr 1*. As is shown in Table 3, this semicoordinate pattern is independent of the gene location of the *try* lesion. Although only one representative mutant strain is listed for each *try* gene, this phenomenon has been observed consistently in many other experiments with other auxotrophs.

It appears certain that the differences in enzyme activity observed between repressed and derepressed extracts are due exclusively to the specific regulation of enzyme formation by tryptophan and not the result of trivialities inherent in the experimental procedures. Control experiments have shown that under the con-

TABLE 3
DEREPRESSION OF THE TRYPTOPHAN BIOSYNTHETIC ENZYMES IN VARIOUS *try* AUXOTROPHS

Mutant strain	—Multiplicity of Derepression—				
	ANase	PRTase	IGPSase	TSase-B	TSase-A
<i>tryA 148</i>	—	184	95	94	98
<i>tryB 479</i>	121	—	28	27	33
<i>tryE 2</i>	134	117	—	43	41
<i>tryD 9</i>	142	126	79	—	77
<i>tryC 89</i>	142	161	65	59	—

Cultures of the auxotrophs possessing derepressed enzyme levels were prepared by growth under conditions of tryptophan limitation in a chemostat as described in *Materials and Methods*. Cells possessing repressed enzyme levels were prepared by growth in the presence of excess tryptophan (25 µg/ml). The multiplicity of derepression for each enzyme was calculated by dividing the specific activity of the extract of chemostat-grown cells by that of the repressed cells.

ditions of assay all the enzymes exhibit normal linear kinetics with respect to time of incubation and extract concentration, whether present in repressed or derepressed preparations. This fact and the results of dilution experiments indicate that no selective lability exists among the enzymes when present at high or low levels in extracts. Furthermore, the results of mixing experiments have excluded the possibility that inhibitors of any of the enzyme activities might be present in either the repressed or derepressed extracts.

Polarity mutations in gene *tryA*: The finding of two distinct coordinate units of expression in the *try* gene cluster in *S. typhimurium*, while consistent with evidence for two units of gene expression,⁷⁻⁹ was somewhat unexpected in view of the apparent contiguity of the genes on the chromosome and of the investigations in *E. coli* which indicate a classical operon structure.³⁻⁶ To study further the functional relationships of the five *try* genes, an analysis of the occurrence and effects of polarity mutations in gene *tryA* was undertaken. A group of 22 independently isolated *tryA* mutants has been examined. By the use of deletion mapping, it has been possible to assign each mutant to one of seven separate regions of the *tryA* gene and to arrange these seven mutant groups in a linear map (Fig. 2). The results of the enzymatic analysis of these mutants grown under conditions of repression and derepression (Table 4) have verified that all lack ASase and have led to the following conclusions concerning polarity.

(1) Of the 22 *tryA* mutations, 16 exhibit detectable polarity effects; that is, the levels of the enzymes controlled by the four remaining intact tryptophan genes are significantly lower than those of controls grown under the same conditions. The other six *tryA* mutations appear to be nonpolar.

(2) The polar and nonpolar mutations are not distributed randomly throughout the *tryA* gene. The polar types tend to cluster toward the operator-proximal end of the gene while the nonpolar types predominate at the operator-distal end. This is evident in Figure 2.

(3) There is a distinct gradient of degree of polarity, which is related to the position of the mutations in the *tryA* gene. This phenomenon is most evident

FIG. 2.—Deletion mapping of mutations of the *tryA* gene of the tryptophan gene cluster in *S. typhimurium*. The procedures and strains used were as described in *Materials and Methods*. In earlier reports, the *supX* locus was referred to as *su leu500*,^{8, 11, 26} and deletion strain *supX 22* as strain 4CT1.^{8, 9, 26} Those mutants labeled *P* exhibit polar effects on the expression of the remaining *try* genes; those labeled *NP* have no polar effect.

from examination of the derepressed enzyme activities listed in Table 4. The polar mutations of group I, closest to the operator (Fig. 2), exhibit the most severe pleiotropic effects, while those of groups II through VII display progressively decreasing levels of polarity effects.

(4) Although the polarity effects encompass all four remaining tryptophan genes, the degree of the effects is not completely coordinate, in contrast to other polarity studies;¹⁸ that is, the expression of *tryB*

TABLE 4
EFFECT OF POSITION ON THE ACTIVITY OF POLARITY MUTANTS OF GENE *tryA*

Mutant group	No. of mutants	Repressed Enzyme Activities Expressed as % of Standard				Derepressed Enzyme Activities Expressed as % of Standard Derepressed Activity			
		PRTase	IGPSase	TSase-B	TSase-A	PRTase	IGPSase	TSase-B	TSase-A
Nonpolar	6	102	120	112	106	100	100	100	100
Polar I	2	<10	40	40	40	0.9	3.6	4.0	4.2
Polar II	3	<10	40	40	40	1.9	4.3	5.1	5.6
Polar III	8	<10	40	40	40	4.2	9.0	13	13
Polar IV	1	<10	40	60	60	16	30	36	38
Polar V	1	<10	50	60	70	39	34	40	46
Polar VII	1	50	80	—	80	68	67	—	56

Repressed and derepressed extracts were prepared as described in *Materials and Methods*. In those groups which consist of more than one mutant strain, the value listed is the mean enzyme activity of the mutants in that group. The *standard repressed activity* assumed for each enzyme was the activity detected in fully repressed cultures of wild-type strain LT2. These activities (in terms of 0.1 μ mole of substrate converted or of product formed/mg protein/20 min) were: PRTase, 0.14; IGPSase, 0.24; TSase-B and TSase-A, 0.59. The nonpolar mutants, as determined by repressed activity, occur in regions V, VI, and VII of *tryA* (Fig. 2) and are combined in the table. The *standard derepressed activity* assumed for each enzyme was the mean derepressed activity of these nonpolar mutant strains. These activities (in terms of the multiplicity of depression over the *standard repressed activity*) were: PRTase, 160; IGPSase, 98; TSase-B, 94; TSase-A, 95.

is more severely limited than that of *tryE*, *tryD*, and *tryC*. The expression of these latter three genes is, however, affected in a coordinate manner. Under repressed conditions *tryB* activity is not detectable (<10% of the control activity) in all the polar mutants of groups I–V, while *tryE*, *tryD*, and *tryC* activities are always present at about 40–60 per cent of the control. A similar disproportionality can be seen in the activities of the corresponding derepressed cultures.

(5) It appears that genes *tryE*, *tryD*, and *tryC* possess a basal level of expression (at about 40% of the fully repressed level) which cannot be reduced by even the most extreme polar mutations of *tryA*. While the polar effects of mutations of groups I through IV on the expression of genes *tryE*, *tryD*, and *tryC* are quantitatively quite distinct in derepressed cultures (reduced approximately 95% in groups I and II, 90% in group III, and 65% in group IV), the corresponding effects in repressed cultures are essentially identical and never exceed a 60 per cent reduction in activity.

Discussion.—The most obvious explanation for the existence of two individually coordinate units of expression in the *try* gene cluster in *S. typhimurium* (*tryA-tryB* and *tryE-tryD-tryC*) is that these units represent two distinct but contiguously linked operons which respond independently to regulation by tryptophan. Indeed this was suggested in the results of preliminary studies of deletion mutations into the *tryA* (operator) end of the cluster.¹⁹ However, the finding that polarity mutations in *tryA* are pleiotropic not only for *tryB* but also for *tryE*, *tryD*, and *tryC* (Table 4) indicates that this simple two-operon model is probably not correct. A more extensive investigation of the operator deletion strains studied by Margolin and Mukai¹⁹ has yielded results^{8, 9} which also support this conclusion. A model which appears to reconcile all the data will be presented in detail elsewhere. But, in brief, it proposes a unique type of gene organization that includes (a) the existence of a single operator region at the *tryA* terminus of the gene cluster through which regulation of all the genes by tryptophan is mediated, and (b) the arrangement of the genes into two separate units (*tryA-tryB* and *tryE-tryD-tryC*) with respect to the initiation of gene expression. It has already been shown that the genetic element involved in repressor recognition (the operator) is distinct from the element governing initiation of gene expression (the promoter).²⁰ Thus, our model envi-

sions the organization of the *try* genes into a genetic unit which is somewhat more complex than a simple operon since it possesses two discrete promotor-like elements, one necessary for the expression of *tryA-tryB*, the other of *tryE-tryD-tryC*.

One manifestation of such a gene organization might be the existence of two sets of coordinately controlled enzymes as has been observed here (Tables 1 and 3). But it is clear from the polarity data (Table 4) that expression of the *tryE-tryD-tryC* unit is not completely independent of the expression of the *tryA-tryB* unit. This semidependency may be explained by invoking the popular ideas that polarity mutations primarily interfere with the translation process and that the translation of nascent mRNA is necessary for its release from the DNA template.²¹⁻²³ It would follow, then, that polar mutations in *tryA* are pleiotropic for *tryE-tryD-tryC*, as well as for *tryB*, if the translation of the nascent mRNA coding *tryA-tryB* is a prerequisite for release of that portion of nascent mRNA coding *tryE-tryD-tryC*. With this assumption the finding that the expression of *tryB* is more severely affected by the *tryA* polar mutations is to be expected. The polar effects would be exerted on *tryB* at two levels, namely, by limitation of the release of nascent mRNA coding *tryA-tryB* and by retardation of the translation of the mRNA which subsequently is liberated. However, since the *tryE-tryD-tryC* unit possesses a separate site for initiation of expression, the effects of *tryA* polar mutations on this unit would occur only at the level of mRNA release. It should be emphasized that with this hypothesis no distinction is made concerning whether transcription of the *try* genes in *S. typhimurium* results in the formation of one polycistronic mRNA containing information for all the genes, as reported for *E. coli*,⁴ or of two distinct mRNA's, one coding for *tryA-tryB* and the other for *tryE-tryD-tryC*. In the former case, the promotor-like elements proposed here would function as specific sites for the attachment of ribosomes; in the latter case, they would serve as initiation sites for the action of the DNA-dependent RNA polymerase.

The discovery that genes *tryE*, *tryD*, and *tryC* possess a basal level of activity that is unaffected by even the most stringent polarity mutations in *tryA* (Table 4) suggests, in terms of the above hypothesis, that normally in the cell a limited rate of transcription of the *try* genes into mRNA is maintained which does not require translation by ribosomes for liberation from the DNA template. This low level of mRNA production might correspond to the transcription process which is believed to take place concomitantly with DNA replication,²⁴ if indeed such a phenomenon does occur.²⁵

The gradient of polarity effects found here in the *try* system points up a direct correlation between the proximity of the polar mutation to the operator and the degree of its pleiotropy. A similar situation has been demonstrated in a study of nonsense mutations in the lactose operon,¹⁰ indicating that this may be a general phenomenon with a common mechanism. It has not yet been determined which of the polar mutations examined here are of the nonsense type. However, none of the strains exhibiting polar effects were found to deviate from the gradient described. We note that no nonpolar mutations have yet been found in the operator-proximal region of *tryA*. The significance of this requires a more detailed analysis of the nature of the mutations in this region.

The ASase-less polarity mutants of *E. coli* studied by Somerville and Yanofsky⁵ probably correspond to the more operator-proximal *tryA* mutant strains described

in this report. Since these workers measured only TSase activity in determining the extent of the pleiotropy, it remains to be seen if the polar effects in *E. coli* display the same peculiar semicoordinacy as found here.

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Abbreviations: 5-MT, 5-methyl tryptophan; Tris, tris (hydroxymethyl) aminomethane; EDTA, ethylenediaminetetraacetic acid; EtOAc, ethyl acetate; ASase, anthranilate synthetase; PRTase, phosphoribosyl transferase; IGPSase, indoleglycerol phosphate synthetase; TSase-A and TSase-B, A and B proteins of tryptophan synthetase, respectively; mRNA, messenger ribonucleic acid.

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