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Naturally Occurring Sites Within the *Shigella dysenteriae* Tryptophan Operon Severely Limit Tryptophan Biosynthesis

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We investigated the structural, functional, and regulatory properties of the *Shigella dysenteriae* tryptophan *(trp)* operon in transduction hybrids in which the *cysB-trp* region of *Escherichia coli* is replaced by the corresponding region from *S. dysenteriae*. Tryptophan biosynthesis was largely blocked in the hybrids, although the order of the structural genes was identical with that of *E. coli*. Nutritional tests and enzyme assays revealed that the hybrids produced a defective anthranilate synthetase (ASase). Deletion mapping identified two distinct sites in *trpE*, each of which was partially responsible for the instability and low activity of ASase. We also discovered a pleiotropic site *trpP*(S) that maps outside the structural gene region and is closely linked to the *S. dysenteriae trp* operator. *trpP*(S) reduced the rate of *trp* messenger ribonucleic acid synthesis, and consequently *trp* enzyme levels, 10-fold relative to wild-type *E. coli*. In recombinants in which the structural genes of *E. coli* were under the control of the *S. dysenteriae* promoter, enzyme levels were also reduced 10-fold. In some fast-growing revertants of the original hybrids, the rates of *trp* messenger ribonucleic acid synthesis and levels of tryptophan synthetase were restored to values characteristic of wild-type *E. coli*. Thus, the Trp auxotrophy associated with the *S. dysenteriae trp* operon derives from the combination of a defective ASase and decreased expression of the entire operon imposed by *trpP*(S).

Members of the genus *Shigella* are closely related to *Escherichia coli*. They share susceptibility to some of the same bacteriophages (30), and both intergenic (8) and intragenic (11, 40) recombinations have been observed in crosses of *S. dysenteriae* and *E. coli*. Measurements of interspecific deoxyribonucleic acid (DNA)-DNA hybridization bolster claims of their kinship (2, 31).

Focusing on the genes of the tryptophan synthetic pathway, a comparison of the *S. dysenteriae* and *E. coli* tryptophan synthetase α proteins has disclosed essentially complete homology for the first 50 residues (27). Interspecific ribonucleic acid (RNA)-DNA duplexes formed between *S. dysenteriae trp* messenger RNA (mRNA) and *E. coli trp* DNA are only slightly less stable than the homologous *E. coli* RNA-DNA duplexes (7). Additionally, we have recently found that the *E. coli trp* repressor recognizes the *S. dysenteriae trp* operator, and vice versa (32).

Despite these documented similarities, the ecology and biochemistry of *E. coli* and *S. dysenteriae* are quite disparate. *E. coli* is a normally innocuous permanent resident of the gut in members of many vertebrate taxa, whereas *S. dysenteriae* is a transient, and often traumatic, visitor specific to man (22). Perhaps the temporary nature of its sojourn relieves *Shigella* of the necessity for metabolic self-sufficiency that *E. coli* needs in order to cope with the "feast and famine existence" (23) of intestinal life. Wild-type strains of *E. coli* will grow on a minimal salts medium supplemented with a carbon source such as glucose, but *S. dysenteriae* has multiple nutritional requirements. Our laboratory strain, Sh16, fails to grow in a minimal-glucose medium supplemented with vitamins and all 20 amino acids, but will grow in a nutrient medium.

Evidence points to the conclusion that *S. dysenteriae* is losing previously competent biochemical pathways. The enteric genera most closely affined with *Shigella*, such as *Escherichia*, *Salmonella*, and *Klebsiella*, are generally capable of synthesizing the substances they require for growth on a minimal-glucose medium. It is probable, therefore, that synthetic pathways evolved in a common ancestor of these genera, and that some pathways were subsequently lost by *Shigella*.

Direct support for this idea comes from the vestigial lactose and *trp* operons of *S. dysenteriae*. *S. dysenteriae* is unable to ferment lactose, yet most strains code for a β-galactosidase simi-

lar to the *E. coli* enzyme (40), although the turnover number of the *S. dysenteriae* enzyme is 5- to 10-fold lower than that of *E. coli*. The Lac⁻ character arises because *S. dysenteriae* behaves both phenotypically and genetically as a *lacY* deletion (11, 29).

The structural genes of the *S. dysenteriae trp* operon are in the same order as in the *E. coli* operon (7, 8); nonetheless, *S. dysenteriae* strains are deficient in tryptophan synthesis (8). This partial Trp auxotrophy is also shown by transduction hybrids in which the *cysB-trp* region of *S. dysenteriae* is introduced into *E. coli*. These hybrids grow very slowly on minimal-glucose unless tryptophan is provided. Anthranilic acid will also appreciably stimulate growth, suggesting that it is the conversion of chorismate to anthranilate that is impaired in the tryptophan pathway (8). We describe here experiments to determine the nature and location of the alterations leading to decreased function of the *S. dysenteriae trp* operon.

MATERIALS AND METHODS

Bacterial strains. All parental bacterial strains are from the collection of C. Yanofsky. The Sh16 strain of *S. dysenteriae* came originally from S. E. Luria. The *E. coli* strains used are derivatives of W3110 with the following exception: W1485 wild type; strain *Δ[tonB trpAE8]* (from W3101). All of the *E. coli* strains originated with K-12. Hybrid strains generated during the course of this work are listed in Table 1.

Growth media. Our minimal medium is that described by Vogel and Bonner (42). Liquid minimal medium contained 1.0% glucose. Tryptophan was supplemented at 50 μg/ml. In some experiments the minimal medium also contained 100 μg of phenylalanine and tyrosine per ml and 1 μg of *p*-aminobenzoic acid and *p*-hydroxybenzoic acid per ml (TPV minimal). Solid minimal medium contained 0.2% glucose and 1.5% agar (Difco). Where noted, solid media also contained 30 μg of cysteine per ml and 0.5% acid casein hydrolysate (ACH), which supplies all the amino acids except tryptophan. Tryptophan or tryptophan precursors were added to solid media at the following concentrations: tryptophan, 20 μg/ml; indole, 10 μg/ml; anthranilic acid, 30 μg/ml. L-broth and L-agar were prepared according to Lennox (26).

TABLE 1. *List of strains generated in this study*

Strain designation	Properties[a]
SDEC11	*S. dysenteriae-E. coli* transduction hybrid: partial Trp auxotroph
SDEC11TP	Faster growing Trp⁺ revertant of SDEC11
SDEE1	SDEC11 by *trpE9829-9851* transductant: *trpP(E) trpE⁺*
SDEE2	SDEC11 by *trpE9829-9851* transductant: *trpP(S) trpE⁺*
SDEE3	SDEC11 by *trpE9829-9851* transductant: *trpP(S) trpE* mutant
5MAR1	SDEC11 by *trpR cysB trpΔE5* transductant: *trpR Cys⁺ trpP(S) trpΔE5*
W5MAR1	W1485 by 5MAR1 transductant: Trp⁺ wild type
5MAR10	5MAR1 by *cysB* W3110 transductant: identical with 5MAR1

[a] *trpP(E)* and *trpP(S)* refer to the promoter regions of the *trp* operons of *E. coli* and *S. dysenteria*, respectively.

Transduction procedures. Lysates of P1*kc* and a clear mutant of P1 (P1*clr*) were made as described by Lennox (26). Transductions were performed basically according to Yanofsky and Lennox (46). P1*kc* was exposed to 5 min of ultraviolet irradiation at 41 ergs/s per mm² before infection and used at a multiplicity of 2 to 3 phage per bacterium. P1*clr* was not irradiated and was used at multiplicities ranging from 0.05 to 0.5. With either phage, cells were centrifuged after the 20-min adsorption period and resuspended in minimal medium or 0.1 M sodium citrate, pH 7.0. Appropriate dilutions of the resuspensions were plated on selective media.

Selection of *S. dysenteriae-E. coli* hybrids. P1*clr* grown on *S. dysenteriae* was used to transduce *E. coli cysB Δ[tonB trpAE12]*, in which the entire *trp* operon is deleted. Before the transduction, the recipient bacteria were incubated at 50 C for 20 min, a treatment designed to destroy a possible heat-labile DNA restriction system (33). A small number of Cys⁺ indole growers appeared when the cells were plated on minimal-glucose agar containing indole. All of these isolates grew very slowly on minimal-glucose agar or minimal-ACH agar. Strain SDEC11 is a typical representative of these hybrid recombinants.

Reversion tests. Spontaneous revertants were scored after plating 5×10^7 or 5×10^8 cells on minimal-ACH agar and incubating for 24 h at 37 C (for strain SDEC11) or 41 C (for strain SDEC11TP). Mutagen plates were made in the same way, with mutagens seeded as crystals, except that a loopful of ethyl methane sulfonate (EMS) was spotted, and ICR-191A was applied to a 1-cm-diameter sterile filter disk as a 0.05-ml aliquot of a 1-mg/ml solution. Revertants appeared as discrete colonies at the boundary of the zone of killing. All mutagen plates were wrapped in foil to prevent exposure to light.

Construction of *trpR* strains. The *cysB-trp* region of strains able to grow on indole was introduced by transduction into *trpR* (45) *cysB Δ[tonB trpAE97]*, a strain in which the entire *trp* operon is deleted. Cys⁺ indole growers were selected on minimal-glucose agar containing indole. Strain *trpR cysB trpΔE5* was constructed by crossing strain *trpΔE5* by strain *trpR cysB Δ[tonB trpAE97]*, selecting anthranilate growers, and screening for Cys⁻.

Growth of cells and enzyme assays. Cells for assay were grown in liquid minimal-glucose or TPV minimal-glucose, with or without tryptophan supplementation. Cultures of 100 ml were grown with vigorous swirling at 37 C. Turbidities were recorded using the red filter (no. 66) of a Klett-Summerson

colorimeter. Cells were harvested by centrifugation at densities of 5×10^8 to 7×10^8 cells/ml, corresponding to 100 Klett units. The cell pellets were washed with cold 0.85% saline and then frozen for subsequent assay. Crude extracts were prepared by thawing and resuspending frozen pellets in 1.0 ml of 0.1 M tris(hydroxymethyl)aminomethane (Tris), pH 7.8, and lysing by sonication. Cell debris was removed by centrifugation.

The NH_3-stimulated reaction of anthranilate synthetase (ASase) was assayed according to Zalkin and Kling (47), with the modifications of Jackson and Yanofsky (21). The reaction mixture consisted of 48 mM $(NH_4)_2SO_4$, 10 mM magnesium acetate, 1 mM dithiothreitol, and 50 mM triethanolamine hydrochloride, pH 8.9, in a final reaction volume of 0.5 ml. A 10-μl amount of a 5-mg/ml chorismic acid solution was added to each reaction just before assay.

The glutamine-stimulated activity of the ASase complex was assayed by the method of Ito and Crawford (18), except that the reaction mixture contained 5 mM L-glutamine, 2 mM magnesium acetate, 1 mM mercaptoethanol, 500 μg of bovine serum albumin per ml, and 50 mM Tris, pH 7.8, in a reaction volume of 0.5 ml. A 10-μl amount of the chorismate solution was added just before assay.

Phosphoribosyl anthranilate transferase (PRTase) was assayed according to Ito and Crawford (18), but the reaction mixture was altered to include 5 μM anthranilic acid, 1 mM phosphoribosyl pyrophosphate, 2 mM magnesium acetate, 1 mM dithiothreitol, and 25 mM Tris, pH 7.8, in a reaction volume of 0.5 ml.

The indole to tryptophan activity of tryptophan synthetase (TSase) was measured as described by Smith and Yanofsky (41). For all of the reactions, 1 unit of enzyme activity is defined as the conversion of 100 nmol of substrate in 20 min at 37 C; enzyme specific activities are given as units per milligram of protein. Protein was determined by the method of Lowry et al. (28).

***trp* mRNA determinations.** Cultures for labeling were grown to 3×10^8 to 5×10^8 cells/ml in liquid minimal-glucose supplemented with tryptophan. Aliquots of 10 ml were incubated for 30 s at 37 C with 100 μCi of [^{3}H]uridine (28 Ci/mmol). Incorporation was stopped by pouring cells over crushed, frozen overkill medium (7). Cell lysis, RNA purification, and hybridization were basically as described by Denney and Yanofsky (7), except that 10 μg of DNA per filter was used, and the 16-h hybridization at 67 C was followed by both a 10-min ribonuclease treatment at 37 C and a further 45-min incubation at 67 C in fresh hybridization buffer. Total labeled RNA was measured by precipitating with cold 5% trichloroacetic acid collecting the precipitate on filters. Dried filters were counted in a Packard liquid scintillation spectrometer. The DNA of transducing phage *ϕ80h pt trpEA* (5) and the control phage *ϕ80h* was purified according to Morse and Yanofsky (35).

Materials. The tetrasodium salt of phosphoribosyl pyrophosphate, pyridoxal phosphate, and 5-methyl- and 5-fluorotryptophan were all obtained from Sigma Chemical Co. 5-Methylanthranilate (5MA) was from K & K Laboratories. Mutagens were obtained from the following sources: EMS and hydroxylamine hydrochloride from Eastman Chemicals; 2-aminopurine and 5-bromodeoxyuridine from Calbiochem; and *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (NTG) from Aldrich Chemicals. ICR-191A was kindly provided by H. J. Creech. Chorismic acid was prepared by the method of Gibson (12). All other chemicals were of reagent grade.

RESULTS

Derivation of *S. dysenteriae-E. coli* hybrids. Transductant SDEC11 has the entire *trp* operon, the *cysB* locus, and perhaps the intervening segment of the chromosome from *S. dysenteriae* strain Sh16 recombined into *E. coli* strain W3110 *cysB Δ[tonB trpAE12]* (Fig. 1). The growth characteristics of all the transductants from the same cross were similar; strain SDEC11 is representative. Strain SDEC11 grew as well as *E. coli* W3110 on minimal-glucose agar if tryptophan, indole, or anthranilic acid was added, but had a doubling time in liquid minimal-glucose four times longer than strain W3110 if neither tryptophan nor one of its precursors was present. The capacity of anthranilate to increase the growth rate of SDEC11 implies, as Eisenstein and Yanofsky (8) proposed, that the tryptophan pathway in *S. dysenteriae* is partially blocked at a step before anthranilate. Since the defect is either within

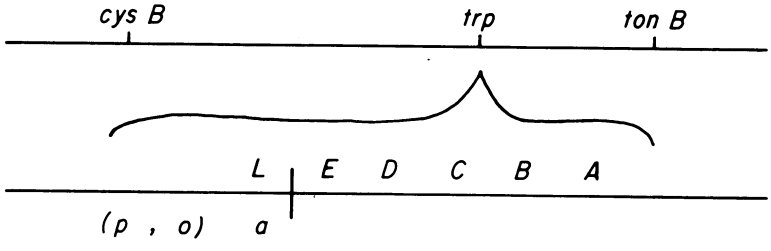

FIG. 1. *Diagram of the cysB-trp region of the E. coli chromosome. The promoter, operator, attenuator, and leader region are represented as p, o, a, and L, respectively, and the structural genes are lettered E through A. The map is not drawn to scale.*

the *trp* operon or cotransduced with it, ASase is the enzyme most likely to be altered.

Reversion of strain SDEC11. Tryptophan-independent revertants of strain SDEC11 arose spontaneously at a frequency of approximately 10^{-6}. Strain SDEC11TP, a representative of the revertant class that grows fastest on minimal-ACH agar, had a generation time in minimal-glucose liquid medium equivalent to that of W3110. On minimal-ACH agar incubated at 37 C, colonies of strain SDEC11TP were slightly smaller after 24 h than W3110 colonies. This difference was greatly exaggerated if incubation was at 41 C: strain SDEC11TP was essentially Trp^- at 41 C. This temperature sensitivity was reversed completely by adding anthranilic acid to the agar.

We tested for stimulation of reversion of strain SDEC11 with the acridine half-mustard frameshift mutagen ICR-191A and the base substitution mutagens NTG, EMS, hydroxylamine hydrochloride, 2-aminopurine, and 5-bromodeoxyuridine. Of these, only NTG increased the rate of reversion of strain SDEC11 to Trp^+.

In contrast, at the restrictive temperature at 41 C no spontaneous Trp^+ revertants of strain SDEC11TP per 10^9 cells plated were detected on minimal-ACH agar. However, NTG and EMS, the only mutagens used that cause transversions, did stimulate reversion of strain SDEC11TP to Trp^+ at 41 C. The other mutagens listed above were ineffective.

Constitutive *trp* enzyme levels in SDEC11 and SDEC11TP. The *trp* operons of strains W3110, SDEC11, and SDEC11TP were transduced into strain *trpR cysB Δ[tonB trpAE97]*, replacing the *cysB-tonB-trp* deletion region of the recipient. In these transductants we could measure constitutive *trp* enzyme production in strains with the same *trpR* allele. Enzyme assays on crude extracts of these strains revealed two major differences between strain W3110 and the hybrids (Table 2). ASase activity, in either the NH_3- or glutamine-stimulated reaction, was not detectable in the *trpR* strain with the SDEC11 *trp* operon. The *trpR* strain with the SDEC11TP *trp* operon had an ASase activity 2% that of *trpR* W3110. Our suspicion of a defective ASase was therefore corroborated by the enzyme data. The second difference was a severe reduction in the levels of enzymes specified by operator-distal genes in the *trp* operon from SDEC11. The PRTase and TSase β_2 specific activities of crude extracts of the *trpR* recombinant with the *trp* operon of SDEC11 were only 2 and 18%, respectively, those of *trpR* W3110 extracts. The effect of the reversion in strain SDEC11TP was profound in restoring the levels of distal gene products: PRTase and TSase β_2 increased to 34 and 108% of the *trpR* W3110 levels.

***trp* mRNA synthesis in the hybrids.** RNA-DNA hybridization was used to determine whether enzyme activities reflected the rate of *trp* mRNA synthesis. The good homology between the *trp* operons of *S. dysenteriae* and *E. coli* (7) enabled us to use the DNA of *ϕ80-trp* transducing phage carrying the *E. coli trp* operon to detect *trp* mRNA of strains SDEC11 and SDEC11TP. In the experiment summarized in Table 3, a 30-s [^{3}H]uridine pulse was used to measure RNA synthesis specifically. This labeling period is too short for degradation to take a significant toll of labeled mRNA (37), unless there is a polar mutation in the *S. dysenteriae trp* operon that decreases the mRNA levels corresponding to the distal region of the gene containing the mutation (14, 36). Strain *trpR* W3110 and the *trpR* derivative of SDEC11TP made *trp* message at approximately the same rate. However, a striking difference was seen between the *trpR* SDEC11 and *trpR* SDEC11TP recombinants. Although both strains grew with a 70-min generation time at 37 C in liquid minimal-glucose supplemented with tryptophan, the *trpR* derivative of

TABLE 2. *Constitutive enzyme specific activities in E. coli and S. dysenteriae-E. coli hybrids*[a]

Strain	ASase (NH_3)	ASase (gln)	PRTase	TSase β_2
trpR W3110	4.7	1.4	1.4	12.5
trpR SDEC11	0	0	0.032	2.2
trpR SDEC11TP		0.026	0.47	13.5

[a] Specific activities were measured in crude extracts from late log-phase cultures grown in TPV minimal-glucose + tryptophan.

TABLE 3. *Pulse-labeled trp mRNA from trpR E. coli and hybrid strains*[a]

Strain	Counts/min hybridized to DNA of *ϕ80h trpEA*	% *trpEA* mRNA
trpR W3110	8,400	0.458
trpR SDEC11	1,250	0.050
trpR SDEC11TP	7,900	0.343

[a] Cells growing exponentially on minimal-glucose + tryptophan were labeled with [^{3}H]uridine for 30 s. *trp* mRNA in purified RNA samples was determined by hybridization to 10 μg of alkali-denatured *ϕ80h trpEA* or *ϕ80h* DNA immobilized on nitrocellulose filters. The *ϕ80h* filters trapped only 50 counts/min with RNA from each culture. The counts from *ϕ80h* filters were subtracted from the *ϕ80h trpEA* counts before calculating the percent of *trpEA* message. Total pulse-labeled RNA was determined by counting 5% cold trichloroacetic acid precipitates of 10-μl aliquots of purified RNA.

SDEC11TP made seven times as much *trp* mRNA as did the *trpR* SDEC11 strain.

Deletion mapping of the proximal portion of the *S. dysenteriae trp* operon. Based on the enzyme data for strains with the *trp* operons of SDEC11 and SDEC11TP, we were convinced that *S. dysenteriae* has one or more alterations in *trpE* that are responsible for lowering the ASase activity. If these alterations were polar, they could account for the low distal enzyme levels and reduced amount of *trp* mRNA in strain SDEC11.

We mapped the *trpE* sites by crossing strain SDEC11 with a series of mutants with deletions entering *trpE* from the operator-distal side, selecting Trp$^+$ recombinants on minimal-ACH agar. All of the deletions used except Δ*[tonB trpAB17]*, whose left-hand end point is in *trpB*, are shown in Fig. 2. The frequency of Trp$^+$ recombinants was calculated from the ratio of transductants on minimal-ACH agar versus minimal-ACH agar supplemented with either indole or anthranilic acid (Table 4). All the deletion strains except the one containing Δ*[tonB trpAE1]* yielded some Trp$^+$ recombinants with strain SDEC11. Notably, strains with *trpΔLD102* and *trpΔLD2* (19), deletions that remove *trpE* and extend into the leader region that precedes *trpE* (3), still generated Trp$^+$ recombinants, albeit at a low frequency. These results demonstrate that a site external to the structural gene region is involved in the Trp$^-$ character of strain SDEC11; replacement of this site by its *E. coli* homologue establishes the Trp$^+$ phenotype. We designate this site *trpP*(S).

Deletion mapping also defined two sites in the distal half of *trpE* that influenced growth in the absence of exogenous tryptophan. The Trp$^+$ transductants were divided into three groups by the application of two criteria (see Table 4): the ability to grow on minimal-ACH agar at 41 C and the ability to grow on minimal-ACH agar containing 0.7 μg of DL-5-methyltryptophan (5MT) per ml at 37 C. This low concentration of 5MT inhibited the growth of strains partially blocked in tryptophan synthesis. The 5MT test is meaningless if the recipient has a *trpR* or *trpO^c* mutation. With these reservations for the interpretation of the 5MT screening, the three groups of transductants are comprised as follows: those growing neither at 41 C nor on 5MT; those growing at 41 C but not on 5MT; and those growing under both conditions. Deletions with end points to the left of the Δ*[tonB trpAE87]* left-hand terminus give only the first class of recombinant. Δ*[tonB trpAE87]* and Δ*[tonB trpAE8]* give the first two classes, and Δ*[tonB trpAE10]* and Δ*[tonB trpAB17]* give all three. Our model for the operator-proximal portion of the *S. dysenteriae trp* operon is diagramed in Fig. 3.

Enzyme levels in recombinants. The *cysB-trp* region of representative isolates of the different types of recombinants obtained with each deletion recipient were crossed into strain

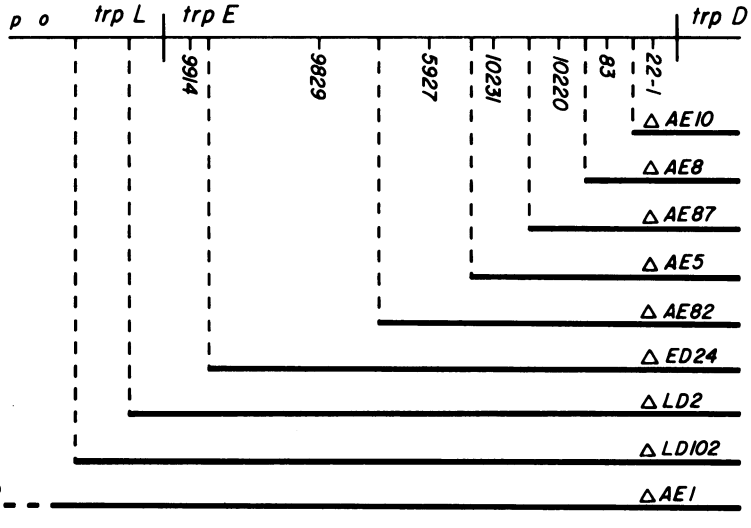

FIG. 2. *Genetic map of trp operon deletions. The horizontal bars represent the portion of the chromosome that is absent. Point mutations that delineate deletion end points (44) are shown at the top of the figure. All the deletions except Δ[tonB trpAE1] stop short of the operator and promoter. The left-hand end point of Δ[tonB trpAE1] is unknown, but the deletion removes all the leader and probably the promoter and operator as well. The detetions in strains Δ[tonB trpAE12] and Δ[tonB trpAE97] are similar to those in Δ[tonB trpAE1].*

trpR cysB Δ[tonB trpAE97]. We assayed *trp* enzymes in the resulting *trpR* derivatives. The three major patterns of enzyme activity observed are presented in Table 5. These patterns correlate with the three groups of transductants classified according to growth phenotype. Class I produces high levels of ASase and distal enzymes and grows at 41 C and on 5MT in its $trpR^+$ form; this class is similar to strain W3110. Class II produces intermediate levels of ASase and high levels of distal enzymes, and $trpR^+$ derivatives grow at 41 C but not on 5MT. Class III produces low levels of ASase and high distal enzyme levels, and the $trpR^+$ form does not grow at 41 C or on 5MT; this class behaves like strain SDEC11TP. These enzyme data support our model for the structure of the *S. dysenteriae trp* operon.

TABLE 4. *Frequency of tryptophan prototrophs among transductants of SDEC11 into E. coli trp deletion mutants*

Transduction recipient	min⁺/ind⁺[a] (or min⁺/ant⁺)	Growth at 41 C[b]	Growth on minimal-ACH[b] + 0.7 μg of 5MT/ml
trpΔAB17	0.23	+	+
trpΔAE10	0.13	+	+
trpΔAE8	0.092	+	0
trpΔAE87	0.075	+	0
trpΔAE5	0.046	0	0
trpΔAE82	0.021	0	0
trpΔAE1	<0.002	NA[c]	NA
trpR trpΔED24	0.035	0	+
trpΔLD2 trpΔA229	0.0089	0	0
trpOc2 trpΔLD102 trpΔA229	0.0059	0	+

[a] Ratio of the number of transductants per milliliter of the infection mixture growing on the restrictive (minimal-glucose [min⁺]) and nonrestrictive (indole [ind⁺]- or anthranilate [ant⁺]-containing) media after 24 h at 37 C. For recipients that could already grow on indole, the nonrestrictive plates contained anthranilic acid instead of indole. Frequencies were averaged for separate experiments.

[b] +, One or more of the transductants could grow at 41 C or on 5MT; 0, none of the transductants could grow.

[c] NA, None available.

Promoter region of the *S. dysenteriae trp* operon also reduces enzyme levels in the *E. coli trp* operon. We wanted to ascertain whether *trpP*(S), the *trp* operon promoter region of *S. dysenteriae*, acts independently of any peculiarities of the *S. dysenteriae* structural genes. The cross shown in Fig. 4 was utilized to generate recombinants in which the promoter, operator, leader, and proximal half of *trpE* were of *S. dysenteriae* origin and the remainder of the *trp* structural genes from *E. coli*. Trp⁺ transductants were selected on minimal-ACH agar and then screened for growth at 41 C and on 5MT. We were compelled to identify recombinants of the genotype we sought *[trpP*(S), $trpE^+$*]* by measuring enzyme activities of isolates with different growth properties.

The strains designated SDEE1, SDEE2, and SDEE3 in Table 6 exhibited the three patterns of enzyme activity found among *trpR* derivatives of the Trp⁺ recombinants from the cross of strain SDEC11 by strain *trpE9829-9851*. Strain SDEE1 had enzyme levels indistinguishable from those of wild-type *E. coli* strain W3110 (see Table 2). Strain SDEE2 showed the uniform reduction in all enzyme levels we anticipated for *trpP*(S) $trpE^+$. Strain SDEE3 seemed to have *trpP*(S) and either the $trpE_x$ or $trpE_y$ site from SDEC11.

The *trpP*(S) phenotype persists in a strain carrying *trpΔE5*. The *trpP*(S) phenotype of strain SDEE2 may depend on the interaction of the *trpP*(S) site and the proximal region of *trpE*, both of which came from strain SDEC11. We examined this possibility by constructing a strain in which *trpP*(S) occurs in an operon with *trpΔE5* (15). The *trpΔE5* deletion lies entirely within *trpE* and removes most of the gene. It recombines weakly with the two most operator-proximal *trpE* point mutations,

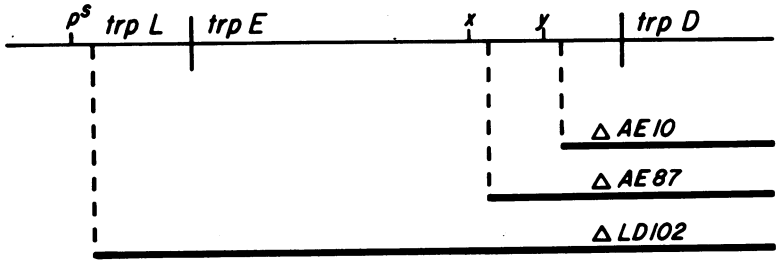

FIG. 3. *Schematic model for the proximal portion of the S. dysenteriae trp operon. Three sites are indicated: p^s[trpP(S)] is a pleiotropic site that decreases expression of the entire operon; X and Y are two sites within trpE that contribute to the inactivation of S. dysenteriae ASase. The most intrusive deletions recombining with each of the three mutations are shown below the chromosome.*

TABLE 5. *Enzyme specific activities of prototrophic transductants from crosses of SDEC11 by various deletion mutants*[a]

Recipient	Recombinant class[b]	ASase (NH_3)	PRTase	TSase β_2	No. of isolates
trpΔAE10	I	3.88 ± 0.88[c]	0.89 ± 0.22	14.7 ± 0.89	7
	II	1.29 ± 0.40	0.67 ± 0.24	15.8 ± 0.81	6
	III	0.21 ± 0.04	0.38 ± 0.13	15.0 ± 0.78	2
trpΔAE8	II	1.47 ± 0.21	0.61 ± 0.15	16.3 ± 0.54	8
trpΔAE87	II	1.43 ± 0.19	0.76 ± 0.15	16.3 ± 1.2	9
	III	0.31 ± 0.06	0.56 ± 0.26	15.8 ± 2.1	3
trpΔAE5	III	0.25 ± 0.07	0.66 ± 0.25	19.0 ± 2.0	3
trpΔAE82	III	0.44	0.81	18.6	1
trpΔED24	III	0.19 ± 0.05	0.34 ± 0.11	15.9 ± 2.8	3
trpΔLD2	III	0.45	0.87	20.0	1
trp0[c]2 trpΔLD102[d]	III	0.066 ± 0.031	0.16 ± 0.05	11.2 ± 2.2	5

[a] Specific activities were measured in crude extracts of *trpR* strains grown in TPV minimal-glucose + tryptophan.

[b] Recombinant classes are based on patterns of enzyme activity: I, high ASase, high distal enzyme (similar to strain W3110); II, intermediate ASase, high distal enzyme; III, low ASase, high distal enzyme (similar to strain SDEC11TP). Representatives of each recombinant class found in every cross are included, except class III recombinants with *trpΔAE8*.

[c] Standard deviations calculated for all recombinant classes with two or more members.

[d] *trp0[c]2 trpΔLD102* transductants were not made *trpR* because we wanted to test whether they still carried the *trp0[c]2* mutation.

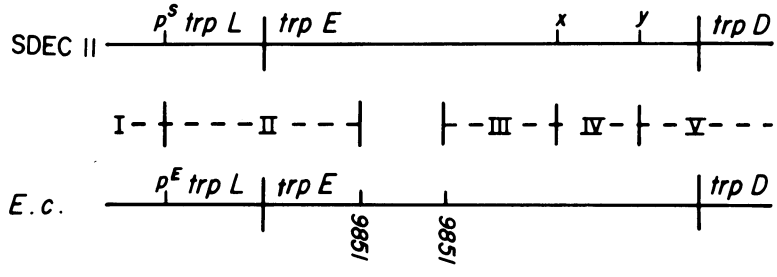

FIG. 4. *Diagram of the cross between strain SDEC11 (top line) and the double nonsense mutant trpE9829-9851 (bottom line), in which Trp⁺ recombinants were selected. Different patterns of double or quadruple crossovers within the five chromosomal regions demarked by roman numerals may produce recombinants with distinctive phenotypes. The following three crossovers are represented among Trp⁺ recombinants we have examined: II-III (SDEE1), I-III (SDEE2), and either I-IV or I-III-IV-V (SDEE3). p^S is from trpP(S); p^E is from trpP(E).*

trpE9777 and *trpE9914*, which have been mapped by RNA sequencing 11 to 15 and 25 nucleotides, respectively, from the initiation codon for the *trpE* polypeptide (4).

Strain SDEE2 was crossed with strain *trpR cysB trpΔE5*, and Cys⁺ transductants were selected on minimal-glucose agar containing tryptophan. Trp⁻ colonies were identified by replication to minimal-glucose agar and further screened by replication to minimal-glucose agar supplemented with anthranilate and to TMF minimal-glucose agar. TMF agar contains 1 μg of L-tryptophan per ml, 100 μg of 5-methylanthranilate (5MA) per ml, and 20 μg of DL-5-fluorotryptophan per ml. TMF completely inhibits the growth of *trpE* mutants that make high constitutive levels of the *trpD* through *trpA* gene products, because 5MA is converted to the toxic tryptophan analogue 5MT. The low concentration of tryptophan in TMF supports the growth of Trp auxotrophs that do not convert large amounts of 5MA to 5MT. 5-Fluorotryptophan enhances the sensitivity of cells to 5MA.

Strain *trpR trpΔE5* would not grow on TMF agar. We anticipated that the low enzyme levels associated with *trpP*(S) would render strain *trpR trpΔE5* resistant to 5MA. Of 1,100 Trp⁻ isolates, only two grew on anthranilate and on TMF. One of these, strain *trpR* 5MAR1, had the enzyme levels expected for a *trpR trpP*(S) *trpΔE5* bacterium (Table 7). Assays with control strains *trpR* W5MAR1 and *trpR* 5MAR10 indicated that the low *trp* enzyme levels in *trpR*

5MAR1 were caused by a site linked to the *trp* operon, presumably *trpP*(S). Thus, we conclude that the *trpP*(S) phenotype prevails in the absence of any substantial portion of *trpE*.

Mapping of *trpP*(S) relative to *trpO*^{c}2. The *trpO*^{c}2 mutation leads to constitutive expression of the *E. coli trp* operon at 70 to 80% of the *trpR* level. *trpO*^{c}2 recombines with *trpΔLD102* at a significant frequency, and *trpO*^{c}2 *trpΔLD102* double mutants have been made (20). Strain *trpO*^{c}2 *trpΔLD102* served as a recipient in a transduction cross with strain SDEC11 as donor. We selected Trp$^+$ recombinants on minimal-ACH agar. Figure 5 is a schematic diagram of this cross.

This selection requires that *trpE* and *trpD* from strain SDEC11 are introduced, and that the *trpP*(E) allele (the promoter region of the *trp* operon of *E. coli*) from the recipient is retained. *trpO*^{c}2 can be scored as an unselected marker, because the *trp* operon of strain SDEC11 is known to be *trpO*$^+$ (32). Trp$^+$ transductants were streaked to minimal-ACH agar (incubated at 41 C) and minimal-ACH agar containing 0.7 μg of 5MT per ml (incubated at 37 C). Recombinants having the *trpO*^{c}2 mutation should grow on 5MT; *trpO*$^+$ recombinants should not. Neither *trpO*^{c}2 nor *trpO*$^+$ recombinants should grow at 41 C, since both will have the temperature-sensitive phenotype conferred by *S. dysenteriae trpE*. The ratio of 5MT-sensitive to -resistant colonies presumably represents the percentage of recombinants between *trpΔLD102* and *trpP*(S) that are also recombinant for *trpO*^{c}2 and *trpP*(E).

Table 8 shows that *trpO*^{c}2 was retained with *trpP*(E) in 80% of the transductants. We measured enzyme levels for tryptophan-grown cultures of transductants scored phenotypically as *trpO*^{c} or *trpO*$^+$. The enzyme data given in Table 9 confirm our assignments of *trpO*^{c} and *trpO*$^+$. The high frequency of *trpP*(E) *trpO*^{c}2 transductants indicates that *trpP*(E) is located either between *trpO*^{c}2 and the left-hand terminus of *trpΔLD102* or closely to the left of *trpO*^{c}2.

Unexpectedly, 3 out of 30 transductants grew well at 41 C. We can not satisfactorily explain their origin.

TABLE 6. *Enzyme specific activities of Trp$^+$ recombinants from SDEC11 × trpE9829-9851 cross*[a]

Recombinant	ASase (gln)	PRTase	TSaseβ_2
trpR SDEE1	1.62	1.37	11.9
trpR SDEE2	0.13	0.098	1.67
trpR SDEE3	0.010	0.040	1.76

[a] Specific activities measured in crude extracts of *trpR* derivatives of recombinant strains grown in TPV minimal-glucose + tryptophan.

TABLE 7. *Enzyme specific activities of trpR trpΔE5 and the SDEC11 × trpR trpΔE5 recombinant 5MAR1*[a]

Strain	ASase (NH_3)	PRTase	TSaseβ_2
trpR trpΔE5	0	1.66	15.6
trpR 5MAR1	0	0.11	1.4
trpR W5MAR1	4.6	1.27	13.2
trpR 5MAR10[b]	0	0.17	2.0

[a] Specific activities were measured in crude extracts of cultures grown in TPV minimal-glucose + tryptophan. 5MAR1 is a strain in which the 5MAR1 *trp* operon is replaced by the W1485 *trp* operon.

[b] Strain in which the *cysB-trp* region of W3110 is replaced by the corresponding region of 5MAR1.

DISCUSSION

Strain SDEC11 is a transduction hybrid in which the entire tryptophan operon of strain Sh16 of *S. dysenteriae* has replaced the *trp* operon of *E. coli* W3110. Strain SDEC11 is a virtual Trp auxotroph; normal growth is restored by addition of tryptophan, indole, or anthranilic acid. We have identified two sites, designated *trpE*$_x$ and *trpE*$_y$ in *trpE* of the hybrid, that result in partial inactivation of the *trpE* polypeptide. We have also discovered a pleiotropic site [*trpP*(E)] in the promoter-operator region of the *trp* operon that reduces both *trp* mRNA synthesis and *trp* enzyme levels 5- to 10-fold relative to W3110 or a Trp$^+$ revertant of SDEC11, strain SDEC11TP. The pleiotropy of *trpP*(S), its high spontaneous reversion rate, and the failure of mutagens other than NTG to enhance reversion remind us of the insertion mutations found in the galactose operon of *E. coli* (16, 39), in the lactose operon (10), and in bacteriophage lambda (17).

The combination of *trpP*(S) and the low activity of the *S. dysenteriae* ASase is required to severely limit growth in the absence of tryptophan or tryptophan precursors. SDEC11TP, which apparently arose from SDEC11 by a spontaneous reversion of *trpP*(S), has the same growth rate as *E. coli* W3110 in minimal-glucose liquid medium at 37 C, even though strain SDEC11TP still makes the *S. dysenteriae trpE* protein. At 41 C, however, SDEC11TP requires anthranilate for good growth, suggesting that the *S. dysenteriae* ASase is temperature sensitive. Recombinants having the W3110 *trp* promoter-operator region of strain W3110 in tandem with the *trpE*$_x$ and *trpE*$_y$ sites of *S. dysenteriae trpE* behave like SDEC11TP. Conversely, in strain SDEE2 the *trpP*(S) site occurs in a *trp* operon containing an *S. dysenteriae-E. coli* recombinant *trpE* that codes for a fully active *trpE* protein. Strain SDEE2 is Trp$^+$ at

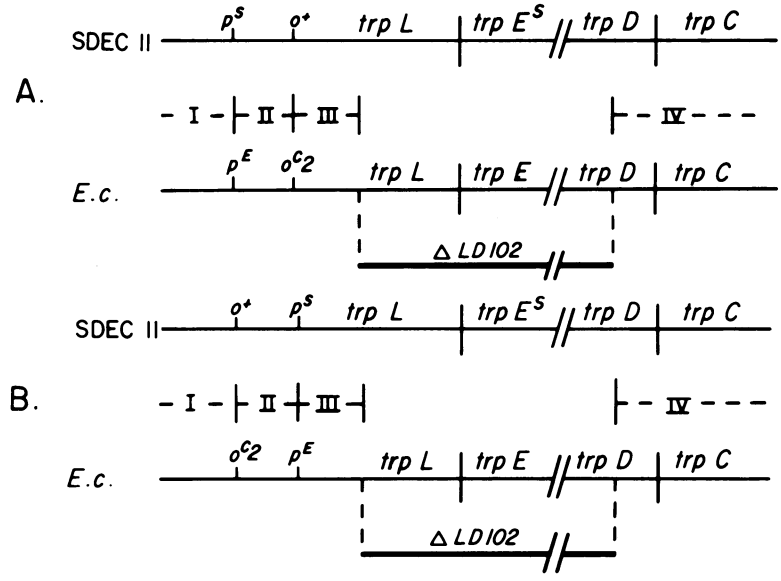

FIG. 5. *Cross between SDEC11 (top line) and [trpO^{c}2 trpΔLD102] (bottom line) diagramed for both possible orders of p^S[trpP(S)] and trpO^{c}2. (A) For the order p^S-O^{c}2-E, the ratio of p^E[trpP(E)] o$^+$ E^S/p^E o^c E^S among Trp$^+$ recombinants should equal the ratio of region II-IV to region III-IV double crossovers. (B) For the order O^{c}2-p^S-E, the ratio of p^E o$^+$ E^S/p^E o^c E^S should equal the ratio of I-II-III-IV quadruple crossovers to III-IV double crossovers.*

TABLE 8. *trp$^+$ transductants from the cross of SDEC11 by trpO^{c}2 trpΔLD102*

Genotype	No. of isolates	Percent	Colony size[a]		
			37 C	41 C	5MT[b]
trpP(E)[c] *trpO^{c}2 trpE*(S)[d]	23	77	Large	Tiny	Medium
trpP(E) *trpO$^+$ trpE*(S)	4	13	Large	Tiny	None
trpP(E) *trpO^{c}2 trpE*(S)	1	3	Large	Medium	Large
trpP(E) *trpO$^+$ trpE*(S)	2	7	Large	Medium	Small

[a] Determined from streaks on minimal-ACH agar after 48 h.
[b] Minimal-ACH agar containing 0.7 μg of 5MT per ml.
[c] *trpP*(E) is the promoter region of *E. coli*.
[d] *trpE*(S) and *trpE*(E) are the corresponding structural genes of *S. dysenteriae* and *E. coli*.

either 37 or 41 C, although strain *trpR* SDEE2 produces all *trp* enzymes at 10 to 15% of the level of strain W3110 *(trpR)*.

Although our data do not order *trpP*(S) relative to *trpO^{c}2*, we have shown that *trpP*(S) is tightly linked to *trpO^{c}2*. In light of this fact, one interpretation of our data is that *trpP*(S) represents a modification, perhaps an insertion, within the *trp* promoter that reduces message initiations without grossly disturbing normal regulation of the operon. However, we can not rule out the possibility that *trpP*(S) may act as a leaky transcription stop (5). Since *trpP*(S) recombines with *trpΔLD102*, *trpP*(S) cannot directly affect a site analogous to the transcription attenuator that occurs in the distal third of the leader region of the wild-type *E. coli trp* operon (1), since *trpΔLD102* removes the attenuator site.

The regulatory behavior of the *S. dysenteriae-E. coli* transduction hybrids reported by Eisenstein and Yanofsky (8) is conveniently incorporated into our model for the *S. dysenteriae trp* operon. They found four types of Trp$^+$ recombinants generated by crossing strain Sh/S of *S. dysenteriae* into *E. coli trp* mutants. One class of hybrids was composed of slow growers whose growth was stimulated by anthranilic acid. This class produced high derepressed levels of TSase when grown in tryptophan-free liquid minimal-glucose medium. These are probably like strain SDEC11. The other three classes grew as fast as wild-type *E. coli* on minimal-glucose medium and were not significantly stimulated by anthranilate, but were distinguishable by the extent to which they derepressed when they were grown without tryptophan: there were high, intermediate, and

TABLE 9. *Enzyme specific activities of trp0^c and trp0^+ transductants from the SDEC11 × trp0^c2 trpΔLD102 cross*[a]

Strain	ASase (NH_3)	PRTase	TSase β_2
W3110	0.05–0.10	0.046	0.56
trpR W3110	4.7	1.4	12.5
trpP(E) *trp0^+* *trpE*(S)[b]	0	0.005	0.59
trpP(E) *trp0^c2* *trpE*(S)	0.066	0.16	10.1

[a] Specific activities were measured in crude extracts of cultures grown in TPV minimal-glucose + tryptophan.

[b] See Table 8, footnotes *c* and *d*, for description of symbols.

low *trp* enzyme producers. Derepression will be greatest for hybrids in which tryptophan synthesis is least efficient (38, 43). We believe that the three types of anthranilate nonresponders they described correlate with our three major classes of Trp$^+$ transductants from the crosses of strain SDEC11 by *E. coli trp* deletion mutants.

The reversion of naturally occurring auxotrophic characters has been observed in several bacterial groups. The requirements for sulfur-containing amino acids in *Pasturella pestis* (9) and for a number of amino acids in various *Salmonella* strains (25) are reversible by spontaneous mutation. More recently, it was shown that seven of twelve amino acid requirements and three of four vitamin requirements of *Lactobacillus casei* can be eliminated by mutagenically induced reversions (34). Thus, a growing body of evidence suggests that in many bacteria, genes coding for the enzymes of dysfunctional biochemical pathways are often retained essentially intact. Such cryptic genes may be free to undergo further mutation and thereby serve an important role in the generation of evolutionary diversity (24, 25).

Luria (29) has discussed the mechanism by which the ability to ferment lactose has been lost in *S. dysenteriae*. The *lacY* and *lacA* genes, coding for the permease and transacetylase, have apparently been deleted, and the *lacZ* gene contains a number of mutations that leave the *S. dysenteriae* β-galactosidase only partially active (40). The *lacI* gene, however, specifies a repressor functionally indistinguishable from the *E. coli lac* repressor. Luria suggests that the initial loss of lactose catabolism resulted from the permease deletion, and that mutations accumulated in the *lacZ* gene after the substrates for β-galactosidase could no longer be transported into the cell. A functional *lacI* product was conserved because repression prevented the synthesis of useless β-galactosidase. There are obvious parallels between the *lac* system and the situation we describe here for the *trp* operon.

The functional loss of these two pathways may confer a selective advantage to *S. dysenteriae*, although the existence of good repression in both the *trp* and *lac* operons would appear to minimize the importance of further reductions in enzyme levels or catalytic functions (13). Nevertheless, Zamenhof and Eichhorn (48), working with *Bacillus subtilis*, and D. E. Dykhuizen (Ph.D. thesis, Univ. of Chicago, 1971), working with *E. coli*, have reported experiments in which Trp mutants have a competitive advantage over isogenic Trp$^+$ strains in chemostats supplemented with tryptophan. In strict terms of energy conservation, the most efficient evolutionary solution would appear to be the deletion of superfluous genes (24), yet this has not been the fate of the *S. dysenteriae trp* operon or *lacZ* gene. Of course, such deletions may be selected against on other grounds. The reasons why any type of inactivating mutations would be selected in a normally regulated operon remain unclear, however, and it is certainly possible that the alterations observed in the *trp* and *lac* operons of *S. dysenteriae* have occurred randomly in the absence of a requirement for tryptophan biosynthesis and lactose utilization.

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LITERATURE CITED

1. **Bertrand, K., L. Korn, F. Lee, T. Platt, C. L. Squires, C. Squires, and C. Yanofsky.** 1975. New features of the regulation of the tryptophan operon. Science 189:22–26.
2. **Brenner, D. J., G. R. Fanning, F. J. Skerman, and S. Falkow.** 1972. Polynucleotide sequence divergence among strains of *Escherichia coli* and closely related organisms. J. Bacteriol. 109:953–965.
3. **Bronson, M. J., C. L. Squires, and C. Yanofsky.** 1973. Nucleotide sequences from tryptophan messenger RNA of *Escherichia coli*: the sequence corresponding to the amino-terminal region of the first polypeptide specified by the operon. Proc. Natl. Acad. Sci. U.S.A. 70:2335–2339.
4. **Bronson, M. J., and C. Yanofsky.** 1974. Characterization of mutations in the tryptophan operon of *Escherichia coli* by RNA nucleotide sequencing. J. Mol. Biol. 88:913–916.
5. **de Crombrugghe, B., S. Adhya, M. Gottesman, and L. Pastan.** 1973. Effects of rho on transcription of bacterial operons. Nature (London) New Biol. 241:260–264.
6. **Deeb, S. S., K. Okamoto, and B. D. Hall.** 1967. Isola-

tion and characterization of non-defective transducing elements of bacteriophage $\phi 80$. Virology 31:289-295.
7. **Denney, R. M., and C. Yanofsky.** 1972. Detection of tryptophan messenger RNA in several bacterial species and examination of the properties of heterologous DNA-RNA hybrids. J. Mol. Biol. 64:319-339.
8. **Eisenstein, R. B., and C. Yanofsky.** 1962. Tryptophan synthetase levels in *Escherichia coli, Shigella dysenteriae,* and transduction hybrids. J. Bacteriol. 83:193-204.
9. **Englesberg, E., and L. Ingraham.** 1957. Meiotropic mutants of *Pasturella pestis* and their use in the elucidation of nutritional requirements. Proc. Natl. Acad. Sci. U.S.A. 43:369-372.
10. **Fiandt, M., W. Szybalski, and M. H. Malamy.** 1972. Polar mutations in *lac, gal,* and phage lambda consist of a few IS-DNA sequences inserted in either orientation. Mol. Gen. Genet. 119:223-231.
11. **Franklin, N., and S. E. Luria.** 1961. Transduction by bacteriophage P1 and the properties of the *lac* genetic region in *E. coli* and *S. dysenteria*. Virology 15:299-311.
12. **Gibson, F.** 1970. Preparation of chorismic acid. Methods Enzymol. 17A:362-364.
13. **Hegeman, G. D., and S. L. Rosenberg.** 1970. The evolution of bacterial enzyme systems. Annu. Rev. Microbiol. 24:429-462.
14. **Hiraga, S., and C. Yanofsky.** 1972. Hyper-labile messenger RNA in polar mutants of the tryptophan operon of *Escherichia coli*. J. Mol. Biol. 72:103-110.
15. **Hiraga, S., and C. Yanofsky.** 1972. Normal repression in a deletion mutant lacking almost the entire operator-proximal gene of the tryptophan operon of *Escherichia coli*. Nature (London) New Biol. 237:47-49.
16. **Hirsch, H. J., H. Saedler, and P. Starlinger.** 1972. Insertion mutations in the control region of the galactose operon of *E. coli*. II. Physical characterization of the mutations. Mol. Gen. Genet. 115:266-276.
17. **Hirsch, H., P. Starlinger, and P. Brachet.** 1972. Two kinds of insertions in bacterial genes. Mol. Gen. Genet. 119:191-206.
18. **Ito, J., and I. P. Crawford.** 1965. Regulation of the enzymes of the tryptophan pathway in *Escherichia coli*. Genetics 52:1303-1316.
19. **Jackson, E. N., and C. Yanofsky.** 1972. Internal deletions in the tryptophan operon of *Escherichia coli*. J. Mol. Biol. 71:149-161.
20. **Jackson, E. N., and C. Yanofsky.** 1973. The region between the operator and the first structural gene of the tryptophan operon of *Escherichia coli* may have a regulatory function. J. Mol. Biol. 76:89-101.
21. **Jackson, E. N., and C. Yanofsky.** 1974. Localization of two functions of the phosphoribosyl anthranilate transferase of *Escherichia coli* to distinct regions of the polypeptide chain. J. Bacteriol. 117:502-508.
22. **Keusch, G.** 1974. Ecology of the intestinal tract. Nat. Hist. 83:70-79.
23. **Koch, A. L.** 1971. The adaptive responses of *E. coli* to a feast and famine existence. Adv. Microb. Physiol. 6:147-217.
24. **Koch, A. L.** 1972. Enzyme evolution. I. The importance of untranslatable intermediates. Genetics 72:297-316.
25. **Lederberg, J.** 1947. The nutrition of *Salmonella*. Arch. Biochem. 13:287-290.
26. **Lennox, E. S.** 1955. Transduction of linked genetic characters of the host by bacteriophage P1. Virology 1:190-206.
27. **Li, S., and C. Yanofsky.** 1972. Amino acid sequences of fifty residues from the amino termini of the tryptophan synthetase α chains of several Enterobacteria. J. Biol. Chem. 247:1031-1037.
28. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193:265-275.
29. **Luria, S. E.** 1965. On the evolution of the lactose utilization gene system in Enteric bacteria, p. 357-358. *In* V. Bryson and H. J. Vogel (ed.), Evolving genes and proteins. Academic Press Inc., New York.
30. **Luria, S. E., and J. W. Burrows.** 1957. Hybridization between *Escherichia coli* and *Shigella*. J. Bacteriol. 74:461-476.
31. **McCarthy, B. J., and E. T. Bolton.** 1963. An approach to the measurement of genetic relatedness among organisms. Proc. Natl. Acad. Sci. U.S.A. 50:156-164.
32. **Manson, M. D., and C. Yanofsky.** 1976. Tryptophan operon regulation in interspecific hybrids of enteric bacteria. J. Bacteriol. 126:679-689.
33. **Mojica-a, T., and R. B. Middleton.** 1971. Fertility of *Salmonella typhimurium* crosses with *Escherichia coli*. J. Bacteriol. 108:1161-1167.
34. **Morishita, T., T. Fukada, M. Shirota, and T. Yura.** 1974. Genetic basis of nutritional requirements in *Lactobacillus casei*. J. Bacteriol. 120:1078-1084.
35. **Morse, D. E., and C. Yanofsky.** 1969. A transcription initiating mutation within a structural gene of the tryptophan operon. J. Mol. Biol. 41:317-328.
36. **Morse, D. E., and C. Yanofsky.** 1970. Polarity and the degradation of mRNA. Nature (London) 224:329-331.
37. **Mosteller, R. D., J. K. Rose, and C. Yanofsky.** 1970. Transcription initiation and degradation of *trp* mRNA. Cold Spring Harbor Symp. Quant. Biol. 35:461-466.
38. **Murgola, E. J., and C. Yanofsky.** 1974. Selection for new amino acids at position 211 of the tryptophan synthetase α chain of *Escherichia coli*. J. Mol. Biol. 86:775-784.
39. **Saedler, H., J. Besemer, B. Kemper, B. Rosenwirth, and P. Starlinger.** 1972. Insertion mutations in the control region of the *gal* operon of *E. coli*. I. Biological characterization of the mutations. Mol. Gen. Genet. 115:258-265.
40. **Sarkar, S.** 1966. Properties and regulation of the β-D-galactosidase in *Shigella dysenteriae* and *Escherichia coli-Shigella dysenteriae* hybrids. J. Bacteriol. 91:1477-1488.
41. **Smith, O. H., and C. Yanofsky.** 1962. Enzymes involved in the biosynthesis of tryptophan. Methods Enzymol. 5:794-806.
42. **Vogel, H. J., and D. M. Bonner.** 1956. Acetylornithinase of *Escherichia coli*: partial purification and some properties. J. Biol. Chem. 218:97-106.
43. **Yanofsky, C.** 1971. Protein structure and evolution, p. 191-206 *In* M. L. Marois (ed.), Proc. 2nd Int. Conf. Theor. Physics Biol. Centre National de la Recherche Scientifique, Paris.
44. **Yanofsky, C., V. Horn, M. Bonner, and S. Stasiowski.** 1971. Polarity and enzyme functions in mutants of the first three genes of the tryptophan operon of *Escherichia coli*. Genetics 69:409-423.
45. **Yanofsky, C., and J. Ito.** 1966. Nonsense codons and polarity in the tryptophan operon. J. Mol. Biol. 21:313-334.
46. **Yanofsky, C., and E. S. Lennox.** 1959. Transduction and recombination study of linkage relationships among the genes of the tryptophan operon of *Escherichia coli*. Virology 8:425-447.
47. **Zalkin, H., and D. Kling.** 1968. Anthranilate synthetase. Purification and properties of component I from *Salmonella typhimurium*. Biochemistry 7:3566-3573.
48. **Zamenhof, S., and H. H. Eichhorn.** 1967. Study of microbial evolution through loss of biosynthetic functions: establishment of defective mutants. Nature (London) 216:456-458.