STRUCTURE OF THE *trpC* CISTRON SPECIFYING INDOLEGLYCEROL PHOSPHATE SYNTHETASE, AND ITS LOCALIZATION IN THE TRYPTOPHAN OPERON OF *ESCHERICHIA COLI*[1]

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THE definition of an operon (JACOB and MONOD 1961) implies that it constitutes a unit of both gene structure and gene expression. In *Escherichia coli* the synthesis of the enzymes specific to the tryptophan pathway has been shown to be coordinate (ITO and CRAWFORD 1965) and subject to polarized regulation (SOMERVILLE and YANOFSKY 1965; YANOFSKY and ITO 1966). These observations have confirmed that the earlier reported closely linked sequence of *trp* genes (YANOFSKY and LENNOX 1959) constitute an operon. Since this latter report the complete sequence of enzymatic reactions in tryptophan synthesis by *E. coli*, under the control of five structural genes (*A-E*), has been elucidated (e.g. see YANOFSKY and ITO 1966).

YANOFSKY *et al.* (1964) have reported that the *E. coli trpA* and *B* genes which determine the α and β subunits of tryptophan synthetase are adjacent. One of the purposes of this study was to determine whether the *trpC* gene and the other genes of the tryptophan operon were directly adjacent to one another.

The enzyme indoleglycerol phosphate (InGP) synthetase is the gene product of the *trpC* locus (SMITH and YANOFSKY 1960; SMITH 1965). The single polypeptide chain of which InGP synthetase consists has recently been purified to homogeneity in the ultracentrifuge (CREIGHTON and YANOFSKY 1966; SMITH, unpublished) and shown to carry out the following consecutive reactions in tryptophan biosynthesis:

(1) Phosphoribosyl anthranilic acid (PRA) ——→ 1-(*o*-Carboxylphenylamino)-1-deoxyribulose 5-phosphate (CDRP)
(2) CDRP ——→ InGP

This report offers evidence that the ability to catalyze each of the above reactions is altered by mutations affecting different parts of the InGP synthetase polypeptide chain.

MATERIALS AND METHODS

Organisms: The mutants described in this report were derived from three strains of *E. coli* K-12; Y mel, W1485 and W3110. A number of the tryptophan auxotrophs from Y mel, T-4-3 (now *tryC1*), T-16 (*trpD1*), T-58 (*trpD2*) and T-80 (*trpD3*) were described (SMITH and YANOSKY 1960). The other *trpC* mutants were isolated from the latter two wild strains after ultraviolet

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or nitrosoguanidine treatment and penicillin selection. Phage T1-resistant *trp* deletions were isolated by the procedure of CRAWFORD and JOHNSON (1964) or were generously supplied by DRS. I. CRAWFORD and C. YANOFSKY. Strain 5927E, lacking anthranilate synthetase was a gift of DR. R. SOMERVILLE. A Shigella strain (Sh-16) was used as the sensitive indicator strain for the transducing phage P1*kc*.

Transduction procedures: Phage P1*kc* carrying markers of the donor bacteria was prepared by the confluent lysis technique (LENNOX 1955). Recipient bacteria were harvested by centrifugation from early log phase cultures in L broth (LENNOX 1955), washed in saline and suspended in saline containing 2.5×10^{-3} M $CaCl_2$. Crosses were performed using modifications of the transduction procedure of YANOFSKY and LENNOX (1959).

Qualitative mapping was achieved by spotting a 2 mm loopful of donor lysate (10^{10} to 10^{11} plaque forming units/ml) on a Tris-glucose agar plate (HERSHEY 1955) previously spread with approximately 5×10^9 cells of a T1 resistant *trp* deletion. After 48 hours incubation at 37°C the observation of prototrophic colonies within the lysate spot was recorded as positive evidence of recombination. In the rare instances where any ambiguity in interpretation of results occurred, transductions were performed using the technique employed for quantitative mapping.

Histidine-requiring *trp* mutants were constructed by transducing donor *trp* markers into a *his⁻cysB⁻* recipient and selecting for *his⁻cysB⁺trp⁻* recombinants (YANOFSKY and LENNOX 1959). Precautions taken to reduce the incidence of lysogeny among recipients included ultraviolet irradiation of the transducing lysate to reduce the lytic titer about 90% (transducing titer reduced about 50%) and infecting the recipients with a multiplicity of 0.5 phage per bacterium. Recombinant double mutants were examined for lysogeny by plating 10^7 cells with the Shigella (10^8 cells) in soft agar on L agar plates (LENNOX 1955).

Two-point transduction crosses were carried out by infecting 1 to 2×10^9 *his⁻trp⁻* recipients with 10^{10} donor phage in one ml of L broth containing 2.5×10^{-3} M $CaCl_2$. For closely linked *trp* markers the transduction mixture was increased up to fivefold. Infection was allowed to proceed for 20 minutes to one hour (no appreciable difference) at 37°C, the tubes were chilled and centrifuged and the cells suspended in 1.0 ml dilution fluid (0.03% NaCl, 0.1% peptone, 5×10^{-4} M $MgSO_4$, 10^{-2} M Tris pH7.8, 2.5×10^{-3} M $CaCl_2$). Appropriate dilutions were plated on a minimal-salts medium (VOGEL and BONNER 1956) containing 1.5% agar, 0.2% glucose and supplemented with the amino acid mixture of CRAWFORD and JOHNSON (1964). To score *trp⁺* recombinants each of four plates containing 30 μg/ml L-histidine in addition to the above supplements was seeded with 0.1 ml of the transduction mixture either by spreading or by the pour-plate technique. *his⁺* recombinants were measured on plates containing 40 μg/ml DL-tryptophan. After incubation for 48 hours at 37°C the number of recombinants was recorded. Appropriate controls for reversion and sterility were routine.

The ratio of tryptophan-independent to histidine-independent colonies gives a measure of recombination between two *trp* markers. As reported previously the transduction of the wild-type *trp* and *his* markers occurs in a ratio of about 1.8 (YANOFSKY and LENNOX 1959) for most recipients. I have found that additivity of map distances is somewhat improved by making correction for the transduction of wild-type markers into individual recipients. One of the most important considerations in obtaining reproducible recombination frequencies was found to be the maintainence of an optimal number of recombinant colonies per plate. In a transduction of *trpD3* into *his⁻trpC1*, the number of recipient cells plated was varied over a 13.5-fold range. The observed recombination frequency (*trp⁺/his⁺* × 100) was found to vary from 3.5 to 7.7, with the low values resulting when the number of *trp⁺* recombinants was below 20 per plate or the number of *his⁺* recombinants was over 300 per plate. To obviate the necessity for repeating each transduction numerous times the decision was made that standard optimal conditions for transduction should include counting only plates containing 20 to 300 recombinant colonies. Where these conditions were not met transductions were repeated with appropriate modifications or are indicated as not meeting these conditions.

Three-point crosses employing two *trp* markers in the recipients were performed in basically the same manner with more specific conditions described in RESULTS.

Enzyme activity: Cell-free extracts were prepared and enzymatic activities measured as

described previously (SMITH and YANOFSKY 1962). The InGP synthetase assay with CDRP as a substrate was modified to include incubation for 15 min at 37°C in 10^{-2} M Tris, pH 7.8. The attempts in this laboratory to devise a quantitative assay for the conversion of PRA to CDRP have not proved satisfactory. However a qualitative assay based on disappearance of anthranilate fluorescence catalyzed by PR-transferase has proved useful. PRA is relatively unstable, being readily hydrolyzed to release anthranilic acid. Since most glycosyl transfer reactions are freely reversible, the conversion of anthranilate to PRA by PR-transferase is expected to be reversible. Evidence to support this is offered by the observation that the transfersae reaction is readily inhibited by pyrophosphate and the course of the reaction can be extended by the addition of inorganic pyrophosphatase or PRPP when the fluorescence decrease begins to level off. Thus if PRA is not soon converted to the relatively stable CDRP, after an initial enzyme catalyzed decrease in anthranilate fluorescence there is a gradual return of fluorescence to the original level along with the formation of free anthranilic acid (GARCILASO and SMITH, unpublished). Extracts of *trpC* mutants which accumulate anthranilate and thus lack Reaction 1 (PRA to CDRP) invariably showed a return of anthranilate fluorescence after 15 to 20 minutes in the PR-transferase assay. With mutants which accumulated CDR (lack Reaction 2) there was no return of fluorescence even after 60 minutes of incubation. The ability to stabilize the fluorescence decrease was not a property of PR-transferase since these activities are separated by fractionation of extracts on Sephadex G-200. The elution profile of the stabilizing activity corresponds to the peak of CDRP to InGP activity when a normal extract is fractionated. Since *trpC* mutants which lack the ability to convert CDRP to InGP have the stabilizing activity it is concluded that the stabilizing activity is due to Reaction 1 and that both Reaction 1 and Reaction 2 are catalyzed by InGP synthetase. The presence of CRM (cross-reacting material) in extracts of mutants was detected by a competition assay using rabbit antibody prepared against partially purified InGP synthetase. The antibody both neutralized and precipitated the enzyme and the conditions for assay were similar to those employed in the measurement of tryptophan synthetase CRM (LERNER and YANOFSKY 1957).

RESULTS

Preliminary classification of mutants: During the course of this work over 500 *E. coli trp* mutants were isolated. Since they were selected for growth on indole as well as tryptophan, *trpB* mutants were excluded. Similarly mutants which accumulated indole or indoleglycerol (*trpA*) or those which grew on anthranilic acid (*trpE*) were not further characterized.

Earlier studies (SMITH and YANOFSKY 1960) had shown that mutants which accumulate CDR in liquid culture lack the ability to convert CDRP to InGP (InGP synthetase) and are suspected to be *trpC* mutants. Since the test for the accumulation of CDR is not unequivocal, the strains which gave a positive response for CDR were grouped, for further classification, with those which accumulated anthranilic acid.

The availability of a small number of phage T1 resistant *trp* deletions which accumulated anthranilic acid made feasible a qualitative mapping procedure. Since the deletions would be expected to end in the structural genes of the enzymes involved in the conversion of anthranilate to InGP, recombination between any point mutant and a deletion would indicate that the point mutation was located outside of the deleted gene sequence. The extent to which the deletions employed extend into the *trp* operon is indicated in the lower part of Figure 1. Point mutants were grouped into classes based on their ability to recombine with the series of overlapping deletions and the enzymatic reactions blocked (Table 1). Unfor-

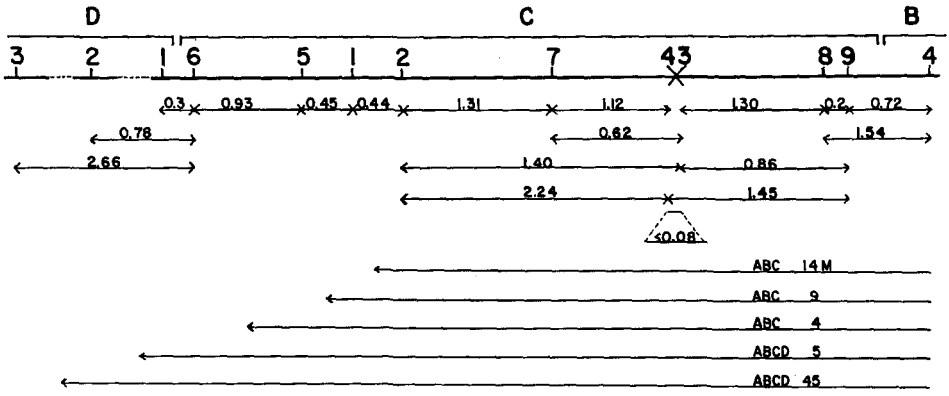

FIGURE 1.—Genetic map of the *trpC* cistron showing its position in the tryptophan operon. Map distances are derived from data in Table 2. The extent to which a number of T1 resistant *trp* deletions extend into the *C* and *D* cistrons is indicated in the lower portion of the figure. Mutants in the group *C5–2* accumulate CDR, while the other *C* mutants shown accumulate anthranilic acid.

tunately, even the smallest deletion (ABC14M) extended through well over half of the *trpC* cistron so that the Class I type (no recombination with any of the deletions indicated) represented the largest number, 132/307. Class II mutants recombine with ABC14M but not the other deletions and were represented by 37 individual isolates. Class III had 47 mutants, Class IV had 22, Class V had 39 and Class VI had 30, all of which were *trpC* or *trpD* mutants. The final distinction between the latter two cistrons was achieved by an examination of enzyme activities in extracts of the mutants and by their orientation in a genetic map.

Mapping of the trpC *cistron. Two-point crosses:* In order to estimate the size of the *trpC* cistron it was deemed desirable to map alleles which were close enough to preclude a high frequency of double exchanges, but yet to keep the number

TABLE 1

Characteristics of trpC *mutant classes*

Class	No. of mutants in class	Recombine with deletions*	Representative mutants	Accumulation products	Steps blocked
I	132	none	C8, 9, 4, 3, 7	anthranilate	1
			C2	anthranilate and CDR	2
II	37	ABC14M	C1	anthranilate and CDR	2
III	47	ABC14M ABC9	C5	anthranilate and CDR	2
IV	1†	ABC14M ABC9 ABC4	C6	anthranilate	1 and 2

* See Figure 1.
† Other strains which locate in this class by deletion mapping are PR-transferase mutants.

of crosses required within a reasonable number. These restrictions were met by setting as the goal the mapping of a sequence of alleles within approximately one map unit of each other and encompassing most of the *trpC* cistron. A series of preliminary screening transductions established the relationship between a number of mutants and several defined markers: *B4*, *C1* and *D1*. Then single mutants were crossed with each other reciprocally as demonstrated in Table 2. Figure 1 shows the genetic map derived from the data in the table. Transductions were generally repeated several times, but in some cases it was still not possible to achieve the standard set as the optimal population density of recombinants per plate (see MATERIALS AND METHODS). The ratio of trp^+ to his^+ recombinants was generally within 10% in multiple determinations and the spread seldom exceeded 20%. When these values were divided by the ratio of trp^+ to his^+ recombinants observed in a cross of wild type into each recipient, the measure of map distance between alleles was usually improved. No adequate explanation of greater than expected deviations in the measured map distance of reciprocal crosses is readily apparent. As seen in Figure 1 the additivity of distances between alleles when crosses extended over larger regions showed deviations from expected values, presumably due to interference. In at least one case the map does not allow an unambiguous interpretation of the orientation of two alleles, *C3* and *C4*.

The *trpC* mutants which accumulate CDR are located in the half of the *C* cistron nearest to *trpD*. A number of other isolates accumulating CDR appear to be closely linked to this group, *C2*, *1*, *5* and are adjacent on both sides to mutants which accumulate anthranilic acid. Site *C6* which presumably locates very near one end of the *C* cistron is the only representative of its type so far characterized. It has been shown to revert, but at low frequency. In two-point crosses and in deletion grouping, mutant *C6* shows no evidence of extensive genetic alterations. At the end of the *C* cistron nearest the *B* cistron there is evidence that other *C* mutants map between *C9* and *B4* (preliminary experiments) and *B4* is not the closest site to the *C* region so far described (CRAWFORD and JOHNSON 1964). To estimate the total map distance of the *C* cistron, I have assumed that it extends approximately half way between the terminal *C* sites indicated and the sites mapped on the neighboring cistrons. With this assumption the *trpC* cistron appears to be 6.3 map units long.

Three-point crosses: With one notable exception, it was possible to order the sites in the genetic map with data derived from two-point crosses. Reciprocal transductions between mutants *C3* and *C4* failed to give any significant number of prototrophs above those observed on reversion controls in spite of the use of 5× transduction mixture. As the data derived from crosses of *C3* and *C4* with other markers did not permit a decision as to their relative order recourse was made to three-point crosses. Double mutants containing an anthranilate synthetase defect, *5927E*, along with the *C* marker were constructed by transduction. Reciprocal crosses were performed in the usual manner with a 5× transduction mixture but recombination between *C3* and *C4* did not occur, as evidenced by the absence of colonies on plates supplemented with anthranilic acid. As a control on the method, mutants *C9* and *C6*, representing the opposite ends of the *C* cistron,

TABLE 2

Mapping summary

Donor	Recipient	Percent *trp*$^+$/*his*$^+$	No. of determinations	Recipient correction†	Corrected map distance	Average
B4	C9	1.14 ± 0.12	2	1.84	0.62	0.72
C9	B4	1.78 ± 0.18	2	2.17	0.82	
B4	C8	2.61 ± 0.10	2	1.68	1.55	1.54
C8	B4	3.35 ± 0.32	4	2.17	1.54	
C9	C8	0.49*	1	1.61	0.30	0.20
C8	C9	0.19 ± 0.05*	2	1.84	0.10	
C9	C3	1.49 ± 0.09	2	1.66	0.90	0.86
C3	C9	1.52 ± 0.30	2	1.84	0.83	
C8	C3	2.65 ± 0.21	4	1.66	1.59	1.30
C3	C8	1.70 ± 0.01	2	1.68	1.01	
C9	C4	2.49 ± 0.10	2	1.52	1.64	1.45
C4	C9	2.32 ± 0.18	2	1.84	1.26	
C3	C4	<0.20*	1	1.52	<0.13	<0.08
C4	C3	<0.05 ± 0.01*	2	1.66	<0.03	
C3	C7	0.90 ± 0.11*	3	2.04	0.44	0.62
C7	C3	1.34 ± 0.27	2	1.66	0.81	
C4	C7	1.53 ± 0.15	2	2.04	0.75	1.12
C7	C4	2.27 ± 0.01	2	1.52	1.49	
C3	C2	3.18 ± 0.22	4	1.95	1.63	1.40
C2	C3	1.95 ± 0.05	2	1.66	1.17	
C4	C2	4.27 ± 0.16	3	1.95	2.19	2.24
C2	C4	3.49 ± 0.41	2	1.52	2.29	
C7	C2	2.69 ± 0.09	2	1.95	1.38	1.31
C2	C7	2.54 ± 0.05	2	2.04	1.24	
C2	C1	0.89 ± 0.12	2	1.68	0.53	0.44
C1	C2	0.67 ± 0.12	2	1.95	0.34	
C1	C5	1.00 ± 0.00	3	2.23	0.45	0.45
C5	C1	0.75 ± 0.16	2	1.68	0.45	
C5	C6	1.80 ± 0.40	2	1.51	1.19	0.93
C6	C5	1.50 ± 0.10	2	2.23	0.67	
C6	D1	0.50 ± 0.08*	2	1.75	0.29	0.30
D1	C6	0.45 ± 0.10*	5	1.51	0.30	
C6	D2	1.46 ± 0.20	2	1.63	0.90	0.78
D2	C6	0.98 ± 0.06	2	1.51	0.65	
C6	D3	4.70 ± 0.30	2	2.19	2.14	2.66
D3	C6	4.80 ± 0.10	2	1.51	3.18	

* Estimated values since number of recombinants per plate not within optimal range.
† Ratio of *trp*$^+$ to *his*$^+$ recombinants observed in transduction with phage grown on wild-type cells.

TABLE 3

Three-point cross to establish order of trpC *mutants*

Cross	No. of colonies on anthranilic	No. of colonies on minimal	Percent anth+
C9 ——→ 5927E *C3*	52	31	60%
C6 ——→ 5927E *C3*	101	28	28%

Transduction of *C* mutants into a *trp* double mutant carrying an *E* cistron marker and *C3*. Recombinants were selected on minimal medium supplemented with anthranilic acid and then half of the colonies were tested for growth on minimal medium. Similar results were obtained when marker *C3* was replaced by *C4*.

were transduced into the same two double mutants. The results of two of the transductions are shown in Table 3. The data indicate the feasibility of the method since as expected the *C6* site would appear to be located between 5927*E* and *C3* while the *C9* site is outside of this region. In addition, the data offer additional evidence of the order of sites as 5927*E*–*C6*–*C3*–*C9* which had been determined by quantitative mapping. The decision as to whether *C3* and *C4* represent identical sites on the genetic map awaits future experiments.

Enzyme activities of mutants: The final determination of the placement of mutants in the *C* or *D* cistron was based on an examination of the enzymatic activities demonstrable in cell-free extracts of the mutants. Table 4 shows that one group of anthranilate-accumulating mutants clearly lack PR-transferase activity but have InGP synthetase. Since they are located in a small area of the genetic map, they are considered to represent alterations of the *trpD* cistron. The

TABLE 4

Enzyme activities of mutants

	InGP synthetase			
Mutant	CDRP→ InGP (2) μmoles/hr/mg	PRA→ CDRP (1)	CRM μmoles/hr/mg	PR-Transferase μmoles/hr/mg
A2	5.48	+	5.5*	4.08
B4	4.12	..	....	2.89
C9	0.341	—	0.406	...
C8	0.056	—	0.031	...
C3	0.003	—	0.009	1.36
C4	0.007	—	0.019	1.28
C7	0.032	—	0.031	...
C2	0	+	0.088	2.02
C1	0	+	0.064	1.91
C5	0	+	0.002	...
C6	0	—	0	0.69
D1	0.68	+	....	0
D2	0.52	+	....	0
D3	0.60	..	....	0
T15E	0.15	..	....	0.045
W1485	0.056	..	....	0.037

* Extract of mutant A2 was used as source of InGP synthetase to standardize antibody preparation.
Enzyme activities in extracts of derepressed mutants measured as described in MATERIALS AND METHODS. The blank spaces indicate that the assay was not done.

explanation for the behavior of *trpC* mutants, in accumulating either anthranilate or CDR, became apparent with the appreciation that highly purified InGP synthetase catalyzed two sequential reactions, PRA→CDRP→InGP (see Introduction). Thus mutants *C1, 2, 5*, which accumulate CDR, lack the ability to carry out the usual measured activity of InGP synthetase, the conversion of CDRP to InGP. They, as well as other CDR accumulators, form an altered enzyme as evidenced by cross-reaction with antibody to InGP synthetase, but still retain the ability to convert PRA to CDRP.

trpC mutants which accumulate anthranilic acid in liquid culture usually retain activity in the reaction CDRP to InGP but at greatly reduced levels. Based on the qualitative assay of stabilizing PR-transferase activity they are void of the ability to convert PRA to CDRP and thus possess an altered InGP synthetase. The amount of antigenic activity (CRM) retained is similar to the activity level in Reaction 2. These mutants all map in that portion of the cistron delimited by *C9* to *C7*. Mutant *C6* again proves unique in being the only strain so far examined which has neither enzymatic nor antigenic activity. The low level of PR-transferase activity in *C6* may be an indication of an antipolar effect. With the exception of mutant *C6* then, the *trpC* cistron seems to be divided into two regions; the part mapping nearest the *B* cistron determining that portion of InGP synthetase involved in Reaction 1, while the part near the *D* cistron codes for the activity of Reaction 2.

DISCUSSION

Analysis by transduction has demonstrated recombination between adjacent nucleotides (HENNING and YANOFSKY 1962), but as pointed out by GUEST and YANOFSKY (1965), the frequencies measured can only be considered approximate. A similar conclusion is derived from the demonstration here of the marked variability in observed recombination frequency depending simply on the number of recombinants plated. Thus measurement of very small map distances, even by transduction, can be subject to considerable error. It is not apparent what the optimal map distance for measurement may be, but it is clear that to achieve comparative results in transduction analysis the experimental procedure should be standardized.

The mapping of the *trpC* cistron is, of course, subject to the errors inherent in the experimental method but there is good reason to believe that with the criteria employed the genetic map presented is a fair representation of the structure of the cistron. With the estimate of total length of the *trpC* cistron as 6.3 map units and the knowledge that InGP synthetase consists of a single chain of about 435 amino acids (CREIGHTON and YANOFSKY 1966), a ratio of 69 amino acids per map unit can be calculated. This value is in good agreement with the ratio of 63 amino acids per map unit calculated from the size of the tryptophan synthetase *A* cistron (4.2 map units) and α subunit of 267 amino acids (YANOFSKY *et al.* 1967). With the total length of the *C* cistron equal to 6.3 map units, the area mapped between the terminal markers *C6* and *C9* should represent some 85% of the gene.

That the markers at the extremities of the *trpC* cistron map within less than one unit of known markers on adjacent cistrons makes it extremely improbable that any genes, other than those specifying enzyme primary structure are interposed through the sequence *trpB, C, D*. Although the sequence of *trp* genes in *Salmonella typhimurium* is the same as in *E. coli* (BLUME and BALBINDER 1966), other studies have led to the interpretation of an operator gene interposed between *trp* genes (MARGOLIN and MUKAI 1964). The *trpA* cistron of *E. coli* has been located adjacent to the *B* cistron (YANOFSKY *et al.* 1967) and if a similar relationship exists between the *trpD* and *E cistrons*, the tryptophan operon would appear to consist of a continuous linear array of structural genes, *E* to *A*. An operator locus may be either part of or immediately adjacent to the *E* gene (SOMERVILLE and YANOFSKY 1965).

InGP synthetase appears to be unique in the tryptophan pathway enzymes in not requiring the presence of another protein component for activity. It has been shown that anthranilate synthetase and PR-transferase are normally tightly bound (ITO and YANOFSKY 1966) and the tryptophan synthetase reaction is catalyzed by an association of α and β subunits (GOLDBERG *et al.* 1966). The ability to catalyze two consecutive reactions in tryptophan synthesis may also prove a characteristic of *E. coli* InGP synthetase different from the other enzymes in the pathway. Evidence has not yet been presented to show whether CDRP is formed as a free intermediate in *E. coli* during the conversion of PRA to InGP or whether it might be an enzyme bound intermediate as is indole in the conversion of InGP to tryptophan by tryptophan synthetase.

The observation that mutants which have lost one of the enzymatic activities of InGP synthetase locate in specific regions of the genetic map corresponding to which activity is altered has also been reported in Salmonella (BLUME and BALBINDER 1966). This behavior may be intepreted in at least several ways. It may mean that these mutants represent alterations in nucleotide codons which define particular amino acids at or near the active sites for the two reactions. Since this explanation involves two active sites on the enzyme and since the same types of mutations spread over large regions of the map, it is not considered the most probable. A second interpretation involves the visualization of a single polypeptide chain whose conformation when changed in the half of the molecule nearest the amino terminus results is loss of capacity to act on PRA, while a change in the carboxyl-half causes decreased catalytic activity on CDRP. The orientation of the carboxyl end of InGP synthetase with the nucleotide sequence of the *trpC* gene nearest the *B* gene has been deduced from the relationship between the α subunit of tryptophan synthetase and the *A* cistron (YANOFSKY *et al.* 1967). The carboxyl terminal sequence of normal and mutant InGP synthetases is being examined currently.

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SUMMARY

In the tryptophan operon the *trpC* cistron specifies the enzyme InGP synthetase. One or both of the reactions catalyzed by the enzyme can be altered by mutation and depending on the activity lost, the mutants are located in corresponding parts of the genetic map. The size of the *trpC* cistron as determined by transduction analysis is estimated to be 6.3 map units, and 85% of this distance is located between the two most distant markers studied. Evidence suggests that the *trp* operon of *E. coli* consists of a linear sequence of five structural genes, *E* to *A*, immediately adjacent to one another.

LITERATURE CITED

BLUME, A., and E. BALBINDER, 1966 The tryptophan operon of *Salmonella typhimurium*. Fine structure analysis by deletion mapping and abortive transduction. Genetics **53**: 577–592.

CRAWFORD, I. P., and L. M. JOHNSON, 1964 Mutants of *Escherichia coli* defective in the B protein of tryptophan synthetase. II. Intragenic position. Genetics **49**: 267–278.

CREIGHTON, T. E., and C. YANOFSKY, 1966 Indole-3-glycerol phosphate synthetase of *Escherichia coli*, an enzyme of the tryptophan operon. J. Biol. Chem. **241**: 4625–4637.

GOLDBERG, M. E., T. E. CREIGHTON, R. L. BALDWIN, and C. YANOFSKY, 1966 Subunit structure of the tryptophan synthetase of *Escherichia coli*. J. Mol. Biol. **21**: 71–82.

GUEST, J. R., and C. YANOFSKY, 1965 Amino acid replacements associated with reversion and recombination within a coding unit. J. Mol. Biol. **12**: 793–804.

HENNING, U., and C. YANOFSKY, 1962 Amino acid replacements associated with reversion and recombination within the *A* gene. Proc. Natl. Acad. Sci. U.S. **48**: 1497–1504.

HERSHEY, A. D., 1955 An upper limit to the protein content of the germinal substance of bacteriophage T2. Virology **1**: 108–127.

ITO, J., and I. P. CRAWFORD, 1965 Regulation of the enzymes of the tryptophan pathway in *Escherichia coli*. Genetics **52**: 1303–1316.

ITO, J., and C. YANOFSKY, 1966 The nature of the anthranilic acid synthetase complex of *Escherichia coli*. J. Biol. Chem. **241**: 4112–4114.

JACOB, F., and J. MONOD, 1961 On the regulation of gene activity. Cold Spring Harbor Symp. Quant. Biol. **26**: 193–209.

LENNOX, E. S., 1955 Transduction of linked genetic characters of the host by bacteriophage P1. Virology **1**: 190–206.

LERNER, P., and C. YANOFSKY, 1957 An immunological study of mutants of *Escherichia coli* lacking the enzyme tryptophan synthetase. J. Bacteriol. **74**: 494–501.

MARGOLIN, P., and F. H. MUKAI, 1964 Evidence for two operons in the tryptophan gene cluster in *Salmonella typhimurium*. (Abstr.) Bacteriol. Proc. p. 87.

SMITH, O. H., 1965 Genetic mapping of mutants deficient in indoleglycerol phosphate synthetase (*tryp-C* mutants). (Abstr.) Bacteriol. Proc. p. 29.

SMITH, O. H., and C. YANOFSKY, 1960 1-(*o*-Carboxyphenylamino)-1-deoxyribulose 5-phosphate, a new intermediate in the biosynthesis of tryptophan. J. Biol. Chem. **235**: 2051–2057. 1962 Enzymes involved in the biosynthesis of tryptophan. Meth. Enzymol. **6**: 794–806.

SOMERVILLE, R. L., and C. YANOFSKY, 1965 Studies on the regulation of tryptophan biosynthesis in *Escherichia coli*. J. Mol. Biol. **11**: 747–759.

VOGEL, H. J., and D. M. BONNER, 1956 Acetylornithinase of *Escherichia coli*: partial purification and some properties. J. Biol. Chem. **218**: 97–106.

YANOFSKY, C., and E. S. LENNOX, 1959 Transduction and recombination study of linkage relationships among the genes controlling tryptophan synthesis in *Escherichia coli*. Virology **8**: 425–447.

YANOFSKY, C., B. C. CARLTON, J. R. GUEST, D. R. HELINSKI, and U. HENNING, 1964 On the colinearity of gene structure and protein structure. Proc. Natl. Acad. Sci. U.S. **51**: 266–272.

YANOFSKY, C., and J. ITO, 1966 Nonsense codons and polarity in the tryptophan operon. J. Mol. Biol. **21**: 313–334.

YANOFSKY, C., G. R. DRAPEAU, J. R. GUEST, and B. C. CARLTON, 1967 The complete amino acid sequence of the tryptophan synthetase A protein (α subunit) and its colinear relationship with the genetic map of the *A* gene .Proc. Natl. Acad. Sci. U.S. **57**: 296–298.