A NEW REGULATORY GENE FOR THE TRYPTOPHAN OPERON OF ESCHERICHIA COLI

S. Hiraga, K. Ito, K. Hamada, and T. Yura

Institute for Virus Research, Kyoto University,

Kyoto, Japan

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Although it is generally believed that the cluster of five genes controlling tryptophan biosynthesis in E. coli constitutes a typical operon, little is known about the genes regulating the expression of this operon. Cohen and Jacob (1959) described mutations of a regulatory gene R1,try (trpR, according to the recent recommendations by Demerec et al., 1966) which result in the non-repressible (constitutive) synthesis of enzymes of the tryptophan pathway and in the resistance to 5-methyltryptophan. The present communication reports on isolation and characterization of a novel type of regulatory mutant for the tryptophan operon.

RESULTS AND DISCUSSION

1) Isolation of the mutant. In an attempt to isolate a regulatory mutant similar to i^s mutants of the lactose operon (Jacob & Monod, 1961), an Ftrp strain (KY485) diploid for the cysB-trp region and carries the cysB marker on the chromosome was treated with N-methyl-N'-nitro-N-nitrosoguanidine, and mutants requiring tryptophan for growth were selected by penicillin screening. Two of the mutants obtained by this procedure, designated S-2 and S-5, were used for this study.

Both S-2 and S-5 mutants required either tryptophan or indole but not anthranilic acid for optimal growth. However, the mutants were "leaky" and

could grow slowly in minimal medium. That these mutants still carry the Ftrp episome was shown by their ability to transfer the episome at high frequency to F^- *trpABCDE4* strain deleting the entire tryptophan operon. F^- derivatives of these mutants obtained by acridine treatment also required tryptophan for growth (1 in Table 1). Moreover, when a standard Ftrp episome was reintroduced into these F^- strains, the resulting merozygotes (KY827 and KY828) still required tryptophan (2 in Table 1). These results indicate that the mutations did not occur on the episome, and that the mutants require tryptophan for growth despite the fact that the cluster of tryptophan structural genes is functionally unaltered.

Table 1

Growth characteristics of various strains*

No.	Genotype	Minimal medium supplemented with				
		None	Anth	Ind	Try	5-MT
1	*trpS5*	-	-	+	+	
2	*trpS5* / Ftrp	-	-	+	+	
3	*trpS5* *trpABCDE4* / Ftrp O-1	+			+	?
4	*trpS*$^+$ *trpA* / Ftrp O-1	+			+	?
5	*trpS5* *trpABCDE4* / Ftrp MtrO-1	-			+	
6	*trpS5* / Ftrp MtrO-1	-			+	
7	*trpS*$^+$ *trpA* / Ftrp MtrO-1	+			+	+

* All tests were made by streaking washed cell suspension on agar media. Minimal medium was supplemented with 50 μg/ml of each compound as indicated, except for 5-MT (5-methyltryptophan) where 400 μg/ml was used. Anth, anthranilic acid; Ind, indole; try, tryptophan.

2) *Mapping of mutant S-5*. It was first examined whether the S-5 mutation occurred at or around the structural genes of the tryptophan operon. It was found that although P1*kc* phage grown on wild-type bacteria gave rise to tryptophan-independent transductants at a reasonable frequency with mutant S-5 as a recipient, no cotransduction between *cysB*$^+$ gene and the site of S-5

mutation could be detected (<1%), in contrast to the control experiment in which cotransduction between *trpA*$^+$ and *cysB*$^+$ was observed at high frequency (25-40%) with the same phage preparation.

When Hfr H (*met thi*) strain was mated with an F$^-$ strain carrying S-5 and *thr* markers, all *thr*$^+$ *met*$^+$ recombinants tested (100 colonies) required tryptophan for growth. This shows that S-5 mutation did not occur at the *trpR* locus which is located near the *thr* marker. Hfr CS101 strain was then mated with an F$^-$ S-5 strain carrying appropriate markers, several classes of recombinants were selected, and their unselected markers examined. The results presented in Table 2 suggest that the S-5 mutation is located near the *str* marker. The gene locus for the S-5 mutation is thus named *trpS* hereafter.

Table 2

Mapping of the S-5 mutation on the chromosome*

Selected markers	Markers from Hfr (%)							Number of recombinants tested
	S-5	*malB*	*met*	*ilv*	*xyl*	*str*	*cysB*	
Ilv$^+$ Met$^+$	25	62	0	100	—	25	7	98
Ilv$^+$ StrR	2	1	34	100	1	0	1	100
Trp$^+$ StrR	100	—	46	73	78	0	0	91
Trp$^+$ Met$^+$	100	57	0	54	69	84	8	100

* Mating between Hfr CS101 and F$^-$ KY874 took place for 150 min at 37°C in Penassay broth (Hfr/F$^-$ = 1/10).
Hfr CS101 : S-5$^+$ *malB*$^+$ *met ilv*$^+$ *xyl*$^+$ *str-s cysB*$^+$
F$^-$ KY874 : S-5 *malB met*$^+$ *ilv xyl str-r cysB*

3) *Enzyme activities of the tryptophan operon in S-2 and S-5 mutants.* Crude extracts of S-2 (KY827) and S-5 (KY828) mutants grown under derepressed or repressed conditions were assayed for activities of enzymes of the tryptophan operon. As shown in Table 3, the mutants exhibit approximately 30% of the wild-type activities for anthranilate synthetase (ASase), indoleglycerol

Table 3

Enzyme activities of the tryptophan operon in various strains*

Enzyme source	ASase	InGPSase	TSase		
			B protein	A protein	BA complex
Derepressed					
Wild type (KY485)	100	100	100	100	100
S-2 (KY827)	21-44	36	–	30-32	67
S-5 (KY828)	31-46	30-35	30	25-29	66
MtrO-1 (KY886)	166-180	93-260	–	–	150-220
Repressed					
Wild type (KY485)	4-10	4-5	15	4-12	8-25
S-2 (KY827)	5-10	13	15	4-14	18
S-5 (KY828)	5-10	–	–	4	13
MtrO-1 (KY886)	74-130	60-115	–	–	60-115

* Cells grown in minimal medium E containing 0.2% Casamino acids and 50 µg/ml L-tryptophan were harvested at late log phase, centrifuged, resuspended in the same medium with L-tryptophan at 2 µg/ml ("derepressed") or 50 µg/ml ("repressed") and aerated for 2 hrs at 37°C. Enzyme assays were performed essentially as described by previous workers (e.g. Matsushiro et al. 1965). Activities with derepressed wild-type extract were set at 100 for each enzyme.

phosphate synthetase (InGPSase), and A and B proteins of tryptophan synthetase (TSase) when grown with a limiting amount of tryptophan, and the production of these enzymes were further repressed by an excess tryptophan. These results suggest that the mutation somehow restricts the expression of the whole tryptophan operon under normally derepressed condition. The data also seem to explain the observed ability of the mutants to grow on indole as well as on tryptophan. Although the precise mechanism of the effect of the mutation is not clear at present, it appears that a cytoplasmic product of the trpS gene is involved in the control of the expression of the tryptophan operon, since the operon located on the Ftrp episome as well as that on the chromosome is similarly affected.

5) The site of action of the trpS gene product. To isolate a mutant at

the iste of action of the trpS gene product, an S-5 mutant carrying the tryptophan operon only on the episome (KY870) was treated with nitrosoguanidine and was plated on minimal agar with appropriate supplements to select for tryptophan-independent revertants. Among the 138 revertants tested, two strains (O-1 and O-2) gave rise to cys$^+$ trp$^+$ recombinants at high frequency when cross-streaked against cells of F$^-$trpS5 cysB. Upon transfer of the Ftrp episome from strain O-1 or O-2 to F$^-$trpS5 recipients, prototrophic merozygotes were obtained at high frequency suggesting that O-1 and O-2 mutations occurred on the episome. Furthermore, when the Ftrp episome carrying O-1 or O-2 mutation was transferred into F$^-$trpA2 trpS$^+$ strain, the resulting merozygotes (KY886 or KY887) did not require tryptophan for growth. It thus appeared likely that O-1 and O-2 mutations affect the response of the tryptophan operon towards the product of the trpS gene, resulting in the normal expression of the operon whether the strain carries trpS5 or its wild-type allele (3 and 4 in Table 1). However, it has not been determined whether O-1 or O-2 mutation affected the response of these strains to the repressing effects of tryptophan.

Unfortunately, O-1 and O-2 mutants were very unstable and could not be recovered from slants or stocks after 1 month in the cold. The survivors in the stock cultures all turned out to require tryptophan for growth (5 and 6 in Table 1), and it seemed as though the Ftrp episome had lost the O-1 or O-2 mutation during the storage.

Interestingly enough, however, strain KY886 or KY887 that carries the wild-type allele at the trpS locus and had previously received the Ftrp episome from strain O-1 or O-2 was found to be resistant to 5-methyltryptophan (7 in Table 1). That this character is due to an alteration on the episome and not on the chromosome was shown by the parallel transfer of the resistance to 5-methyltryptophan and the episome itself to cells of another F$^-$ strain. The mutated episome no longer gave rise to tryptophan-independent merozygotes when transferred to F$^-$trpS5 cells, however. The mutants

carrying the mutated episome will therefore be referred to as MtrO-1 and MtrO-2, derived respectively from O-1 and O-2 strains.

Syntheses of all the enzyme of the tryptophan operon in strain MtrO-1 are only weakly repressible by tryptophan (Table 3). The resistance to 5-methyltryptophan was also found to be cotransduced with the trpA gene at high frequency by phage P1kc. Further studies with this strain including its relation to trpS, trpR and other 5-methyltryptophan resistant mutants are in progress.

Mutants similar to trpS5 reported above have been independently isolated in the laboratory of Dr. A. Matsushiro (personal communication).

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