REGULATORY MUTANTS OF THE TRYPTOPHAN OPERON OF *SALMONELLA TYPHIMURIUM*[1]

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Received February 2, 1970

THE tryptophan (*trp*) operon of *Salmonella typhimurium* consists of five structural genes regulated as a unit by tryptophan (BLUME and BALBINDER 1966; BAUERLE and MARGOLIN 1966a, 1966b; BLUME, WEBER and BALBINDER 1968). The biosynthetic reactions involved and the order of the genes (see Figure 1), as well as the regulation of the *trp* operon have been shown to be identical in *Escherichia coli* (ITO and CRAWFORD 1965; MATSUSHIRO *et al.* 1965; YANOFSKY and LENNOX 1959). Regulatory mutants which permit constitutive synthesis of the tryptophan enzymes have been isolated in *E. coli* by selecting for strains which are resistant to 5-methyltryptophan, a structural analogue of tryptophan (COHEN and JACOB 1959; HIRAGA 1969). In this paper we deal with the isolation and characterization of regulatory mutants for the *trp* operon of *S. typhimurium*.

Constitutive mutants for the *trp* operon of Salmonella were obtained by selecting for resistance to either of two tryptophan analogues: 5-methyltryptophan or 6-fluorotryptophan, both of which inhibit bacterial growth. It has been known for some time that 5-methyltryptophan acts by inhibiting the activity of anthranilate synthetase (MOYED 1960). As we shall see, and as other workers have reported (ITO, HIRAGA and YURA 1969) this analogue as well as 6-fluorotryptophan is also capable of mimicking the role of tryptophan as a co-repressor. The regulatory mutants we have found have been classified into at least four different genetic groups, of which two correspond to regulatory mutations already described in *E. coli* and two belong to previously unreported classes.

MATERIALS AND METHODS

Nomenclature: The nomenclature used for the bacterial mutant strains is the one proposed by DEMEREC *et al.* (1966).

Bacterial strains: the *trp* mutants employed in this work have been previously described. (BLUME and BALBINDER 1966; BAUERLE and MARGOLIN 1966b; BALBINDER, BLUME, WEBER and TAMAKI 1968). Those listed in Table 1 were employed to isolate various regulatory mutations. Others were employed in mapping (see Figure 2). Strains SO-82 and SO-114 (Table 1) are anthranilic acid-utilizing revertants of the polar mutant *trpA49* and contain deletions in the *trpA* gene (BALBINDER *et al.* 1968). The various *supX* strains employed in mapping were obtained from P. MARGOLIN, and have also been described (MARGOLIN and BAUERLE 1966). The two strains

[1] Research supported by National Science Foundation grants GB-4903 and GB-7296.

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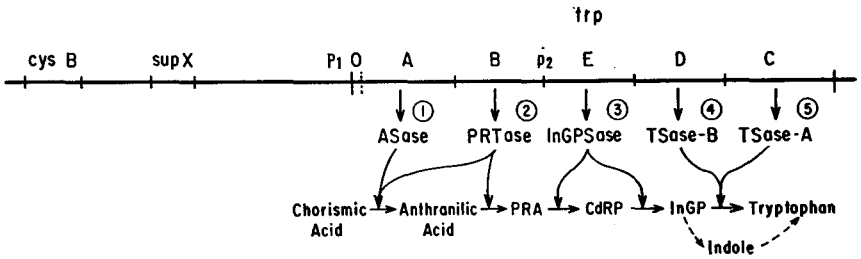

FIGURE 1.—The tryptophan (*trp*) operon of *Salmonella typhimurium*. The circled numbers refer to the sequence in which the products of the five *trp* structural genes participate in the biosynthesis of L-tryptophan. The capital letters denote the various *trp* genes. *trpA* codes for anthranilate synthetase (ASase) and *trpB* codes for phosphoribosyl transferase (PRTase), the components of a protein aggregate which carries out the first two steps of L-tryptophan biosynthesis. Free PRTase, however, can carry out the conversion of anthranilic acid to N-5′-phosphoribosyl anthranilate (PRA). Indoleglycerol phosphate synthetase (InGPSase), the *trpE* gene product catalyzes reactions three and four: PRA to 1–(O-carboxyphenylamino)-1-deoxyribulose-5-phosphate (CdRP) and this intermediate to indole-3-glycerol phosphate (InGP). Tryptophan synthetase (TSase) components B(β) and A(α) are required for the conversion of InGP to tryptophan. Mutants for the *trpA* gene are capable of utilizing anthranilic acid or indole, instead of tryptophan, as a growth factor. Mutants for the genes *trpB*, *trpE* or *trpC* cannot grow on anthranilic acid supplement but they will grow on indole. P1 represents the *trp* promoter and P2 the low efficiency "promoter-like" element first described by BAUERLE and MARGOLIN (1966a). *O* indicates the *trp* operator (*trpO*). Deletion of *supX* suppresses the mutation *leu500* (MUKAI and MARGOLIN 1963). *cysB* mutations lead to a requirement for cysteine.

TABLE 1

Strains used as sources of regulatory mutations

Strain	Genotype	Type of mutation	Polarity index	Source
....	*trpA49*	NS	97	LT7
....	*trpA81*	FS	95	LT2
....	*trpA111*	NS	83	LT2
....	*trpA47*	NS	86	LT7
....	*trpA8*	MS	0	LT7
SO-82	*trpA513*	DL	0	*trpA49*
SO-114	*trpA49 trpA512*	double mutant NS plus distal DL	<10	*trpA49*
SO-151	*cysB403 pro-24 met-365 purB210 pyrC197*	*trp*+ prototroph	..	LT2
....	*cysB403*	*trp*+ prototroph	..	LT2

Symbols: NS, nonsense mutation; FS, frameshift; MS, missense; DL, deletion.
The polarity index refers to the intensity of the polar effect of each NS and FS mutation. It is expressed as the percent reduction in the rate of synthesis of the TSase β and α components from the maximum (standard) rate shown by nonpolar mutants, under conditions of derepression (BLUME, WEBER and BALBINDER 1968).

at the bottom of Table 1 are *trp*+ prototrophs but carry other genetic markers as indicated. They are from the collection of the late DR. M. DEMEREC. The following multiple mutants were used in the construction of strains carrying various combinations of markers: *trpA8 cysB12*, *trpA8 trpC3 cysB12*, and *trpB883 cysB12*. The first two are also from the collection of DR. DEMEREC, the third one was obtained from DR. COLIN STUTTARD.

Culture media: Nutrient broth (Difco) was used as a routine complex medium. The defined minimal medium of VOGEL and BONNER (1956) supplemented with 0.005% acid hydrolyzed casein (SMITH and YANOFSKY 1962) and 0.2% glucose was used routinely (M medium). Difco agar (1.5%) was added when solid medium was required. Appropriate supplements were added when required, as will be indicated. The analogues 5-methyltryptophan (5MT) and 6-fluorotryptophan (6FT) were always added at a final concentration of 100 μg/ml. 5MT was obtained from K & K Rare Chemicals, Plainview, N. Y. and 6FT from Aldrich Chemical Company, Milwaukee, Wisconsin.

Genetic mapping: All genetic crosses were performed by transduction mediated by phage P22. The methods employed in the preparation and storage of transducing lysates have been described (BLUME and BALBINDER 1966). When used in crosses, analogue-resistance markers were always unselected. Recombinant colonies were suspended in 0.5 ml of sterile 0.9% saline and tested by streaking onto minimal agar supplemented with the proper analogue (see *Media*, above). Growth was scored after 24 and 48 hr of incubation at 37°C.

Induction of mutations: Some of the regulatory mutations were obtained following treatment of the bacteria with various chemical mutagens by the spot-test technique (BALBINDER 1962). Others were of spontaneous origin. Reversions to prototrophy were also induced using the spot-test method. The mutagens used are listed in the legend to Table 2.

Construction of merodiploids: The F′*trp* factor (F 71) of SANDERSON and HALL's (1970) strain SU-694 was transferred to *trpE95*, a strain carrying a deletion in *trpE*, and *trpE95/trpE95* homogenotes were isolated as indole-requiring segregants which were capable of transmitting the *trpE95* marker to a recipient carrying a deletion of the entire *trp* operon. These, in turn, were used as donors in crosses to *trpO trpA* mutants and phototrophic merodiploids were selected. The heterogenotic nature of the prototrophs was ascertained by their ability to transfer *trpE95* to a deletion-carrying strain (as above), and give rise to *trpO trpA* segregants at low frequency ($< 1\%$).

Cell growth and enzyme assays: As a rule repressing conditions consisted of growth to late log phase on M medium supplemented with 50 μg/ml L-tryptophan, and derepressing conditions of overnight growth (18–20 hr) in the same medium supplemented with a limiting amount (5 μg/ml) of tryptophan. All incubation was carried out on a rotary shaker at 37°C. In some cases where we wanted to determine only the ability of certain strains to derepress rather than the maximal derepressed enzyme levels, the bacteria were grown under standard repressing conditions (above), harvested by centrifugation, washed and resuspended in M medium without tryptophan, and incubated on a rotary shaker at 37°C for 4 hr. The procedure employed to obtain crude extracts for assay of the tryptophan biosynthetic enzymes has been described (BALBINDER *et al.* 1968). The assay procedures for InGPSase, and both components of TSase (see Figure 1) have been described (BLUME and BALBINDER 1966). ASase and PRTase were assayed fluorometrically by a modification of the procedure of ITO and CRAWFORD (1965). This consisted of a continuous assay in a Turner fluorometer equipped with a temperature control door and circulating water bath set at 37°C, and a Rustrak recorder. Activation light of 320 mμ was obtained using a combination of Corning #7–54 and Wratten #34 filters. The fluorescence emission (410 mμ) was detected using Corning filter #5–58. In the ASase assay anthranilic acid formation was measured and in the PRTase assay anthranilic acid disappearance was measured. The standard ASase reaction mixture contained 10 mμmoles of chorismic acid prepared by the procedure of GIBSON (1968), 10 mμmoles L-glutamine, 4 μmoles of $MgSO_4$, 50 μmoles KPO_4 buffer (pH 8.2), water and enzyme to a final volume of 1.0 ml. The PRTase reaction mixture contained 2 mμmoles of anthranilic acid, 0.5 μmoles of 5-phosphoribosyl 1-pyrophosphate, and $MgSO_4$, KPO_4 buffer, water and enzyme as above.

TABLE 2

Origins of various strains carrying trp *regulatory mutations*

Strain	Genotype	Strain in which isolated and mode of origin
SO-127	O517 A111	trpA111, by mutation - DES
SO-128	R520 A111	" " "
SO-156	O522 A8	trpA8 " spontaneous
SO-155	R521 A8	" " "
SO-159	O525 A49	trpA49 " "
SO-161	O527 A49	" " "
SO-160	OA526	" " "
SO-162	OA528	" " "
SO-61	A49 A515	" " "
SO-66	A49 A516	" " "
SO-165	A47 R531	trpA47 " "
SO-167	R533 A47	" " "
SO-168	A81 A534	trpA81 " "
SO-171	A81 A537	" " "
SO-119	O518 A49 A512	SO-114 " "
SO-120	O519 A49 A512	" " "
SO-205	T542 A49 A512	" " "
SO-296	O578 A^+	SO-151 " NA
SO-316	O598 A^+	" " AP
SO-330	R612 A^+	" " ICR
SO-314	T596 A^+	" " AP
SO-337	T619 A^+	" " ICR
SO-363	T645 A^+	" " AP
SO-368	T650 A^+	" " DES
SO-206	O517 A^+	SO-127, reversion A111→A^+
SO-197	R533 O517 A^+	cross SO-167 X SO-206
SO-204	R533 O527 A^+	" SO-167 X SO-189 (*)
SO-548	O518 A^+	" SO-119 X supX38
SO-546	O519 A^+	" SO-120 X supX38
SO-547	O522 A^+	" SO-156 X "
SO-549	O525 A^+	" SO-159 X "
SO-542	O519 A8	" trpA8 cysB12 X SO-546
SO-544	O518 A8	" " X SO-548
SO-545	O525 A8	" " X SO-549
SO-141	R520 A^+	" SO-128 X trp^+
SO-121	R520 A8	" SO-143 X trpA8
SO-143	R520 A^+ cysB403	" SO-128 X cysB 403
SO-203	R533 A^+	" SO-167 X SO-187 (*)
SO-139	T542 A^+	" SO-205 X trp^+
SO-138	R531 A^+	" SO-165 X trp^+
SO-190	R532 A^+	" SO-166 X trp^+
SO-514	O518 A^+ cysB 12	" SO-292 X trp^+
SO-292	O518 A49 A512 cysB12	" SO-119 X trpB883 cysB12
SO-517	O517 A^+ C3	" trpA8 trpC3 cysB12 X SO-206

RESULTS

Isolation of trp *regulatory mutants*: Constitutive mutants for the *trp* operon were isolated by selecting for 5MT- or 6FT-resistant derivatives of the various strains listed in Table 1. 5MT is known to mimic the role of tryptophan as a feedback inhibitor of ASase (MOYED 1960), and we found this also to be true of 6FT (CORDARO, unpublished). To avoid the recovery of too many feedback-resistant mutants of ASase, some of our regulatory mutations were isolated in the *trpA* mutants listed in Table 1. In these cases, anthranilic acid (10 μg/ml) was present in the selective medium. In cases where the *trypA* marker present in analogue-resistant isolates was not desired, it was substituted with the wild-type allele by transduction or by reversion. When analogue resistant mutations were isolated in the *trp* prototrophic strains listed at the bottom of Table 1, their growth requirements were supplied at a final concentration of 10 μg/ml. The origins of various strains described in this paper are given in Table 2.

Anthranilic acid is capable of partially overcoming the inhibitory effects of 5MT and 6FT on bacterial growth. At the concentrations of anthranilic acid and analogues we used routinely (10 μg/ml and 100 μg/ml, respectively), the extent of this relief of inhibition was found to depend upon the levels of the *trp* enzymes, in particular PRTase, of each individual strain. Thus *trpA* polar mutants (carrying nonsense or frameshift mutations) were completely inhibited under our conditions while the growth of nonpolar mutants was only slowed down. As part of the ASase–PRTase complex (see Figure 1), PRTase is subject to feedback inhibition by tryptophan or its analogues but is not normally inhibited when free (BAUERLE and MARGOLIN 1966b; CORDARO, unpublished). Since the presence of a nonsense or frameshift mutation in *trpA* results in the absence of ASase, strains carrying these mutations have only free PRTase. Thus, inhibition of PRTase activity does not appear to be the mechanism whereby the analogues prevent bacterial growth. Table 3 shows that both 5MT and 6FT act like tryptophan in preventing derepression of the *trp* enzymes. These observations suggest that both analogues can act as false co-repressors and agree with the report of ITO, HIRAGA and YURA (1969) that 5MT represses synthesis of *trp* m-RNA in *E. coli*. This is further supported by the finding that all 5MT- and 6FT-resistant mutants isolated in *trpA* strains were derepressed for the *trp* enzymes (i.e., *trpR520 trpA8*, bottom, Table 3). We shall return to this question in the discussion. Of the two analogues, 6FT was the stronger growth inhibitor in agreement with the observation (Table 3) that it inhibits derepression of the *trp* enzymes more efficiently than 5MT. Resistant mutants isolated against one of the analogues were cross-

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For reasons of convenience, the *trp* designation has been omitted in the genotypes listed in this table and subsequent ones. Unless specifically indicated, it will be understood that all alleles listed are in *trp* genes. In all crosses included in this table, the recipient is given first. The procedures employed for obtaining regulatory mutations are described under METHODS. The mutagens used were: AP, 2-aminopurine; ICR, 3-chloro-7-methoxy-9-(3[chloroethyl]amino-propylamino) acridine dihydrochloride; DES, diethyl sulfate; NA, nitrous acid. In addition to these mutagenic agents, some regulatory mutations not described in this table but included in the totals in Table 5 were induced with N-methyl-N′-nitro-N-nitrosoguanidine. (•) For information on these strains see Table 8.

TABLE 3

Repression of the tryptophan biosynthetic enzymes by 5-methyltryptophan and 6-fluorotryptophan

Strain (genotype)		Culture conditions	Enzyme Levels (relative sp. act.) PRTase	βTSase
R⁺ A8	1.	Minimal + tryptophan (50 μg/ml)	1	1
R⁺ A8	2.	Minimal + 5-methyltryptophan (100 μg/ml)	4	4
R⁺ A8	3.	Minimal + 6-fluorotryptophan (100 μg/ml)	1.7	2
R⁺ A8	4.	Minimal (no supplements)	42	18
R520A8	5.	Minimal + tryptophan (50 μg/ml)	79	37

The enzyme levels are given as relative to those of repressed wild type taken as unity. Cases 1 and 5 in the table represent standard repressing growth conditions (See METHODS). For 2, 3, and 4, cells were grown under standard repressing conditions to late log phase, harvested by centrifugation, washed twice in sterile saline solution and resuspended in each of the indicated media. After 4 hr of incubation on a rotary shaker at 37°C without detectable growth, they were harvested and crude extracts obtained as indicated under METHODS. The difference in the multiplicities of derepression of PRTase and βTSase is a consequence of the presence of the low-efficiency "promoter-like" element P2 between the genes *trpB* and *trpE* (BAUERLE and MARGOLIN 1966a; MARGOLIN and BAUERLE 1966).

resistant to the other, again indicating a common mechanism of action for both analogues.

Determination of linkage of regulatory mutations: The co-transducibility of mutations to analogue resistance with the *trp* operon was determined in crosses against several of the deletions shown in Figure 2. Two types of crosses were employed: (1) the recipient carried deletions of the proximal portion of the *trp* operon including *trpA*, and (2) the recipient carried deletions in the distal portion of the *trp* operon not including *trpA*. Both types of crosses would yield exclusively sensitive recombinants in the case of non-cotransducible mutations. In the case of co-transducible mutations, analogue-resistant recombinants would appear: for mutations located within the segment deleted in the recipient strain they would be the only kind of recombinants obtained; for mutations located outside of the

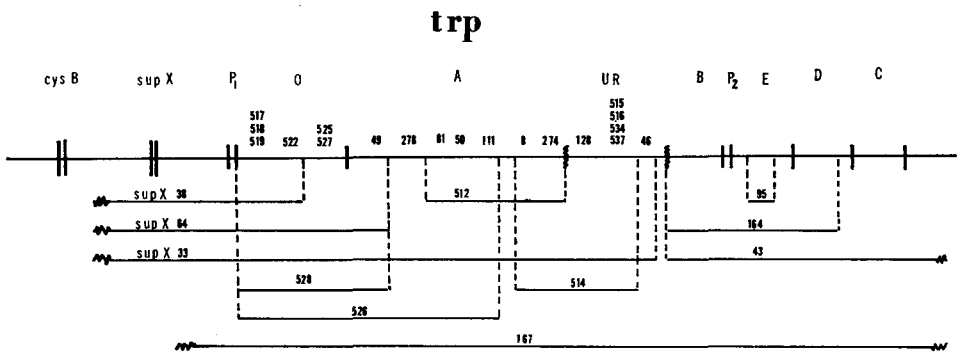


FIGURE 2.—A map of the *trp* operon. The position and extent of various deletions used in mapping is indicated, as well as the location of other mutations mentioned in the text. The "unusual" region (U.R.) located between *trpA* and *trpB* is defined by a group of mutations deficient in both ASase and PRTase activities (BAUERLE and MARGOLIN 1966b; BALBINDER, BLUME, WEBER and TAMAKI 1968) and may correspond to the C-terminal portion of *trpA* or the N-terminal portion of *trpB*.

TABLE 4

Determination of cotransducibility of constitutive mutations and the trp *operon*

Cross R X D	Selective Medium	Recombinants A^-S	A^-R	A^+S	A^+R	Total
I. Co-transducible mutations						
A) Crosses to deletions covering trpA						
supX33 X 0517 A111	MAA	0	200	0	0	200
supX33 X 0522 A8	"	0	20	0	0	20
ABEDC 167 X 0578 A^+	M	-	-	0	100	100
ABEDC167 X 0598 A^+	M	-	-	0	100	100
B) Crosses to deletions distal to trpA						
BEDC43 X 0517 A111	M	-	-	196	4	200
BED164 X 0517 A111	M	-	-	192	8	200
E95 X 0517 A111	M	-	-	194	6	200
II. Unlinked mutations						
A) Crosses to deletions covering trpA						
supX33 X R521 A8	MAA	200	0	0	0	200
supX33 X R520 A111	"	200	0	0	0	200
supX33 X R531 A47	"	180	0	0	0	180
supX33 X T542 A49 A512	"	100	0	0	0	100
ABEDC167 X R612 A^+	M	-	-	100	0	100
ABEDC167 X T596 A^+	"	-	-	100	0	100
B) Crosses to deletions distal to trpA						
BEDC43 X R520 A111	M	-	-	75	0	75
BED164 X R520 A111	M	-	-	104	0	104

Bacteria infected with transducing lysate were plated on the selective media indicated (about 10^8 bacteria/plate). In all these crosses resistance to 5MT was unselected and recombinants were tested for resistance or sensitivity to the analogue as described under METHODS. Symbols: R, recipient; D, donor; M, minimal medium; MAA, minimal medium supplemented with 10 μg/ml of anthranilic acid; A^-S, anthranilic requirer 5MT-sensitive recombinant; A^-R, anthranilic requirer 5MT-resistant; A^+S, prototroph sensitive; A^+R, prototroph resistant; (—) recombinants not recovered under selective conditions employed.

deleted segment both resistant and sensitive recombinants would occur. In this fashion we could obtain information about the general location of co-transducible mutations to analogue resistance. The results of several crosses are given in Table 4. These clearly distinguish between linked and unlinked mutations and indicate that the former map in the proximal portion of the *trp* operon.

Among the mutations showing linkage to *trp* we were able to distinguish three genetically distinct types: (1) one group mapping at the proximal extremity of *trpA*, most of them within *supX38*, (Figure 2) and having the characteristics of mutations of the *trp* operator (*trpO*), (2) mutations which recombined with *supX38* and *supX64* and probably represent alterations of ASase to feedback resistance, and (3) mutations of an entirely novel type which grew better on anthranilic acid supplement when 5MT was present (5MT dependent). Of the group showing no linkage to *trp*, we found two genetically distinct groups; (1) one showing very close linkage to *thr* (*trpR*) and (2) a group not mapping near *thr* and whose location has not yet been established (*trpT*). The characterization of these different types of mutations will be presented later. Their distribution among the various strains employed as sources of regulatory mutations is shown in Table 5. Each regulatory mutant was isolated as a single colony on 5MT- or 6FT-selective agar. In the case of spontaneous mutants, these were from platings of different cultures of the same strain. Mutagen-induced mutants were taken from colonies appearing on the selective agar after the addition of the mutagen (see METHODS). Thus, we feel confident that each mutant is of independent origin.

Reversion to prototrophy in the presence of regulatory mutations: In general, the presence of a regulatory mutation in *trpA* strains did not affect the frequency

TABLE 5

Distribution of regulatory mutations according to strain of origin

Strain (or genotype)	Linked to trp			Not linked to trp		Total
	trpO	ASR	MTD	trpR	trpT	
trpA49	4(2 DL)	0	2	0	0	6
trpA81	0	0	8	0	0	8
trpA111	1	0	0	12	0	13
trpA47	0	0	0	3	0	3
trpA8	3	0	0	1	0	4
SO-82	0	0	0	0	6	6
SO-114	10	0	0	1	2	13
SO-151	24	7	0	5	32	68
cysB403	23	5	0	7	4	39
Totals	65	12	10	29	44	160

The numbers of mutants of each type isolated and analyzed are given. Symbols: DL, deletion; ASR, feedback-resistant ASase; MTD, 5MT-dependent mutants.

TABLE 6

Frequencies of reversion to prototrophy of trpA111 *in the presence and absence of constitutive mutations*

Strain	Genotype	Mutagen		
		Sp.	DES	NG
---	R^+ O^+ A111	16	600	3000
SO-128	R520 O^+ A111	15	600	3000
SO-127	R^+ O517 A111	263	3000	3000

The numbers represent prototrophic revertants observed per 10^8 cells plated. The procedure used to obtain revertants is described under METHODS. Symbols: Sp, spontaneous; DES, diethyl sulfate; NG, N-methyl-N'-nitro-N-nitrosoguanidine.

of reversion to prototrophy of the *trpA* markers. There was only one exception to this rule. The mutation *trpA111* showed a higher reversion frequency in the presence of the mutation *trpO517* than in its absence (Table 6). This does not seem to be due to the presence of a class of revertants which might only appear in a derepressed strain (i.e., a missense triplet producing an enzyme capable of a low level of activity) since no increase in mutation frequency was observed in the presence of *trpR520*, a mutation which leads to a higher level of constitutive synthesis than *trpO517* as we shall see later. The meaning of this observation is not clear at present.

We noticed that some revertants to tryptophan independence in analogue-resistant derivatives of *trpA* mutants differed among themselves in their re-

TABLE 7

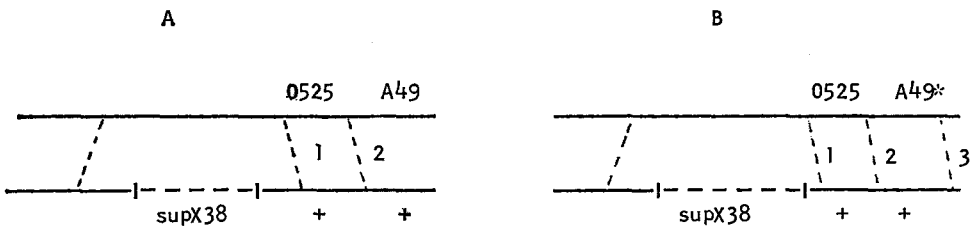
Phenotypes of prototrophic revertants of trpO trpA *strains*

Strain	Genotype	Prototrophic revertants			
		MTR	MTRA	MTS	Total tested
---	O^+ A49	0	0	140	140
SO-159	O525 A49	34	108	2	144
SO-161	O527 A49	51	113	0	164
---	O^+ A111	0	1	208	209
SO-127	O517 A111	83	227	0	310

Prototrophic revertants of the strains indicated were tested for sensitivity or resistance to 5MT as described under METHODS. Symbols: MTR, resistant to 5MT; MTRA, conditional-resistant to 5MT (requires anthranilic acid for resistance); MTS, sensitive to 5MT.

TABLE 8

Crosses of revertants of trpO525 trpA49 *and* trpO527 trpA49 *against the deletion* supX38



Cross-over Event				1	2	3	
Recombinant Phenotype				MTS	MTRA	MTR	Total
Cross	Donor	Donor Genotype	Donor Phenotype				
A	SO-159	0525A49	AAU-MTR	3	57	0	60
A	SO-161	0527 A49	AAU-MTR	2	128	0	130
B	SO-187	0525 A49*	P-MTRA	4	196	0	200
B	SO-189	0527 A49*	P-MTRA	2	198	0	200
B	SO-186	0525 A49*	P-MTR	1	16	83	100
B	SO-188	0527 A49*	P-MTR	3	8	139	150

In all crosses *supX38* was the recipient. After exposure to transducing phage, infected bacteria were plated on minimal agar supplemented with 10 μg/ml of leucine. The reason for adding this supplement was that the recipient *supX* strain carried the mutation *leu500* which is suppressed by the deletion of the *supX* gene (MARGOLIN and BAUERLE 1966), and restoration of this gene by transduction would result in a requirement for leucine. Recombinants were then tested on media containing leucine in addition to other supplements as described under METHODS. Symbols: AAU, anthranilic acid utilizer; P, prototroph; other symbols as in the legend to Table 7. The asterisk (*) indicates a reversion of the *trpA49* mutation.

sistance to analogues. Some examples are shown in Table 7. At least two types of prototrophs were recovered among revertants of the *trpO trpA* strains indicated in Table 7: (1) fully resistant to 5MT, and (2) resistant to 5MT only in the presence of anthranilic acid (conditional-resistant revertants). In one case (strain SO-159) two fully sensitive revertants were also obtained, but there is some doubt as to whether these were true revertants and not contaminants. A detailed analysis of some revertants of SO-127 (*trpO517 trpA111*) showed that the reversions only involved the *trpA111* mutation leaving *trpO517* unaffected,

and that some reversions could alter the response of the ASase–PRTase complex to L-tryptophan inhibition (CORDARO and BALBINDER, in press). A study of some revertants of SO-159 and SO-161 (see Table 7) leads to the same conclusions.

The results of crosses between revertants of the two *trpO trpA49* strains shown in Table 7 and the deletion *supX38* are given in Table 8. Both *trpO525* and *trpO527* map outside of the region covered by *supX38* as these results indicate. In this respect they differ from the other *trpO* mutations analyzed. Some implications of this observation will be discussed later. Since both SO-159 (*trpO525 trpA49*) and SO-161 (*trpO527 trpA49*) were derived from *trpA49* (Table 2) and are identical to each other in every characteristic thus far investigated, it is likely that *trpO525* and *trpO527* are independent occurrences of the same mutation. As the table shows, when the donors in these crosses were conditional-resistant revertants (SO-187 and SO-189) two recombinant types were recovered: 5MT-sensitives and conditional resistants. The same results were obtained when the original *trpO trpA49* double mutant strains were used as donors (SO-159 and SO-161), but when the donors were fully resistant revertants (SO-186 and SO-188) three recombinant classes were obtained: the same two as in the preceding crosses plus a fully resistant class. These results suggested that in conditional-resistant revertants the *trpA49* nonsense codon has back-mutated to the wild-type condition and in full resistant revertants a new codon has been created at the *trpA49* site which is responsible for a feedback-resistant ASase. This was tested with the ASase of strains SO-188 and SO-189. The results, shown in Table 9, confirmed the conclusion drawn from the genetic analysis. The reason why reversion of *trpA49* to the wild-type *trpA*$^+$ allele in the presence of *trpO527* or *trpO525* leads only to conditional rather than full resistance to 5MT, seems to be the low constitutive level of expression conferred by these *trpO* mutations. This will be discussed later.

Linked mutations: the tryptophan operator (trpO): Among the analogue resistant mutations linked to *trp*, one group was found to map predominantly

TABLE 9

Feedback inhibition of ASase in two prototrophic revertants of trpO527 trpA49 *which differ in their resistance to 5MT*

Concentration L-tryptophan	Strain phenotype	SO-188 MTR	SO-189 MTRA	LT2 (wild type)
		Percent inhibition of ASase activity		
10^{-3} M		94	—	—
5×10^{-4} M		83	—	—
10^{-4} M		30	96	100
5×10^{-5} M		13	91	95
10^{-5} M		9	38	60
10^{-6} M		—	7	10
None		0	0	0

Assay procedure for ASase is given under METHODS. Symbols for phenotypes are the same as in Table 7; (—) assay not performed.

TABLE 10

Mapping trpO *mutations against the deletion* supX38

Donor (genotype)	Selective Medium	Recombinants (phenotypes) A^-R	A^+R	A^-S	A^+S	Total
0517 A111	MAA	176	24	0	0	200
0A526	"	320	0	0	0	320
0A528	"	200	0	0	0	200
0522 A8	"	85	5	0	0	90
0522 A8	M	-	430	-	0	430
0518 A49 A512	"	-	384	-	0	384
0519 A49 A512	"	-	432	-	0	432
0525 A49	"	-	262	-	18	280
0527 A49	"	-	128	-	2	130

In all crosses *supX38* was the recipient. Bacteria infected with transducing phage were plated on the indicated media containing 10 μg/ml of leucine (see legend to Table 8). Symbols are the same as in Table 4. In this table full and conditional 5MT-resistant recombinants are considered as one class.

within the segment covered by the deletion *supX38*. This deletion was isolated by MARGOLIN and BAUERLE (1966) who found that it removed the *trp* promoter (P1, Figure 1) and probably the *trp* operator (*trpO*) but did not extend into *trpA*. Table 10 gives the results of crosses of various *trpO trpA* mutants against this deletion. Only in the last two crosses, involving the analogue-resistant mutations *trpO525* and *trpO527*, were sensitive recombinants obtained. All the other crosses yielded exclusively resistant recombinants. These results clearly show that *trpO525* and *trpO527* lie outside the segment deleted by *supX38* while the remaining *trpO* mutations map within this segment and suggest that *supX38* deletes P1 but only part of *trpO*, a result compatible only with the order P1–*trpO*–*trpA*. This problem is considered in a separate publication (CALLAHAN, BLUME and BALBINDER 1970).

Two of the mutants we analyzed probably carry deletions extending from *trpA* to *trpO*. These are *trpOA526* and *trpOA528*. In the first place, these mutations could not revert to prototrophy. Second, they failed to give rise to prototrophic recombinants in crosses to *supX38* (Table 10, second and third crosses) as well as various *trpA* point mutations (Figure 2). Also, in crosses to *trpO trpA*$^+$ donors no 5MT-sensitive recombinants were observed in a total of over 4,000

TABLE 11

Recombination between trpO *mutations*

Donor (genotype)	Recombinants		
	A^+S	A^+R	Total
0519 A49 A512	0	416	416
0522 A8	2	494	496
0525 A49	19	307	326

The strain SO-514 *(trpO518 cysB12)* was the recipient, and it was transduced with lysates grown on each donor strain. Prototrophic recombinants were selected on minimal medium and tested for resistance to 5MT as described under METHODS. Symbols: as in Table 4.

prototrophic recombinants tested. Although these mutations have the characteristics expected of *trpOA* deletions, we cannot exclude the possibility that they represent "insertions" of some genetic material. This is further discussed in another publication (CALLAHAN, BLUME and BALBINDER 1970).

Table 11 shows that recombination between *trpO* mutations does occur. On the basis of these results we can define at least three sites within *trpO*. A preliminary map of *trpO* based on the data in Tables 10 and 11 is shown in Figure 2. In this map, *trpO522* has been arbitrarily placed to the right of *trpO518*. Our present results do not allow for an unambiguous positioning of these two mutations with respect to each other.

The recombination frequencies observed between *trpO* mutations in Table 11 (about 0.4% between *trpO522* and *trpO518*; about 6% between *trpO525* and *trpO518*) as well as those between *trpO525* or *trpO527* and *supX38* shown in Table 10 (about 6.5% between *trpO525* and *supX38*, for example) are rather high. The significance of this observation is difficult to assess in the absence of additional data. It may be an indication that the size of *trpO* is, indeed, quite large as reported for the *trp* operator of *E. coli* (HIRAGA 1969).

Table 12 gives the levels of TSase (β and α components) observed under repression and derepression conditions. Several features of this table deserve some comment. First, the constitutive levels determined by the various *trpO* mutations are rather low ranging from a 2–3 fold increase over basal level (SO-545) to a 10–13 fold increase (SO-206, SO-517). Second, these strains are capable of derepressing in the absence of endogenously synthesized tryptophan (SO-127, SO-517, SO-156, etc.) or in the presence of a *trpR* mutation (SO-197, SO-204). In contrast, the two deletions *trpOA526* and *trpOA528* (SO-160, SO-162) produce constitutive enzyme levels which are as high as those of fully derepressed nonpolar auxotrophs (see *trpA8*, second from bottom in Table 12) and this constitutive level does not change under derepression conditions. These data indicate that most *trpO* mutations still retain some capacity for repressor recognition and that this capacity is completely lost when *trpO* is deleted. This, plus the fact that recombination between *trpO* mutations can be observed suggests that these are

TABLE 12

Levels of β and α TSase in various trpO *mutants*

Strain	Genotype	Enzyme levels			
		Repressed		Derepressed	
		β TSase	α TSase	β TSase	α TSase
SO-127	0517 A111	1.2	1.1	36	30
SO-206	0517 A^+	12	10	16	10
SO-517	0517 A^+ C3	13	12	60	90
SO-197	R533 0517 A^+	42	44	52	44
SO-161	0527 A49	5	4	4	3
SO-189	0527 A^+	4.5	3	-	7
SO-204	R533 0527 A^+	31	33	36	35 (*)
SO-542	0519 A8	10	9	33	33 (*)
SO-156	0522 A8	7	7	30	30 (*)
SO-544	0518 A8	7	8	33	30 (*)
SO-545	0525 A8	3	2	-	-
SO-160	OA526	59	75	65	70 (*)
SO-162	OA 528	76	68	73	70 (*)
Controls					
-	O^+ A8	1	1	70	90
-	Wild type (LT2)	1	1	6	5

The origins of the strains included in this table are given in Table 2. Enzyme levels are expressed as relative to repressed wild type taken as unity. These levels, as well as those given in other places throughout this paper, represent an underestimate by approximately a factor of 2 of P1 initiated expression. The reason for this is that under repression conditions, the last three genes of the *trp* operon are expressed both from P1 and P2 (BAUERLE and MARGOLIN 1966a; MARGOLIN and BAUERLE 1966). Strains marked with an asterisk (*) were only allowed to derepress for 4 hr on minimal medium in the absence of tryptophan and are not fully derepressed. All others were grown overnight on limiting tryptophan (see METHODS). Repression conditions of growth were as described under METHODS; (—) assay not performed.

the result of point mutations. No method for studying reversion to *trpO*$^+$ has been found thus far. HIRAGA (1969) has also observed in *E. coli* that *trpO* mutants are partially constitutive to varying degrees.

The presence of a polar mutation interferes with full expression of a *trpO* mutation but this is corrected by replacing the polar mutation with a nonpolar one

or with the wild-type allele (compare first three strains in Table 12). In this regard the mutations *trpO527* and *trpO525* are exceptional in that they show the same level of expression in the presence of the strongly polar mutation *trpA49* and in its absence. The table gives full data for *trpO527* only (SO-161 and SO-189). Also, although *trpA49* is more strongly polar than *trpA111* (Table 1) constitutive synthesis in *trpO527 trpA49* (SO-161) is higher than in *trpO517 trpA111* (SO-127). These observations raise certain questions about the status of *trpO525* and *trpO527*. We shall return to this problem in the DISCUSSION.

Table 13 shows the results of *cis*-dominance tests for several *trpO* mutations. As can be seen the merodiploids could synthesize constitutive levels of InGPSase (specified by the chromosomal markers) but not ASase (specified by the episomal markers) under repressed conditions. These results show that *trpO* alleles exert their control on the expression of the genes located on the chromosome (*cis* position) and not on those located on the episome (*trans* position). Thus, as has been shown in other systems, *trpO* mutations are *cis* dominant.

Linked mutations to 5MT dependence: One group of mutants at first con-

TABLE 13

Cis-dominance tests for trpO *mutations*

Genotype (endo-/exogenote)	ASase (units/mg protein)	InGPSase (units/mg protein)
$\underline{O^+ A^+ E^+}$ (wild type)	0.02	0.16
$\underline{O^+ A8\ E^+}$	0	0.125
$\underline{O^+ A^+ E95}$	0.02	0
$\underline{O518\ A8\ E^+}$	0	0.4
$\underline{O519\ A8\ E^+}$	0	0.3
$\underline{O522\ A8\ E^+}$	0	0.96
$\underline{O^+ A8\ E^+}$ / F' $\underline{O^+ A^+ E95}$	0.015	0.156
$\underline{O518\ A8\ E^+}$ / F' $\underline{O^+ A^+ E95}$	0.018	0.77
$\underline{O519\ A8\ E^+}$ / F' $\underline{O^+ A^+ E95}$	0.02	0.60
$\underline{O522\ A8\ E^+}$ / F' $\underline{O^+ A^+ E95}$	0.02	0.96
$\underline{O518\ A^+ E^+}$	0.3	0.4
$\underline{O519\ A^+ E^+}$	0.4	0.35
$\underline{O522\ A^+ E^+}$	-	0.93
$\underline{O517\ A^+ E^+}$	0.45	1.2

The enzyme levels are expressed in specific activity units (units/mg protein). Cells were grown under conditions of repression and enzymes assayed in crude extracts as described under METHODS. The procedure used to obtain *trp* merodiploids is also described under METHODS. The levels of InGPSase shown by some of these strains are somewhat lower than expected. We attribute this to difficulties with the assay procedure for this enzyme.

TABLE 14

Comparison of growth rates and enzyme levels of 5MT-sensitive, resistant, and dependent strains

Strain	Genotype	Generation times (minutes)			Enzyme levels			
		LAA	LAAMT	Indole	PRTase		αTSase	
					R	D	R	D
-	O^+ A8	47	90	50	1	200	1	91
-	O^+ A49	180	No Gr	66	<0.25	<0.25	0.6	6
SO-114	O^+ A49 A512	60	No Gr	57	<0.25	30	0.4	70
SO-119	O518A49 A512	54	54	63	<0.25	40	3.0	80
SO-61	O^+ A49 A515	115	73	60	1.3	1.6	3.0	3.0
SO-66	O^+ A49 A516	120	70	54	0.85	-	5.0	3.0
SO-168	O^+ A81 A534	-	-	-	0.9	1.0	2.6	2.0
SO-171	O^+ A81 A537	-	-	-	1.0	1.5	2.4	2.5

Generation times are given in minutes needed for one doubling of the bacterial population. These were determined turbidimetrically with a Klett-Summerson colorimeter. Enzyme levels are relative to repressed wild type taken as unity (see Table 12). Repressing (R) and derepressing (D) growth conditions are described under METHODS. In this case all strains were derepressed by overnight growth in the presence of limiting levels of L-tryptophan. Symbols: LAA, M medium supplemented with 2 μg/ml anthranilic acid; LAAMT, as LAA plus 100 μg/ml 5MT; No Gr = no growth; (—) experiment not performed. For further details see METHODS.

sidered to be 5MT resistant proved to be, on closer analysis, dependent on the analogue for growth. This group is represented in Table 14 by the strains SO-61, SO-66, SO-168, and SO-171. Mutants of this class had several distinctive characteristics in common: (a) they were isolated only in strains carrying extremely polar *trpA* mutations (Table 5); (b) they could not revert to prototrophy; (c) required anthranilic acid for growth but utilized this compound much more efficiently in the presence of 5MT (Figure 3; Table 14); (d) showed constitutive synthesis of the *trp* enzymes (Table 14); (e) unlike *trpO* mutations this constitutive level did not increase upon derepression (Table 14).

Genetic analysis proved that these strains were double mutants: they retained the original polar mutation unchanged and possessed in addition a second mutation (*515*, *516*, *534*, *537*) located in the "unusual" region (Figure 2). Both the original polar mutation and the mutation in the "unusual" region were separated by genetic recombination. A detailed analysis of one of these strains (CALLAHAN and BALBINDER 1969, 1970) indicates that the mutation in the "unusual" region creates a transcription-initiating signal which is independent of *trpO* control. These strains grow better on anthranilic acid supplement when 5MT is present because the analogue, in its capacity as co-repressor, interferes with the normal initiation of transcription at P1 and favors, consequently, the initiation of transcription at the site (or sites) of the transcription-initiating mutations. Under these conditions sufficient PRTase is produced to permit growth on anthranilic acid. In agreement with this interpretation the enzyme levels of SO-61 are elevated when this strain is grown in the presence of anthranilic acid and 5MT

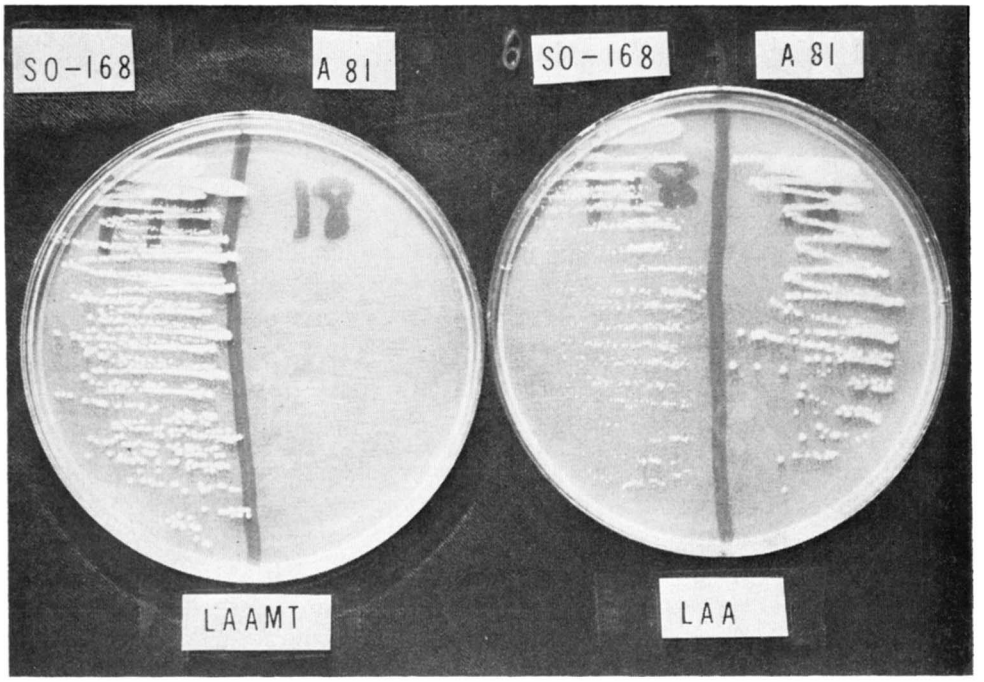


FIGURE 3.—Growth patterns of the 5MT-dependent strain SO-168. The growth of the 5MT-dependent strain SO-168 (*trpA81 trpA534*) is compared to that of the 5MT-sensitive strain *trpA81*, from which SO-168 was derived. As can be seen, *trpA81* grows better than SO-168 on LAA (M medium supplemented with 2 μg/ml of anthranilic acid), but on LAAMT (as LAA, with the addition of 100 μg/ml of 5MT) *trpA81* is completely inhibited while SO-168 grows more efficiently than it does on LAA. Plates had been incubated at 37°C for 48 hr. For further details see METHODS.

(3.6 times over basal level for PRT and 3.4 times over basal level for β-TSase) (CALLAHAN and BALBINDER 1970). It is not yet known whether the several transcription-initiating mutations isolated are different from each other or whether they represent independent recurrences of the same mutation.

Presumed feedback-resistant mutations: A group of 5MT mutations in the *trp* region proved to map within the *trpA* gene since they recombined with both *supX38* and *supX64* (Figure 2). These mutations seem to retain normal repression control of the *trp* operon and are believed to be alterations of ASase to a feedback-resistant form. They arose only in *trp* prototrophs (Table 5). They have not been analyzed further.

Mutations unlinked to the trp operon: trpR *and* trpT. These two groups are genetically different: *trpR* mutations are defined by the fact that they map very close to *thr* on the Salmonella map (about 50% co-transduction), while *trpT* mutations do not map in this region. Their map position has not been determined yet and we cannot exclude the possibility that they may define more than one genetic locus.

The *trpR* mutations are probably equivalent to those in *E. coli* first described

by COHEN and JACOB (1959) since their map position appears to be the same. The *trpR* gene in *E. coli* codes for a protein with the characteristics of the *trp* repressor (MORSE and YANOFSKY 1969b).

Mutations of the *trpT* class have not been reported in *E. coli*. They are clearly not equivalent to the tryptophanyl tRNA synthetase mutations (*trpS*) of *E. coli* (HIRAGA *et al.* 1967; KANO, MATSUSHIRO and SHIMURA 1968; DOOLITTLE and YANOFSKY 1968) since they do not map in the *strA–malA* region of the Salmonella chromosome and differ in this, as well as in other respects, from *trpS* mutations we have isolated (McCANN; unpublished). They are also different from the 5MT-resistant mutations which map near *argG* in *E. coli* and are unaltered

TABLE 15

Levels of β and α TSase in various trpR *and* trpT *mutants*

Strain	Genotype	Enzyme levels			
		Repressed		Derepressed	
		β TSase	α TSase	β TSase	α TSase
SO-128	R520 A111	2.7	2.3	22	27
SO-141	R520 A^+	22	21	30	28
SO-121	R520 A8	24	28	85	-
SO-167	R533 A47	5.2	-	50	80
SO-203	R533 A^+	30	33	33	36
SO-205	T542 A49 A512	1.8	-	30	64
SO-139	T542 A^+	1.8	1.4	4.5	3.3
SO-138	R531 A^+	25	25	-	-
SO-190	R532 A^+	42	27	-	-
SO-337	T619 A^+	-	3.7	-	7.5
SO-363	T645 A^+	-	12.5	-	12.0
SO-368	T650 A^+	-	29	-	28
5MT resistant recombinants from crosses to thrA9					
SO-121 X thrA9		27	23	-	-
SO-138 X thrA9		40	33	-	-

The origins of the strains included in this table are given in Table 2. Enzyme levels are expressed as relative to repressed wild type taken as unity. All strains were derepressed by overnight growth in the presence of limiting L-tryptophan. For further details see legend to Table 12. (—), assays not performed.

in repression of the *trp* enzymes (HIRAGA *et al.* 1968) since (a) *trpT* mutations do not map near *argG* and (b) they synthesize the *trp* enzymes constitutively (Table 15). Finally, *trpT* mutants differ from the aromatic amino acid permease mutants described by AMES and ROTH (1968) in that (a) they are sensitive to azaserine while the permease mutants are resistant to this compound, (b) they excrete tryptophan, (c) *trpT* mutations do not map near *pro*, where the permease mutations have been located.

Table 15 shows the levels of TSase (β and α) of several *trpR* and *trpT* strains. In general *trpR* strains seem to be capable of higher constitutive levels of synthesis than *trpO* mutants (see Table 12) or *trpT* mutants (except for SO-368). As in the case of *trpO* mutants, these constitutive enzyme levels increase upon derepression caused by tryptophan deprivation.

DISCUSSION

A search for regulatory mutations of the *trp* operon of *Salmonella typhimurium* using various Salmonella mutants as sources has yielded four genetically distinct classes. Two of these, *trpO* and *trpR* appear in every respect to be equivalent to the *trpO* and *trpR* mutations described in *E. coli* (HIRAGA 1969; COHEN and JACOB 1954; MORSE and YANOFSKY 1969b). The other two, *trpT* and what we have called 5MT-dependent mutations have not been previously reported.

The existence of three classes of regulatory mutations unlinked to *trp* had been reported earlier (MCCANN and BALBINDER 1969): *trpR*, *trpT*, and *trpV*; the latter two mapping near *purG* and *proAB*, respectively. Subsequent work showed these map positions to be incorrect and for the time being we are considering *trpT* and *trpV* as one class, *trpT*. Beyond what has been presented in the RESULTS section, we have no further information about *trpT* mutations at this time. It is possible that they may be in some way involved in the production of tryptophanyl tRNA synthetase, since in several operons controlling enzymes needed in amino acid biosynthesis, a role for the corresponding tRNA in repression control has been demonstrated (ROTH, ANTÓN and HARTMAN 1966; ROTH and AMES 1966; EIDLIC and NEIDHARDT 1965; FREUNDLICH 1967). This possibility is being tested at present.

The mutations to 5MT dependence are not truly regulatory since they do not define a genetic element normally involved in repression control of the *trp* system. They represent mutations in a structural gene which confer constitutivity by bypassing the normal regulation of the operon. Transcription-initiating mutations have been reported for the *E. coli trp* operon (MORSE and YANOFSKY 1969a) but these were phenotypically 5MT resistant rather than 5MT dependent. Their mutations map within the ASase gene (*trpE* in *E. coli*) while ours are located in the "unusual" region (see legend to Figure 2). Further details are given in a separate publication (CALLAHAN and BALBINDER 1970). The fact that a transcription-initiating mutation located in a structural gene can bypass the normal control over operon expression exerted by the operator is consistent with a model put forward by REZNIKOFF *et al.* (1969) which proposed that operator-bound repressor interferes with the progress of RNA polymerase along the DNA

template thus bringing about repression. One fact supporting this model is that in the *lac* operon the operator maps between the promoter and the first structural gene (IPPEN *et al.* 1968; MILLER *et al.* 1968). The same situation seems to exist in the *trp* operon of *S. typhimurium* (CALLAHAN, BLUME and BALBINDER 1969, 1970). It is then conceivable that transcription-initiating mutations located at the proximal end of the first structural gene close, or adjacent to, the operator may be mistaken for operator mutations. One case of this sort may be that of mutations *trpO525* and *trypO527*. The constitutive expression of the *trp* operon brought about by these mutations is unaffected by the extremely polar mutation *trpA49* (Table 12). The explanation of this anomalous result is not obvious, but one possibility is that *trpO525* and *trpO527* are not mutations of *trpO* but new "promoters" located at the extreme proximal end of *trpA*. In this event, however, they would have to map to the right of *trpA49* (as Figure 2 is drawn), but three-point tests favor their location to the left of this mutation (CALLAHAN, BLUME and BALBINDER 1970). Work on this problem is continuing.

Our observations indicate that 5MT and 6FT inhibit bacterial growth by mimicking the action of tryptophan both as a feedback inhibitor of ASase, as originally reported by MOYED (1960), and as a co-repressor. That 5MT can prevent growth by inhibiting ASase activity is clearly shown by the correlation between the growth behavior of the two classes of revertants of *trpO trpA49* mutants (Tables 7 and 8) and the susceptibilities of their ASases to end-product inhibition (Table 9). ITO, HIRAGA and YURA (1969) showed that 5MT can prevent derepression of *trp* m-RNA in *E. coli* indicating that this analogue could act as a co-repressor. We present evidence in this report consistent with their finding. First, all 5MT- and 6FT-resistant mutants isolated in *trpA* strains are derepressed for the enzymes of the *trp* operon. Second, 5MT and 6FT prevent the derepression of these enzymes (Table 3). It could be argued that this results from a general inhibition of protein synthesis caused by the analogues rather than a specific repression of the *trp* operon. If this were the case, however, we would not expect to find a class of 5MT-dependent mutants such as the ones we have described. These require 5MT in order to utilize anthranilic acid as a growth factor, and this phenotype is caused by the presence of "transcription-initiating" mutations in the "unusual" region. When transcription is initiated exclusively at, or near, the site of one of these mutations (such as *trpA515*) sufficient PRTase is produced to allow the strains to grow on anthranilic acid supplement. In order for this to happen, P1 initiated transcription must be prevented either by depleting P1, or by repressing the *trp* operon (CALLAHAN and BALBINDER 1970). In this latter event, 5MT acts exactly like tryptophan since repression by either of these compounds brings about an increase in PRTase level. Furthermore, if 5MT or 6FT were to interfere with protein synthesis, we would not expect this effect to be reversed by a mutation within a *trp* structural gene.

The distribution of the various classes of analogue-resistant mutations according to their strain of origin (Table 5) is interesting in one respect. It shows that the presence of extreme polar mutations in *trpA*, such as *trpA49* and *trpA81* (Table 1) favor the detection of certain types of constitutive mutations: those

which delete the operator and the site of the polar mutation simultaneously (deletion mutants *trpOA 526* and *trpOA 528*, Figure 2 and Table 12), and transcription-initiating mutations in the "unusual" region. This is due to the fact that the polar mutations cause the synthesis of extremely low levels of PRTase (BAUERLE and MARGOLIN 1966b; BALBINDER *et al.* 1968) and, by interfering with full expression of *trpO* or *trpR* mutations, do not permit these levels to be raised sufficiently to allow growth on anthranilic acid supplement. It is interesting that the two *trpO* mutations isolated in the presence of *trpA49* and not associated with deletions extending into *trpA*, are those which show anomalous behavior: *trpO525* and *trpO527*.

We have pointed out earlier that strains carrying *trpO* mutations and those with *trpOA* deletions make elevated levels of the *trp* enzymes, but while the former are capable of derepressing further the latter are not. This observation suggests that as long as the operator is physically intact it cannot entirely lose, as a result of point mutations, its ability to recognize the repressor. This may be a common feature of genetic elements whose role is not to code for the structure of a protein, but to be recognized by a protein as a signal of some sort. One such element could be the promoter, which represents a signal for RNA polymerase to initiate transcription (JACOB, ULLMAN and MONOD 1964). It is interesting in this regard that mutations of the *lac* promoter (IPPEN *et al.* 1968) which leave this element at least partially intact result in a reduced efficiency of expression but do not totally abolish it.

The excellent technical assistance of Mr. ERNEST LANE, Mrs. TONJA CURRY, Mrs. EVELYN BALBINDER and Miss IRENE GUGGENHEIM is gratefully acknowledged. We thank Dr. KENNETH SANDERSON for his gift of strain SU-694, and Drs. P. MARGOLIN and R. BAUERLE for the *supX* strains used in this investigation.

SUMMARY

Four classes of regulatory mutations for the tryptophan (*trp*) operon of *Salmonella typhimurium* have been obtained by selecting for strains resistant to the tryptophan analogues 5-methyltryptophan and 6-fluorotryptophan. Two of these classes, *trpO* and *trpR*, are probably equivalent to regulatory mutations described for the *trp* operon of *Escherichia coli*. The first class, *trpO* consists of mutations mapping at the extreme proximal end of the *trp* operon and satisfying the criteria for mutations of the *trp* operator. The second class, *trpR*, shows very close linkage with *thr* (about 50% co-transduction) and may be the structural gene for the *trp* aporepressor according to recent work with *E. coli*. The remaining two classes of regulatory mutations have not been previously reported. One class, *trpT* consists of mutations not linked to *trp* or *thr* and whose map position has not yet been determined. The regulatory function they define is unknown. The last class of regulatory mutants shows a dependence on 5-methyltryptophan for the utilization of anthranilic acid as a growth factor. This peculiar phenotype is caused by the creation of a transcription-initiating mutation in a region which corresponds to the C-terminal portion of the first structural gene or the N-terminal portion of

the second structural gene ("unusual" region). All these mutations lead to various levels of constitutive synthesis of the *trp* biosynthetic enzymes.

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