POLARITY AND ENZYME FUNCTIONS IN MUTANTS OF THE FIRST THREE GENES OF THE TRYPTOPHAN OPERON OF *ESCHERICHIA COLI*[1]

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IN operons which are believed to be transcribed into polycistronic messenger RNA molecules, some revertible mutations result in a lowered rate of synthesis of enzymes specified by genes operator-distal to the mutated gene. This phenomenon, termed polarity (FRANKLIN and LURIA 1961; JACOB and MONOD 1961), has been studied in several organisms and a variety of explanations have been proposed for the observed effect on enzyme levels. Mutational changes which cause polarity are now known to have in common the introduction of a polypeptide chain-termination codon within a structural gene of an operon (NEWTON *et al.* 1965; WHITFIELD, MARTIN and AMES 1966; YANOFSKY and ITO 1966). Mutations of the missense type, resulting in amino acid substitutions, do not cause polarity.

In the present study mutants of the first three genes of the tryptophan operon of *Escherichia coli* were characterized. The properties of the mutants and their respective altered proteins or protein fragments revealed interesting features of the corresponding wild-type proteins. Many of the mutants exhibited polarity and were employed in determinations of the shape of the polarity gradient for their respective genes. Additionally, antipolarity (ITO and CRAWFORD 1965; YANOFSKY and ITO 1967), an effect on expression of the gene immediately preceding a gene with a chain termination mutation, was examined in strong polar mutants of several genes of the operon.

MATERIALS AND METHODS

Bacterial strains: Most of the mutants studied were isolated in the W3110 strain of *Escherichia coli* K-12; a few were obtained in strain Ymel. Mutagenesis was performed by treatment with ultraviolet light or ICR191A (3-chloro-7-methoxy-9-(3-[chloroethyl]-amino propylamino)-acridine dichloride); penicillin selection was employed to enrich for Trp auxotrophs. Every mutant studied should represent an independent mutational event because only one mutant of each type was saved from each treated subculture. For polarity determinations, each *trp* operon alteration was introduced into a W3110 background containing a *trpR*$^-$ allele (YANOFSKY and ITO 1966). The *trpR*$^-$ allele employed results in the production of elevated (constitutive) levels of all the *trp* operon enzymes when cells are grown in the presence or absence of excess tryptophan. In

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some cases a *tna*⁻ (tryptophanase-less) marker was present in the genetic background. [*tonB trp*] deletion mutants were isolated as described previously (COUKELL and YANOFSKY 1971). Internal deletion *trp BC27* was obtained from Dr. I. CRAWFORD. Strain *trp C55* was provided by Dr. O. SMITH.

Merodiploid strains were prepared by transferring the Fredericq *cysB–trp colVB* episome (FREDERICQ 1963) bearing the desired *trp* marker into an appropriate *recA⁻cys⁻trp⁻* recipient, selecting for *cys*+. Each chromosomal *trp* marker was introduced into a *thy⁻cysB⁻* [*tonB trpAE*] deletion mutant by replacement of the [*tonB trpAE*] deletion. *recA* was then crossed in from an Hfr *recA⁻thy*+ strain, selecting *thy*+ recombinants. All merodiploids employed were tested to ensure that they could transfer the Fredericq episome, and that the transferred episome had not undergone recombination in the *cysB–trp* region.

The order of genetic elements in the *trp* operon of *E. coli* is *OEDCBA*.

Suppression, mapping, reversion, and polarity: Suppression tests were performed as previously described (YANOFSKY and ITO 1967) by spot test transduction with P1kc lysates prepared on [*tonB trpAE*] deletion strains carrying $su^+_{am}1$ (Ser), $su^+_{am}2$ (Gln), $su^+_{am}3$ (Tyr), $su^+_{oc}4$ (Tyr), two additional and dissimilar ochre suppressors which we designate $su^+_{oc}A$ and $su^+_{oc}B$ (YANOFSKY and ITO 1966), and su^+_{UGA} (Trp) (SAMBROOK, FAN and BRENNER 1967). A mutant which is suppressed by any amber suppressor is considered an amber mutant. A mutant which is not suppressed by any of the amber suppressors but is suppressed by one of the ochre suppressors is classified as an ochre mutant. Several of the suppressors employed were supplied by Drs. A. GAREN and S. BRENNER.

The specialized non-defective *trp* transducing phage (pt's) used in mapping studies were obtained from others or isolated in this laboratory from lysogens of prototrophs or of appropriate [*tonB trp*] deletion mutants. Strain $i\lambda h\phi 80$ pt BA5-2 was provided by Dr. N. FRANKLIN and ϕ80pt CA77 by Drs. S. DEEB and B. HALL. The transduction mapping procedures employing recombination spot tests with [*tonB trp*] deletion mutants or recombination frequency determinations with *his⁻trp⁻* recipients were described previously (YANOFSKY *et al.* 1964; YANOFSKY and ITO 1966). Recombination frequencies were corrected on the basis of His+/Trp+ ratios obtained in control crosses in which P1kc grown on a wild-type donor strain was used to transduce each *his⁻trp⁻* recipient.

Reversion studies were performed by plating 0.05 ml (2×10^8 cells) of an overnight L-broth culture of a washed bacterial suspension on minimal agar with or without a supplement of 0.1 μg/ml L-tryptophan. In nitrosoguanidine (NG) and ICR191A (ICR) tests, 100 μg and 10 μg, respectively, in 0.05 ml, were spotted or spread on one section of the minimal agar surface. Plates were scored for revertants after 3 days of incubation at 37°C. ICR191A was kindly supplied by Dr. H. CREECH.

The extent of polarity in any mutant was determined by calculating the ratio of tryptophan synthetase A or B activity to anthranilate synthetase activity, and comparing the value so obtained with that of an appropriate missense mutant or prototroph control (YANOFSKY and ITO 1966). All strains examined contained the same *trpR⁻* allele and were grown in a medium containing excess tryptophan, as described under enzyme assays.

Enzyme assays: Each *trpR⁻* culture was grown at 37°C with vigorous shaking in minimal medium (VOGEL and BONNER 1956) supplemented with L-tryptophan, L-tyrosine and L-phenylalanine, each at 50 μg/ml, p-aminobenzoic acid and p-hydroxybenzoic acid, each at 1 μg/ml, and 0.2% glucose. Cells were harvested after 4 generations of growth; the cell concentration at the time of harvesting was $5–7 \times 10^8$/ml. The harvested cells were disrupted by sonic oscillation as described elsewhere (YANOFSKY and ITO 1966). The assay procedures for anthranilate synthetase, PRA transferase, InGP synthetase and tryptophan synthetase A and B were essentially as described elsewhere (YANOFSKY and ITO 1966) with the following minor changes: a) The 5-phosphoribosyl-1-pyrophosphate concentration in the PRA transferase assay was 5×10^{-4} M; b) Component I activation by Component II (anthranilate synthetase reaction) was accomplished by mixing extracts containing the two components and incubating at 37°C for 2 min prior to sampling for assays. We have found the latter procedure to be most effective in fully activating Component I.

RESULTS

Characteristics of trpC *mutants:* The *trpC* protein catalyzes the following two sequential reactions in tryptophan biosynthesis and is therefore properly designated N-(5-phosphoribosyl) anthranilate isomerase-indolyl-3-glycerol phosphate synthetase.

(1) N-(5′-phosphoribosyl) anthranilate (PRA)→ 1-(o-carboxyphenylamino)-1-deoxyribulose-5-phosphate (CdRP) (PRA isomerase reaction)

(2) CdRP → indolyl-3-glycerol phosphate (InGP) (InGP synthetase reaction)

The native protein has been obtained in pure form; it is a single polypeptide chain with a molecular weight of 45,000 daltons (CREIGHTON and YANOFSKY 1966). Kinetic analyses indicate that there are two distinct and non-overlapping active sites on the protein molecule, each concerned with one of the above reactions (CREIGHTON 1970). In view of the bifunctional capacity of this enzyme it is not surprising that complementation has been observed with *C*-gene mutants in both *in vivo* and *in vitro* tests (BLUME and BALBINDER 1966; SMITH 1967; CREIGHTON 1970; this report).

The genetic and biochemical properties of the mutants studied are summarized in Figure 1 and Table 1. Mutant sites were grouped unambiguously by deletion mapping and ordered approximately within groups and with respect to mutant sites in other groups on the basis of recombination frequency data. The distance of each mutant site from the origin of *trpC* was estimated as follows: The total recombinational length of *trpC* based on mapping data was taken as 5 units. Since the *trpC* polypeptide has a molecular weight of 45,000 daltons, there should be *ca.* 1,248 nucleotide pairs in *trpC*. Using the ratio 250 base pairs per map unit (1,248 nucleotide pairs/5 map units), the estimated distance in nucleotide pairs from the origin to each mutant site was calculated. This number was then assigned as the mutant number. Although these mutant numbers certainly do not correspond exactly to the nucleotide pair changed by mutation, they do indicate relative location in *trpC* and therefore are considered preferable to random isolation numbers as designations for mutants. Some mutants (e.g., *trpC657* and *trpC700*, Figure 1) give extraordinarily high or low recombination values which are inconsistent with genetic map region assignments provided by deletion mapping. In such cases we consider the region assignment accurate and the location assignment arbitrary. In view of the known effects of single nucleotide pair changes on recombination frequency (YANOFSKY *et al.* 1964; YANOFSKY 1967; NORKIN 1970), mutants which behave aberrantly are to be expected. Despite this difficulty one is obliged to accept the genetic map as a crude approximation of physical distance.

Several of the ICR191 induced *trpC* mutants reverted in response to treatment with ICR or nitrosoguanidine. This behavior has been noted previously in reversion studies with these mutagens (OESCHGER and HARTMAN 1970); in such cases the interpretation given is that the mutant change is a +1 (base pair addition) frameshift which is reverted by a −1 (base pair deletion) frameshift induced by ICR *or* NG treatment (OESCHGER and HARTMAN 1970).

Of the many point mutants examined, including those with chain termination

TABLE 1

Characteristics of trpC *mutants*

Mutant number	Isolation number	Estimated map units from origin	Inducing mutagen	Suppression[a] su^+_{am}	Suppression[a] su^+_{oc}	Reversion ICR	Reversion NG	Accumulation*	Leakiness	Probable mutant type
55	6	.22	UV	—	—	—	—	AA	—	ct
142	10377	.57	UV	+	+	—	+	AA	—	am
315	9905	1.26	UV	+	+	—	+	AA	—	am
385	9870	1.54	UV	+	+	—	+	AA	—	am
426	10243	1.70	UV	+	+	—	+	AA	—	am
486	10295	1.94	UV	+	+	—	+	AA	—	am
658	9875	2.63	UV	—	+	—	+	AA	—	oc
670	9800	2.68	UV	—	—	—	+	CdR	—	ms
675	9771	2.7	UV	+	+	—	+	AA	—	am
700	10398	2.8	UV	+	+	—	±	AA	—	am
726	30–1	2.9	ICR	—	—	+	—	AA	—	fs
755	223–1	3.02	ICR	—	—	+	+	AA	—	fs
756	26	3.02	ICR	—	—	+	+	AA	—	fs
757	10A	3.02	ICR	—	—	—	—	AA	—	fs
782	9941	3.13	UV	—	—	—	+	CdR	—	ms
800	32	3.20	ICR	—	—	+	+	AA	—	fs
801	21–1	3.20	ICR	—	—	+	+	AA	—	fs
802	74	3.21	ICR	—	—	+	—	AA	—	fs
807	63	3.23	ICR	—	—	+	—	AA	—	fs
812	2A	3.25	ICR	—	—	+	+	AA	—	fs
815	10394	3.26	UV	—	—	—	—	AA	—	ct
885	17–1	3.54	ICR	—	—	+	+	AA	—	fs
954	15–2	3.82	ICR	—	—	—	—	AA	+	fs
956	20	3.83	ICR	—	—	+	—	AA	++	fs
1009	15–3	4.03	ICR	—	—	—	—	AA	+	fs
1047	51	4.18	ICR	—	—	+	—	AA	++	fs
1117	9830	4.46	UV	—	—	—	+	AA	—	ms

AA = anthranilic acid
CdR = 1-(o-carboxyphenylamino)-1-deoxyribulose
a = suppressed by one or more amber or ochre suppressors, or by both types of suppressor.
am = amber; oc = ochre; fs = frameshift; ms = missense; ct = chain termination and therefore polar, but the nature of the mutational change is not known.
UV = ultraviolet light
ICR = ICR 191-A
NG = N-methyl-N′-nitro-N-nitrosoguanidine
* PRA spontaneously decomposes to anthranilate and therefore the latter compound is accumulated by mutants blocked in PRA metabolism.

mutations near the end of the gene, only two, both missense mutants, accumulate CdR. In addition, none of the [*tonB trpAC*] deletion mutants listed in Figure 1, including those with termini most operator-distal in *trpC*, accumulates CdR. These findings suggest that some sequence of amino acids at or near the carboxy terminus of the *trpC* polypeptide is essential for PRA isomerase activity, and this sequence is lacking in the mutants mentioned. Alternatively, incomplete *trpC* polypeptides may be produced and then degraded (PLATT, MILLER and WEBER 1970; GOLDSCHMIDT 1970), obscuring their catalytic capabilities. Several mutants

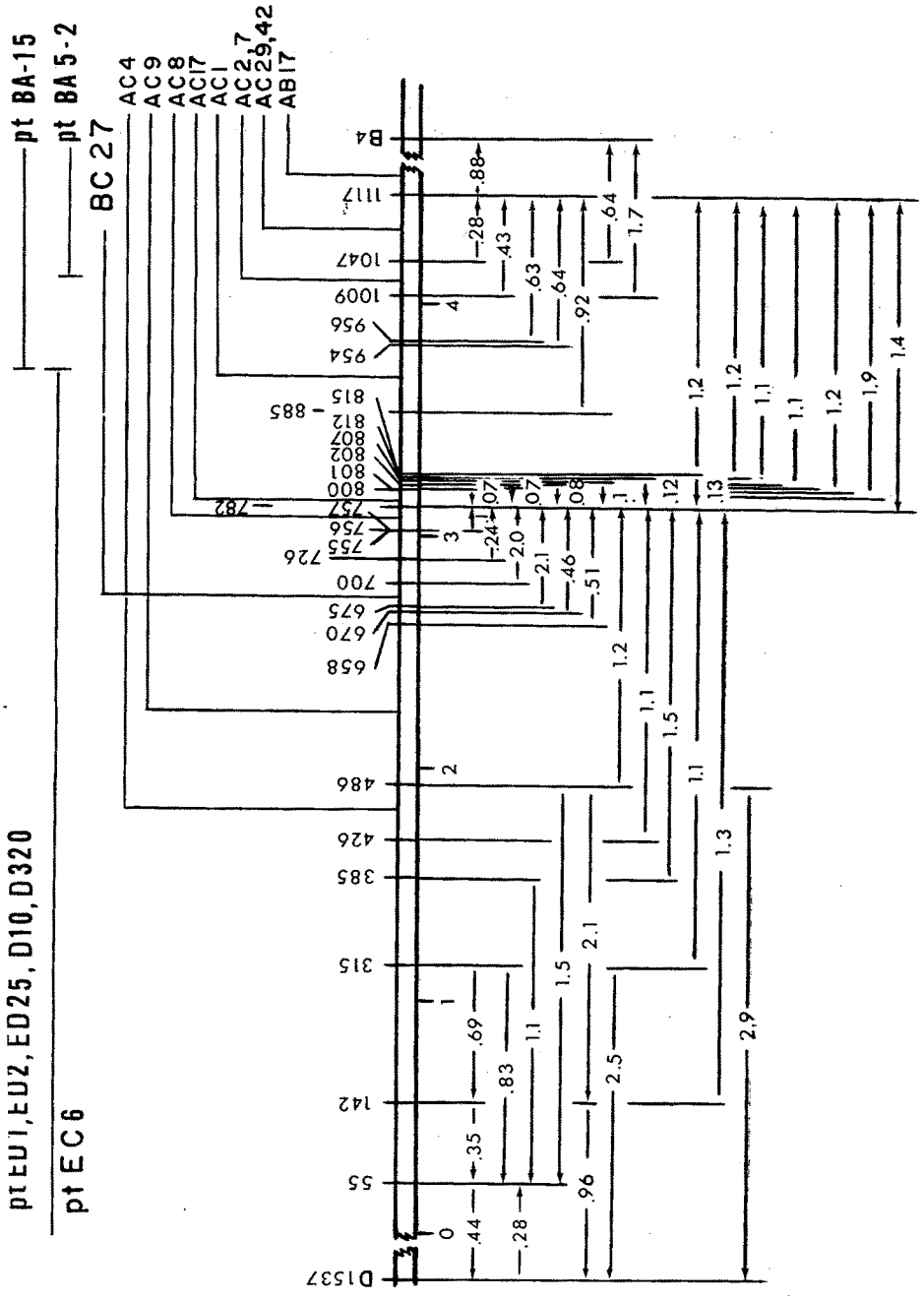

FIGURE 1.—Genetic map of *trpC*. Strains *AB17* and *AC1* through *AC42* carry overlapping [*tonB trp*] deletions, with termini in the *trp* operon as indicated. Since *tonB* is on the *trpA* side of the operon, these deletion mutants lack the entire region between the terminus in the *trp* operon and the terminus in or beyond *tonB*. Strain *BC27* is an internal deletion with termini in *trpC* (shown) and *trpB*. The regions of the *trp* operon *carried by* φ80 or lambda-φ80 transducing phage are indicated by bars. The recombination values presented are based on Trp prototroph frequencies (recipient designated by the head of the arrow), normalized to the frequency of transduction of a *his*$^-$ marker of the recipient.

TABLE 2

Complementation tests with trpC, trpD *and* trpE *mutants*

Chromosome marker	Episome marker								
	E10323am	E10220ms	E5972oc	D159oc	D822fs	D1081ms	D1431oc	C142am	C670ms
E9914oc		—	—				±	±	
E9851oc–E9829am		—	—				±	±	
E5972oc	—	—		+	+		+		
E5947ms	—	—				+			
D159oc	+	+	+		—	—	—	+	+
D822fs		+		—			—		
D928ms		+		—	—	—	—		
D1431oc		+		—	—				
C885fs		+						—	—
C117ms								—	+

+ = growth of the respective merodiploid on minimal agar. All strains are *recA*⁻.

with alterations near the end of the gene are leaky and one, *trpC1047*, has low levels of InGP synthetase activity. Presumably the leaky mutants also have some PRA isomerase activity but the activity must be low, otherwise they would accumulate CdR. Deletion mutants *tonB trpAC17*, *AC1*, *AC2*, *AC7*, *AC29*, and *AC42* also have low levels of InGP synthetase activity. *TrpC1117*, a mutant which lacks PRA isomerase activity, has normal levels of InGP synthetase. We classify this strain as a missense mutant because of this and because in distinction to other *trpC* mutants with alterations near the end of the gene, such as *trpC1047*, it is not leaky. Conceivably, however, it could be a nonsense or frameshift mutant.

Complementation is observed in merodiploids constructed with the two presumed missense mutants *trpC670* and *trpC1117* (Table 2). This is expected because the altered *trpC* polypeptides specified by these mutant genes are fully active in the PRA isomerase, and InGP synthetase reactions, respectively (CREIGHTON 1970). *TrpC670* does not complement *trpC885*, a frameshift mutant altered in the terminal quarter of the *C* gene. Complementation tests with chain termination mutants (*trpC142* and *trpC885*) altered near the beginning and end of the gene, respectively, were negative (Table 2), providing additional confirmation of the conclusion that *trpC* specifies a single polypeptide chain (CREIGHTON and YANOFSKY 1966; CREIGHTON 1970).

To summarize the findings with *trpC* mutants, despite the bifunctional nature of the *trpC* polypeptide, and the fact that missense mutants with alterations in different regions of the molecule have full activity in one of the two reactions, a polypeptide of near normal length may be required for either activity to be expressed fully. However, the strains available do not permit us to draw any conclusions concerning the possibility that a polypeptide consisting solely of the last half of the *trpC* polypeptide would have normal PRA isomerase activity.

The polarity gradient obtained with *trpC* mutants of the various classes which

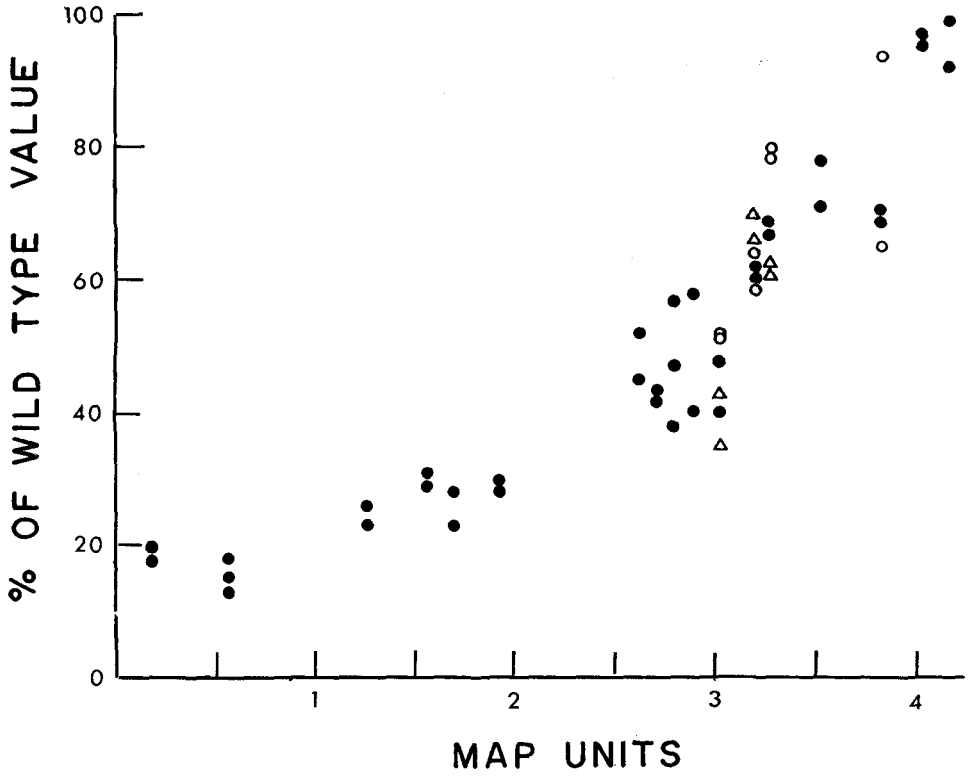

FIGURE 2.—Polarity gradient for *trpC* mutants. The relative specific activity of tryptophan synthetase A or B is plotted *vs.* the estimated map location of the corresponding mutational alteration. Each point represents a polarity determination for a separate culture of a presumed *trpC* chain termination mutant. All strains were *trpR*⁻ and were harvested during logarithmic growth in the presence of excess tryptophan (see METHODS). The different symbols at the same map position represent values for different mutants with alterations at approximately the same map position.

would be expected to exhibit premature chain termination is shown in Figure 2. The strongest polar mutant in *trpC* is about 80–85%polar and the gradient increases progressively until chain termination mutants are encountered which are non-polar. *TrpC* missense mutants are non-polar.

Characteristics of trpD *mutants:* In *E. coli* the *trpD* and *trpE* proteins are components of the anthranilate synthetase–phosphoribosyl anthranilate transferase complex (ITO and YANOFSKY 1969; ITO, COX and YANOFSKY 1969). This complex catalyzes the following reactions in tryptophan biosynthesis:

(1) Chorismate + L-glutamine → anthranilate (anthranilate synthetase reaction)

(2) Anthranilate + 5-phosphoribosyl-1-pyrophosphate → PRA (PRA transferase reaction).

The *trpE* protein, termed Component I of the complex, bears primary responsibility for conversion of chorismate → anthranilate, while the *trpD* protein, Com-

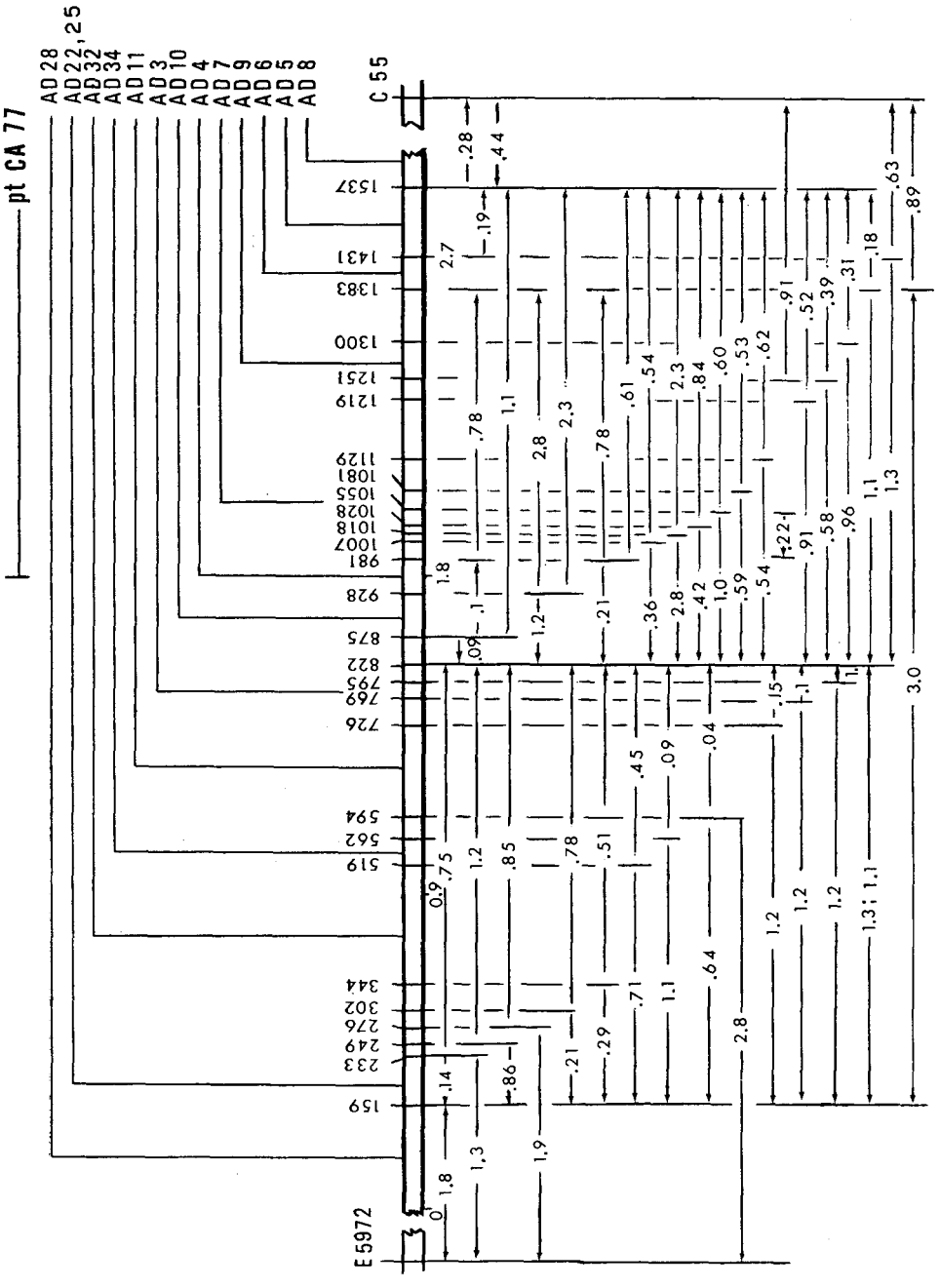

FIGURE 3.—Genetic map of *trpD*. Strains *AD3* through *AD34* carry overlapping [*tonB trp*] deletions with termini in *trpD* as indicated. The region of *trpD* carried by *ptCA77* is indicated by the bar. The recombination values presented are based on Trp prototroph frequencies (recipient designated by the head of the arrow) normalized to the frequency of transduction of a *his*⁻ marker of the recipient.

ponent II, is uniquely concerned with the conversion of anthranilate → PRA. It is believed that *trpE* and *trpD* each specify a single polypeptide chain and that the anthranilate synthetase-PRA transferase complex is a tetramer composed of two chains of each type. The *trpE* polypeptide has been obtained in pure form from *E. coli* (ITO, COX and YANOFSKY 1969) and the *trpE* and *trpD* polypeptides (*E. coli* nomenclature) have been isolated in pure form from *Salmonella typhimurium* (NAGANO and ZALKIN 1970; NAGANO, ZALKIN and HENDERSON 1970). Separated Component I is inactive in the conversion of chorismate + L-glutamine → anthranilate but will catalyze anthranilate formation from chorismate when NH_3 replaces L-glutamine as amino group donor. Separated and complexed Component II are equally active in the conversion of anthranilate → PRA. Thus Component II provides the PRA transferase activity of the anthranilate synthetase–PRA transferase complex and contributes (NAGANO, ZALKIN and HENDERSON 1970), or activates, the site for L-glutamine utilization.

The genetic and biochemical properties of the *trpD* mutants examined in the present study are summarized in Table 3 and Figure 3. Procedures identical to those employed in the approximate ordering of *trpC* mutant sites were used in the construction of the genetic map in Figure 3. The recombinational length of *trpD* based on the data in Figure 3 was taken as 3.15 map units and the number of nucleotide pairs assumed to be *ca.* 1,672 (based on a polypeptide molecular weight of *ca.* 60,000 daltons). These figures are equivalent to a ratio of 530 nucleotide pairs per map unit. As was the case with *trpC* mutants, we have assigned *trpD* mutant numbers on the basis of estimated distances in nucleotide pairs from the origin of the gene. Several *trpD* mutants gave obviously aberrant recombination values, e.g., mutants *trpD928* and *trpD1018*, in comparison with other mutants altered in the same region of the map. The relative locations of the altered sites in these mutants is therefore arbitrary.

Mutants of the amber, ochre, frameshift, unclassified chain termination and missense types were isolated. With the exception of mutants with alterations in the initial segment of *trpD*, all the strains examined accumulated large amounts of anthranilate when grown with a limiting supplement of tryptophan. Since the poor accumulators contain normal levels of Component I, this finding indicates that *in vivo* NH_3 is not used efficiently as an amino group donor. These observations also suggest that *in vivo* a fragment of the *trpD* polypeptide one-fourth of normal length or larger is sufficient to permit L-glutamine utilization. Consistent with this conclusion are the results of enzyme assays (last column, Table 3), which demonstrate that extracts of all but the first group of *trpD* mutants and one exception, *trpD1028*, catalyze anthranilate formation with L-glutamine as amino group donor. It is also evident from Table 3 that extracts of only the *trpD* missense mutants have fully active Component I. The lower values obtained with extracts of other *trpD* mutants may reflect less effective Component II fragments, or different extents of degradation (PLATT, MILLER and WEBER 1970; GOLDSCHMIDT 1970) or synthesis of polypeptide fragments fully active in permitting L-glutamine utilization. The one exceptional mutant mentioned, *trpD1028*, apparently produces a polypeptide *in vivo* which is capable of per-

TABLE 3

Characteristics of trpD *mutants*

Mutant number	Isolation number	Estimated map units from origin	Inducing mutagen	Suppression[a] su^+_{am}	Suppression[a] su^+_{oc}	Reversion ICR	Reversion NG	Accumulation*	Leakiness	Probable mutant type	Relative component I activity,† percent
159	9778	.3	UV	—	+	—	+	faint	—	oc	0
233	57	.44	ICR	—	—	+	—	none	++	fs	0
249	9838	.47	UV	+	+	?	?	+	—	am	0
276	10007	.52	UV	—	—	?	?	+	++	ct	0
302	27	.57	ICR	—	—	+	—	none	++	fs	0
344	75	.65	ICR	—	—	+	—	faint	++	fs	0
519	62	.98	ICR	—	—	+	—	faint	+	fs	0
562	9923	1.06	UV	—	—	—	—	++	—	ms	100
594	64	1.12	ICR	—	—	—	—	++	+	fs	18
726	42	1.37	ICR	—	—	—	—	++	—	fs	23
769	24	1.45	ICR	—	—	+	+	++	—	fs	22
795	35	1.5	ICR	—	—	+	—	++	—	fs	31
822	9885	1.55	UV	—	—	+	—	++	—	fs	34
875	9929	1.65	UV	—	—	+	—	++	—	fs	37
928	10303	1.75	UV	—	—	—	+	++	—	ms	92
981	10497	1.85	UV	—	—	—	—	++	—	ct	37
1007	17	1.9	ICR	—	—	—	—	++	—	fs	16
1018	9924	1.92	UV	+	+	+	+	++	—	am	5
1028	4925	1.94	UV	—	—	+	—	++	—	fs	0
1055	10451	1.99	UV	—	+	—	+	++	—	oc	29
1081	10299	2.04	UV	—	—	—	+	++	—	ms	90
1129	10207	2.13	UV	+	+	—	—	++	—	am	17
1219	9996	2.3	UV	—	—	—	—	++	—	ct	34
1251	10023	2.36	UV	—	—	+	—	++	—	fs	35
1300	16–1	2.46	ICR	—	—	+	—	++	—	fs	26
1383	10500	2.61	UV	—	+	—	+	++	—	oc	51
1431	10401	2.7	UV	—	+	+	+	++	—	oc	33
1537	T16	2.9	UV	—	—	—	+	++	—	?	11

† Component I activity in the absence of added Component II divided by Component I activity in the presence of an added excess of Component II, with L-glutamine as amino group donor.

* Accumulation of anthranilate in culture filtrates.

a = suppressed by one or more amber or ochre suppressors, or by both types of suppressor.

am = amber; oc = ochre; fs = frameshift; ms = missense; ct = chain termination and therefore polar, but the nature of the mutational change is not known.

UV = ultraviolet light

ICR = ICR 191-A

NG = N-methyl-N′-nitro-N-nitrosoguanidine

mitting L-glutamine utilization because the mutant accumulates anthranilate.

To examine further the function of *trpD* polypeptide fragments in L-glutamine utilization, extracts of deletion mutants with termini at different points in *trpD* were also assayed and the culture filtrates of these strains examined for anthranilate accumulation (Table 4). Deletion mutants *tonB trpAD28* through *trpAD25* did not accumulate anthranilate nor did their extracts permit L-glutamine utilization in anthranilate formation. Deletion mutant [*tonB trpAD32*] accumu-

TABLE 4

Anthranilate synthetase activity of extracts of [tonB trpAD] *deletion mutants*

Deletion mutant*	Anthranilate accumulation	Relative Component I activity: Percent of activity with Component II added†
trpR⁻ tonB trpAD28	0	0
trpR⁻ tonB trpAD22	0	0
trpR⁻ tonB trpAD25	0	0
trpR⁻ tonB trpAD32	+	0
trpR⁻ tonB trpAD11	+	53
trpR⁻ tonB trpAD3	+	30
trpR⁻ tonB trpAD4	+	16
trpR⁻ tonB trpAD7	+	18
trpR⁻ tonB trpAD6	+	27
trpR⁻ tonB trpAD12	+	80
trpR⁻ tonB trpAD8	+	14

Cultures were grown in liquid medium with vigorous aeration and harvested during logarithmic growth as described in METHODS.

* Deletion mutants are listed in the same order as their respective deletion termini in *trpD* (see Figure 3).

† Extracts were assayed with and without the addition of Component II, with L-glutamine as amino group donor.

lated anthranilate but a *trpD* fragment could not be detected in enzyme assays. All other *trpD* deletion mutants gave positive results in both tests, despite the fact that in each strain a segment of unknown length from some other protein is probably fused to the remaining amino terminal segment of the *trpD* polypeptide. Comparison of the positions of deletion termini in these mutants with the map locations of *trpD* point mutant sites (Figure 3) indicates that approximately the same point on the map divides anthranilate accumulator-L-glutamine utilizers from nonaccumulator–nonutilizers.

The data in Figure 4 illustrate the saturation of Component I and presumed Component I–[Component II fragment] complexes (slightly active) by free Component II. Since extracts of all *trpD* mutants can be activated to the same specific activity in the anthranilate synthetase reaction by unaltered Component II (see Table 7), it is believed that the anthranilate synthetase activity exhibited by extracts of mutants such as *trpD795* and *trpD594* is due to activation of their Component I by reversible association with a slightly functional Component II fragment, or full activation of a fraction of their Component I molecules by a variable but limiting number of Component II fragments. No attempt was made to distinguish between these alternatives. Activation of the Component I of a [*tonB trpAD*] deletion mutant has been demonstrated (ITO and YANOFSKY 1969). The inset in Figure 4 shows that the Component II of *trpE* missense mutants is generally not readily dissociable from the presumed anthranilate synthetase–PRA transferase complex. In the experiment illustrated, increasing amounts of PRA transferase (present in the various mutant extracts) were added to a constant amount of purified Component I and anthranilate formation from

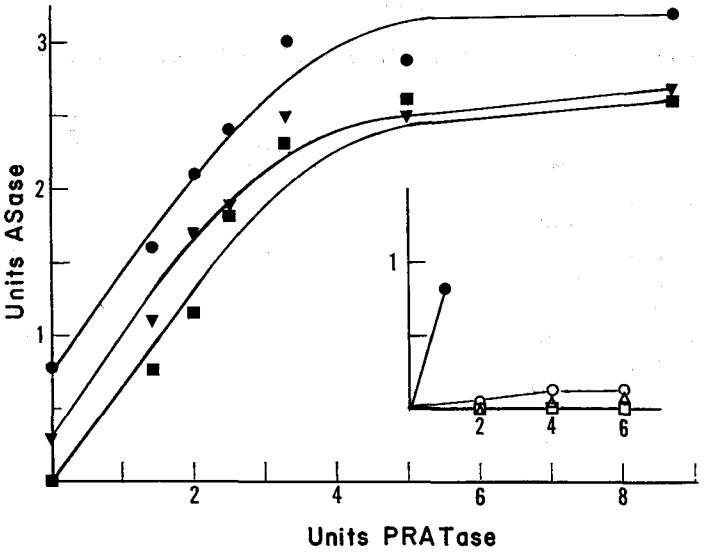

FIGURE 4.—Interactions between anthranilate synthetase-PRA transferase components. Large figure: activation of the Component I of three chain termination mutants (■ = *D159*; ▲ = *D594*; ● = *D795*) by increasing amounts of Component II (PRA transferase). Anthranilate synthetase (ASase) activity was measured. L-glutamine was the sole amino group donor included in the assay mixture (see METHODS). Note that extracts of *D594* and *D795* have activity in the absence of added Component II. Inset: Assay for the dissociation of Component II from the anthranilate synthetase-PRA transferase complexes of presumed *trpE* missense mutants (□ = *ET3*; △ = *E10220*; ○ = *E5947*; ● = *E5972*). Twenty units of Component I were mixed with increasing amounts of extracts of the *trpE* mutants. *trp E5972* is a nonsense mutant, the others are missense mutants. Glutamine was the sole amino group donor included in the assay mixture (see METHODS).

chorismate + L-glutamine was measured. It is evident that dissociation was observed only with the *trpE5947* extract, and then, only to a slight extent. The control strain, *trpE5972*, is a nonsense mutant. If the behavior of the anthranilate synthetase–PRA transferase complex of the three *trpE* missense mutants is typical of the normal complex, association of the subunits of anthranilate synthetase can be considered to be essentially irreversible. It is not known whether the presumed Component II fragments of *trpD* chain termination mutants readily dissociate from Component I.

Several *trpD* mutants of the chain termination type are leaky. Extracts of *trpD233* and *trpD302* were assayed for PRA transferase activity and low, readily detectable levels were found. This activity could be partially responsible for the poor accumulation of anthranilate by these strains. Leakiness of chain termination mutants can be due to reinitiation of polypeptide synthesis at a Met codon near the chain termination codon (SARABHAI and BRENNER 1967). If this is the explanation in this case it is not expressed as polarity relief in the polarity determinations with these mutants (Figure 5); however, only if the reinitiation codon were close to the termination codon would appreciable relief of polarity be

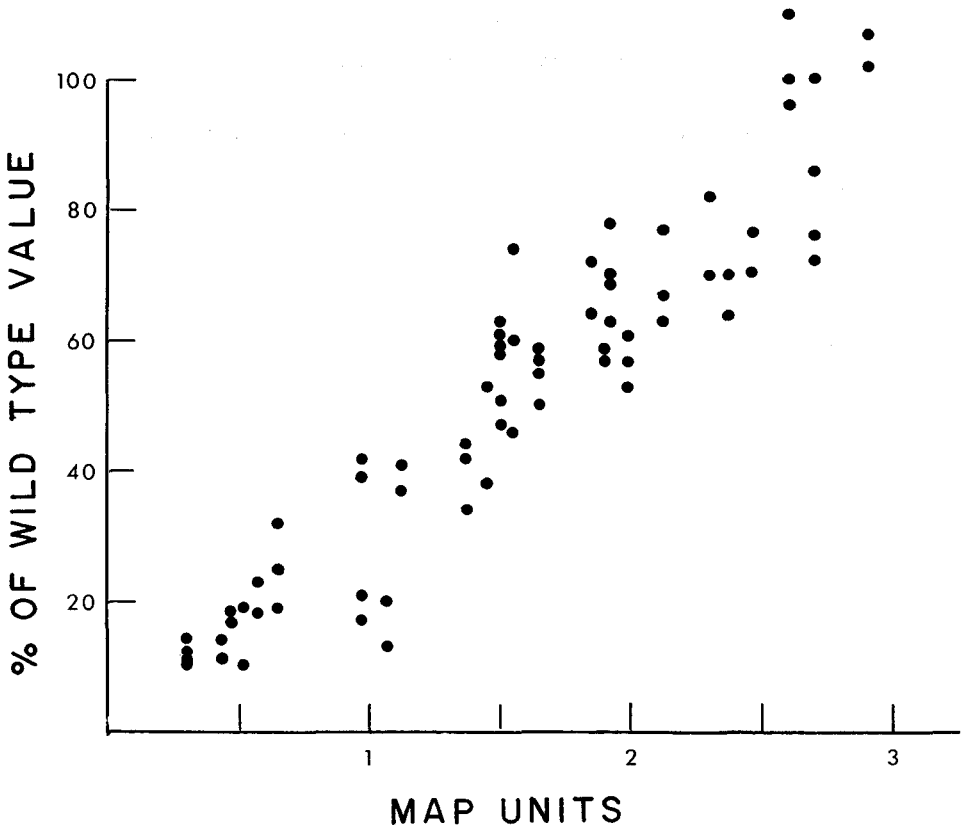

FIGURE 5.—Polarity gradient for *trpD* mutants. The relative specific activity of tryptophan synthetase A or B is plotted *vs.* the estimated map location of the corresponding mutational alteration. Each point represents a polarity determination for a separate culture of a presumed *trpD* chain termination mutant. All strains were *trpR*$^-$ and were harvested during logarithmic growth in the presence of excess tryptophan (see METHODS).

expected (NEWTON 1969; ZIPSER *et al.* 1970). The fact that extracts of leaky *trpD* mutants do not exhibit anthranilate synthetase activity with L-glutamine as amino group donor (an exception is *trpD594*) suggests that a proper initial segment of the *trpD* polypeptide is not present in this strain. The low level of PRA transferase activity could be explained if the terminal two-thirds of the *trpD* polypeptide were sufficient for PRA transferase activity, since leaky chain termination mutants could be producing such fragments. In fact, it is conceivable that some of the leaky *trpD* mutants are auxotrophs because the *trpD* fragment they produce is unable to activate Component I, rather than because they have low levels of PRA transferase activity. Complementation tests with *trpD* missense mutants and chain termination mutants with alterations at opposite ends of the gene support the conclusion that *trpD* specifies a single polypeptide chain (Table 2).

The polarity gradient obtained with *trpD* mutants of the various classes which

TABLE 5

Characteristics of trpE *mutants*

Group	Isolation number	Estimated map units from origin	Inducing mutagen	Suppression[a] su^+_{am}	Suppression[a] su^+_{oc}	Reversion ICR	Reversion NG	Leakiness	Anthranilate sensitivity[b]	Probable mutant type
	9914	.2	UV	—	+	—	+	—	S	oc
I	9777	.55	UV	—	—	+	+	—	S	fs
	10294	.85	UV	—	+	—	+	—	S	oc
	10320	.85	UV	—	—	—	+	—	S	ct
	10323	1.95	UV	+	+	—	+	—	S	am
II	9829	2.05	UV	+	+	—	+	—	S	am
	9809	2.05	UV	+	+	—	+	—	S	am
	9851	3.45	UV	—	+	—	+	—	S	oc
	10385	3.55	UV	—	+	—	+	—	S	oc
III	10381	3.6	UV	—	—	—	—	—	S	ct
	6185	...	UV	—	+	—	+	—	S	oc
	10256	...	UV	+	+	—	+	—	R	am
	5927	3.65	UV	—	—	—	—	—	S	ct
	30–2	4.05	ICR	—	—	+	—	—	R	fs
IV	9773	4.15	UV	—	+	—	+	—	R	oc
	10231	4.3	UV	—	+	—	+	—	R	oc
	9802	...	UV	+	+	—	+	—	S	am
	E3	...	ICR	—	—	—	—	—	R	fs
	9780	5.45	UV	+	+	—	+	—	R	am
V	10237	...	UV	+	+	—	+	—	R	am
	10247	...	UV	—	+	—	+	—	R	oc
	10491	...	UV	+	+	—	+	+	R	am
	10220	6.3	UV	—	—	—	+	—	R	ms
	9903	6.4	UV	—	—	+	—	—	R	fs
VI	9887	6.45	UV	—	—	+	—	—	R	fs
	T3	...	UV	—	—	—	+	—	R	ms
	T15	...	UV	—	—	+	—	—	R	fs
VII	72	6.55	ICR	—	—	+	+	—	R	fs
	31	...	ICR	—	—	+	—	—	R	fs
VIII	83	6.65	ICR	—	—	+	—	—	R	fs
	5984	6.8	UV	—	+	—	+	—	R	oc
IX	5972	6.9	UV	—	+	—	+	—	R	oc
	31–2	7.0	ICR	—	—	+	—	—	R	fs
X	22–1	7.1	ICR	—	—	—	—	—	R	fs
	5947	7.3	UV	—	—	—	—	—	R	ms

[b]S/R = growth completely inhibited or not on minimal agar supplemented with 30 μg/ml anthranilic acid.

a = suppressed by one or more amber or ochre suppressors, or by both types of suppressor.

am = amber; oc = ochre; fs = frameshift; ms = missense; ct = chain termination and therefore polar, but the nature of the mutational change is not known.

UV = ultraviolet light.

ICR = ICR 191-A.

NG = N-methyl-N′-nitro-N-nitrosoguanidine.

would be expected to exhibit premature termination of translation is shown in Figure 5. The strongest polar mutant encountered, *trpD159* is about 85–90% polar and the gradient increases in near-linear fashion until the altered sites in non-polar ochre mutants such as *trpD1383* or *trpD1431* are reached. *TrpD* missense mutants are non-polar.

Characteristics of trpE *mutants:* As mentioned in the section dealing with *trpD* mutants, *trpE* is the structural gene for a polypeptide designated Component I of the anthranilate synthetase–PRA transferase complex (ITO and YANOFSKY 1969; ITO, COX and YANOFSKY 1969). The *trpE* polypeptide has been obtained in pure form and has a molecular weight of *ca.* 60,000 daltons (ITO, COX and YANOFSKY 1969).

The largest group of mutants examined in the present study are altered in *trpE* (Table 5). The classification and mapping procedures employed in the characterization of these mutants are described in the *trpC* and *trpD* sections. Since such a large number of *trpE* mutants were examined and since an unusually large fraction of these gave inconsistent recombination frequency data, the mutants have not yet been assigned mutant numbers which reflect estimated distances from the origin of the gene. Instead, the genetic map has been divided into regions on the basis of recombination tests with deletion mutants and *trp* transducing phage. An attempt was made to order some of the mutants within each group, as can be seen in Figure 6. The recombinational length of *trpE* estimated from the data is 8.4 map units. If we take the length of *trpE* as *ca.* 1,672 nucleotide pairs (based on a polypeptide molecular weight of *ca.* 60,000 daltons), the ratio of nucleotide pairs per map unit is 200.

Mutants of the various chain termination classes were identified, as can be seen in Table 5. Missense assignments were verified by sucrose gradient analyses; only missense mutants contain PRA transferase in a complex which sediments at the same rate as the anthranilate synthetase–PRA transferase complex from wild type; i.e., the PRA transferase of *trpE* chain termination mutants sediments at the same rate as does unassociated Component II (ITO and YANOFSKY 1966; ITO and YANOFSKY 1969). In addition, only missense mutants in *trpE* are non-polar.

Complementation was not observed in merodiploids constructed with chain termination mutants with alterations at opposite ends of the gene, or in merodiploids with missense or with missense and chain termination alterations on chromosome and episome (Table 2), supporting the conclusion that *trpE* specifies a single polypeptide chain.

The shape of the polarity gradient obtained with *trpE* mutants (Figure 7) differs markedly from that observed with *trpD* and *trpC* mutants. Extreme polarity, *ca.* 95%, is characteristic of all chain termination mutants with alterations in the first half of the gene. Beyond the *trpE* midpoint the gradient increases sharply, although non-polar chain termination mutants were not detected. Mutant E5947, the most operator-distal *trpE* mutant studied, is non-polar and is believed to be a missense mutant (see Figure 4), but this assignment is not certain.

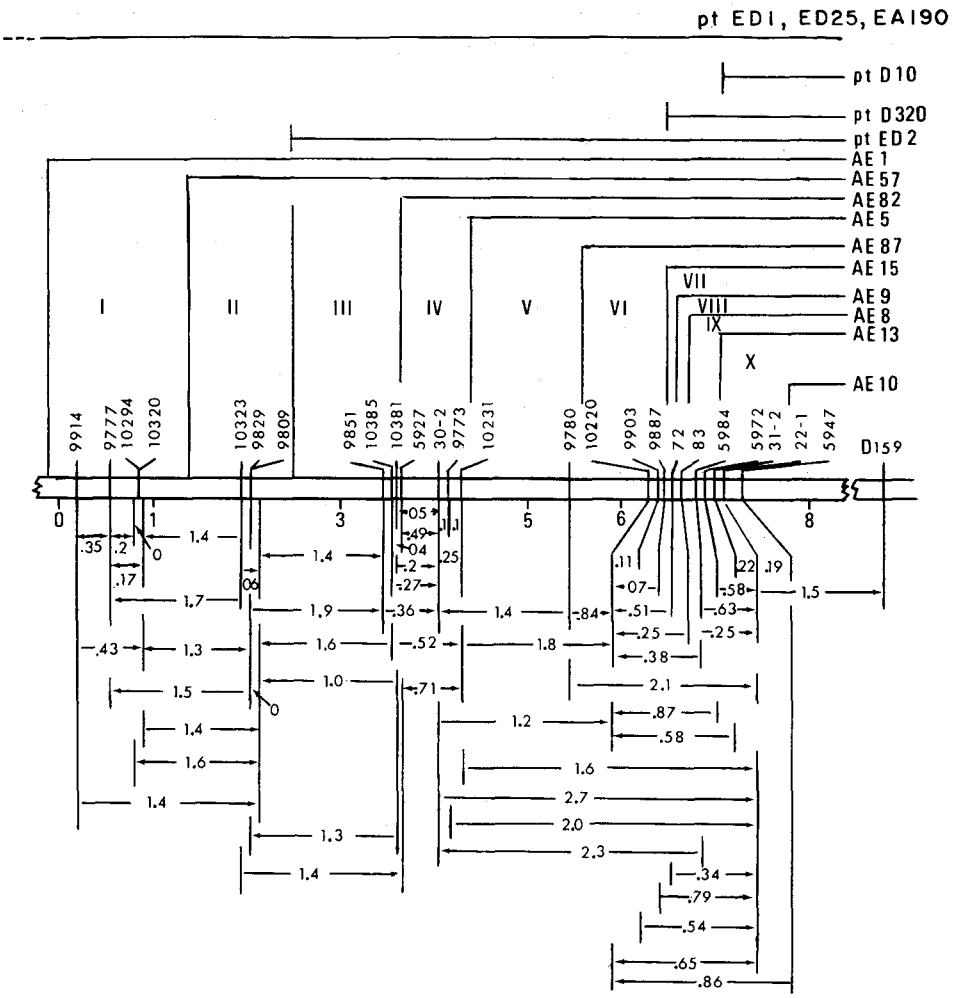

FIGURE 6.—Genetic map of *trpE*. The *E* gene is divided into segments defined by the termini in *trpE* of overlapping [*tonB trpAE*] deletions and in phage *ptED2*. The recombination values presented are based on Trp prototroph frequencies (recipient designated by the head of the arrow) normalized to the frequency of transduction of a *his*$^-$ marker of the recipient.

When *trpE* polar mutants are assayed for PRA transferase activity, the levels detected are unexpectedly low in comparison with the observed levels of tryptophan synthetase A or B (Table 6). It is unlikely that this non-equivalent reduction results from overproduction of the tryptophan synthetase proteins due to transcription initiations at the internal promoter, P2, or is a consequence of both polarity and intiations at P2, because polarity gradients of both PRA transferase and tryptophan synthetase are seen, and because in both weak and strong polar mutants, the disparity is evident (Table 6). We examined the possibility that PRA transferase levels are low in the *trpE* polar mutants because transferase is unstable when it is not associated with Component I of the anthranilate synthe-

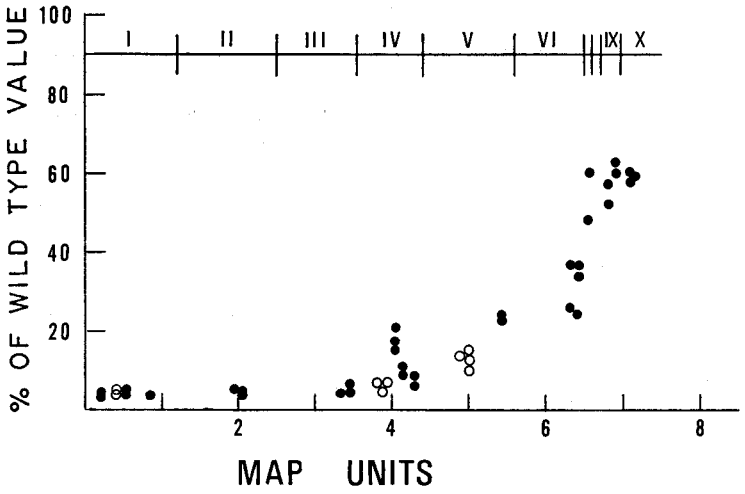

FIGURE 7.—Polarity gradient for *trpE* mutants. The relative specific activity of tryptophan synthetase A or B is plotted *vs.* the estimated map location of the corresponding mutational alteration. The open circles represent polarity values for mutations localized in particular segments but for which recombination frequency data were not obtained. These values are placed at approximately the midpoint of the respective segment. Each point represents a polarity determination for a separate culture of a presumed *trpE* chain termination mutant. All strains were *trpR*⁻ and were harvested during logarithmic growth in the presence of excess tryptophan (see METHODS).

TABLE 6

Non-equivalent polarity in polar trpE *mutants*

Strain	Enzyme activity, percent of wild-type value	
	PRA transferase	Tryptophan synthetase A or B
trpR⁻ *trpE9914*	0.64	3
trpR⁻ *trpE9777*	0.72	4
trpR⁻ *trpE9851*	0.6	4
trpR⁻ *trpE9780*	3.2	23
trpR⁻ *trpE9903*	8.5	33
trpR⁻ *trpE5972*	11	60
trpR⁻ *trpE22–1*	13	62
trpR⁻ *cysB*⁻ *trpE9851*/colVB *trpD159*	0.8	*
trpR⁻ *cysB*⁻ *trpE9851*/colVB *trpD822*	3.8	*
trpR⁻ *cysB*⁻ *trpE5972*/colVB *trpD159*	8.4†	*
trpR⁻ *cysB*⁻ *trpE5972*/colVB *trpD822*	8.0	*

* Values determined but uninterpretable because both chromosome and episome contribute this activity.

† The anthranilate synthetase activity of this extract with glutamine as amino group donor was 13% of that of the haploid wild-type control, also indicating that the PRA transferase level (in this case assayed as Component II) was low.

TABLE 7

Examination of polar trpB, trpC *and* trpD *mutants for antipolarity*

Extract of mutant*	Ratio of gene product activities, percent of control†	
	Polarity	Antipolarity
	A/E	B/E
trpR⁻ A38oc	—	41
trpR⁻ A2oc	—	44
trpR⁻ A96oc	—	78
	A/E	C/E
trpR⁻ B4oc	4	103 ± 4
trpR⁻ B6oc	5	101 ± 3
trpR⁻ B9579oc	4	84 ± 5
trpR⁻ B10081oc	5	90 ± 3
trpR⁻ B10321oc	6	98 ± 9
trpR⁻ B-ICR-1fs	14	106 ± 4
	A/E‡	D/E‡
trpR⁻ C142am	16	85 ± 4
trpR⁻ C315am	24	94 ± 5
trpR⁻ C426am	26	91 ± 4
trpR⁻ C486am	29	111
trpR⁻ C700am	48	91 ± 2
trpR⁻ C755fs	44	92
trpR⁻ C800fs	60	94
trpR⁻ C1047fs	96	86 ± 6
	B/E‡	E‡
trpR⁻ D159oc	12	95 ± 4
trpR⁻ D233fs	13	86 ± 3
trpR⁻ D302fs	21	92
trpR⁻ D344fs	25	94
trpR⁻ D1007fs	58	100
trpR⁻ D1382oc	105	89 ± 5

* All strains examined carried the same *trpR*⁻ mutation.

† The control strain in each case was a *trpR*⁻ *trp* operon missense mutant, or a *trpR*⁻ Trp prototroph. The letters A, B, C, D and E refer to the *trp* gene specifying the polypeptide product which was measured.

‡ Extract assayed in the presence of excess anthranilate synthetase-PRA transferase Component II.

tase–PRA transferase complex. Merodiploids were constructed in which each chromosome has a *trpE* polar mutation and each episome has a chain termination mutation in *trpD*. In these merodiploids the episome contributes Component I of the complex but not Component II. The complex was present in extracts of the merodiploids, but the PRA transferase levels remained low (Table 6). The explanation for the non-equivalence of polarity is not known. Low non-equivalent levels of PRA transferase also have been noted in studies with comparable polar mutants of *S. typhimurium* (BLUME, WEBER and BALBINDER 1968).

A unique property of strong polar *trpE* mutants, mutants with >92% polarity, is their sensitivity to growth inhibition by moderately high concentrations of anthranilate (Table 5). The cause of this inhibition was investigated by con-

structing *recA*⁻ merodiploids in which the chromosome contains a strong polar mutation in *trpE* and the episome contributes functional *trpD* or *trpC*, or both (but not a *trpE* product). These analyses suggest that anthranilate sensitivity results from anthranilate inhibition of the low levels of *trpD* and *trpC* polypeptides in *trpE* polar mutants; i.e., good growth in the presence of high concentrations of anthranilate is obtained only when an extra copy of both *trpD* and *trpC* is introduced. The requirement for greater than minimal levels of *trpD* and *trpC* polypeptides is also evident when growth of merodiploids is examined on minimal agar. As shown in Table 2, *trpD1431* and *trpC142* poorly complemented the strong polar mutants. Furthermore, strong *trpE* polars grow more slowly than weak *trpE* polars on low levels of anthranilate. Thus the defect exaggerated by the presence of high concentrations of anthranilate is also evident under other conditions.

Examination of strong polar mutants for antipolarity: Antipolarity, the reduction of the relative level of the polypeptide specified by the gene of an operon immediately preceding a gene with a chain termination mutation, was previously described for mutants of the *trp* operon, particularly those altered in the initial segment of *trpA*, the last gene of the operon (ITO and CRAWFORD 1965; YANOFSKY and ITO 1966, 1967). Several strong and weak polar mutants in *trpD* and *trpC* isolated in the present study, and strong polar mutants in *trpB*, were examined for antipolarity, following logarithmic growth of *trpR*⁻ derivatives in the presence of excess tryptophan. The data in Table 7 show that under the conditions employed there is no strong antipolar effect except for the *trpA* mutants included as controls (in mutant *trpA38* an ochre codon replaces the codon specifying lysine at position 15 in the *trpA* polypeptide; C. YANOFSKY, unpublished). Antipolarity was detected previously in studies with polar *trp* mutants (YANOFSKY and ITO 1966) but it now appears that, with the exception of *trpA* mutants, the antipolar effect is so slight that it is not possible to show a correlation with the extent of the polar effect.

DISCUSSION

When gene expression is examined in chain termination mutants of polycistronic bacterial operons, polar gradients of distal gene expression are observed within each gene, with the most extreme effects on enzyme formation exhibited by mutants altered in the operator-proximal region of their respective gene. The slope of the polarity gradient characteristic of each gene appears to differ significantly from one gene to another, within an operon, and when genes in different operons are compared (NEWTON *et al.* 1965; BAUERLE and MARGOLIN 1966; FINK and MARTIN 1967; JORDAN and SAEDLER 1967). For example, in the present study we observed a near-linear polarity gradient in *trpD* and a slightly concave gradient in *trpC*. The most extreme polar mutations at the operator-proximal ends of both genes reduced synthesis of enzymes specified by distal genes of the operon by 80–90%, while the most operator-distal chain termination mutations had little or no polar effect. By contrast, in *trpE*, the first gene of the operon, all chain termination mutations in the operator-proximal half of the genetic map

reduced distal gene expression by 95%. [This level of polarity may be an underestimate because the internal promoter, P2, located in the vicinity of the *trpD–trpC* boundary, could be responsible for half or more of this residual synthesis (BAUERLE and MARGOLIN 1967; MORSE and YANOFSKY 1968).] Non-polar chain termination mutations were not detected in *trpE* but this may be due to sample size and the lack of mutants with alterations at the very end of the gene. In other studies, in which a small number of polar *trpB* mutants were examined, the polarity gradient resembled that observed here in *trpE* (YANOFSKY and ITO 1966) and extreme polar mutants (*ca.* 95% polarity) were encountered. Although these polarity gradients are based on genetic maps rather than on physical distances, it seems likely that the differences noted are real and reflect some fundamental dissimilarity in the structures of the respective genes. The difference cannot simply be due to gene size because *trpE* and *trpD* polypeptides are approximately the same length. What could these gradient differences mean within the framework of current proposed hypotheses of the mechanism of polarity in bacteria?

Mechanisms of polarity have been proposed which are based on one or more of the following expectations: a) ribosomes attach efficiently only at or near the 5′ end of a messenger and detach in the untranslatable region as some function of the length of the region; b) ribosome detachment at an introduced chain termination codon is followed by reattachment at low and differing rates at distal initiator codons; c) secondary structure of polypeptide initiator regions of mRNA prevents efficient ribosome attachment unless the immediately preceding region is traversed by ribosomes; d) transcription and translation are to some extent coupled and transcription may terminate when an untranslatable region is being transcribed; e) ribosome-free untranslatable regions are subject to endonucleolytic attack and subsequent exonucleolytic degradation which extends into distal mRNA regions rendering these regions unavailable for translation. Since several of these expectations may obtain, it is difficult to select the single cause of polarity in bacteria, if, in fact, there is but one cause.

Transcription studies with polar mutants would appear to favor explanations d) and e) because mRNA regions operator-distal to a polar chain termination codon are found in reduced amounts (CONTESSE, NAONO and GROS 1966; IMAMOTO and YANOFSKY 1967a; IMAMOTO and YANOFSKY 1967b). The extent of the reduction of distal mRNA correlates roughly with the severity of polarity (IMAMOTO and YANOFSKY 1967a); however, conditions of derepression and severity of amino acid starvation also affect the relative amount of distal mRNA detected (MORSE and GUERTIN 1971b; C. YANOFSKY, unpublished). Studies of the basis of the reduction of distal mRNA regions have given seemingly conflicting results; some findings indicate that the messenger is attacked in the untranslatable region between the terminator (nonsense codon) and the next polypeptide initiator (MORSE and YANOFSKY 1969), while other observations suggest that transcription can terminate prematurely when RNA polymerase encounters a translation terminator which is not immediately followed by an initiator (IMAMOTO, KANO and TANI 1970).

Recently, additional evidence has been obtained consistent with the degradation model for polarity (MORSE and YANOFSKY 1969; MORSE and PRIMAKOFF 1970; MORSE and GUERTIN 1971a; KUWANO, SCHLESSINGER and MORSE 1971). Both polarity of gene expression and reduction of distal mRNA levels are relieved by alterations in a (polarity relief) suppressor gene (MORSE and PRIMAKOFF 1970). Since the polarity relief suppressor gene studied most extensively (MORSE and PRIMAKOFF 1970; MORSE and GUERTIN 1971a) gives equivalent relief of polarity of all classes of chain termination mutants, does not appear to act at the translation level, is recessive to its wild-type allele, and appears to code for an endonuclease (KUWANO, SCHLESSINGER and MORSE 1971), it seems likely that it relieves polarity by reducing nucleolytic attack of the mRNA of polar mutants. Viewed in relation to these observations, the polarity gradient characteristic of each gene could reflect the specificity of the polarity endonuclease(s), the distribution of the ribonucleotide sequence(s) in the untranslatable region which can be attacked, the probability that they will be attacked, and the length of the susceptible untranslatable mRNA region. The rate of presumed 5′ → 3′ exonucleolytic degradation initiated at each endonucleolytic break could also affect the shape of the polarity gradient. In addition, the distribution of internal translation initiators could influence the shape of the polarity gradient (NEWTON 1969). In view of the many variables which would be involved in a degradative mechanism of polarity, and the likelihood that a similarly large number of variables would apply in other proposed mechanisms, it is perhaps not too surprising that the explanation of polarity and polarity gradients remains uncertain.

The ratios of nucleotide pairs per genetic map unit estimated for *trpE, D* and *C* in the present study are 200, 530 and 250, respectively. Comparable values for *trpC, B* and *A* from other studies are 207 (SMITH 1967), 240 (CRAWFORD and SIKES 1970), and 190 (YANOFSKY *et al.* 1967), respectively. The high ratio obtained for *trpD* may be due to the use of very few mutants as reference recipients in determinations of recombination frequencies. Total genetic (recombinational) length increases as small distances are summed; thus the smaller the number of reference recipients, the lower the estimate of the total length of a genetic region. Considering the uncontrollable variables involved in recombination frequency determinations, particularly marker effects, the above values are in reasonably good agreement and certainly exclude an order-of-magnitude variation from gene to gene.

The finding that an appreciable antipolar effect is demonstrable only in *trpA* mutants under our conditions indicates that this restriction on gene expression is by no means a general one. Why strong antipolarity is evident in mutants of other genes of the *trp* operon under other conditions (ITO and CRAWFORD 1965; BALBINDER *et al.* 1968) is not known, nor do we understand the basis of antipolarity in *trpA* mutants or the antipolarity gradient in *trpA* seen under our conditions (YANOFSKY and ITO 1967).

The unexpected property of strong polar mutants in *trpE*—growth inhibition by high concentrations of anthranilate—is concluded to be due to a reduction of the amount of *trpD* and *trpC* polypeptides, and presumed *in vivo* inhibition of

both of these gene products by anthranilate. Whether this inhibition has any biological significance is not known (e.g., see HENDERSON, ZALKIN and HWANG 1970).

The correlation of mutant site location with the properties of the resultant gene product provides important insight into the functions of different regions of *trp* operon polypeptides. The findings with *trpD* mutants suggest that approximately the first quarter of the *trpD* polypeptide is sufficient to permit utilization of L-glutamine as amino-group donor in anthranilate formation from chorismate, in the presence of anthranilate synthetase Component I, the *trpE* gene product (see also, BAUERLE, SECOR and GRIESHABER 1971). In agreement with this conclusion is the recent observation that a fragment of PRA transferase of molecular weight *ca.* 15,000–19,000 daltons produced by proteolytic digestion of the anthranilate synthetase–PRA transferase complex is capable of contributing a functional glutamine site when complexed with anthranilate synthetase Component I (HWANG and ZALKIN 1971). Of further interest is the finding that in *Pseudomonas putida* PRA transferase and the glutamine activation component of anthranilate synthetase are readily separable polypeptides, and the molecular weight of the glutamine activating subunit is 18,000 daltons (QUEENER and GUNSALUS 1970).

In the present study it was noted that some chain termination mutants altered early in *trpD* produced a slightly active gene product. This product does not appear to interact with the *trpE* polypeptide and permit L-glutamine utilization. (However, since the *trpD* mutants mentioned are leaky, it is likely that *in vivo* some anthranilate is formed, but with NH_3 as amino donor.) In view of these observations it is conceivable that the two functions of the *trpD* polypeptide—namely permitting glutamine utilization and converting anthranilate to PRA—are controlled by distinct segments of the polypeptide. The varied phenotypes we observe could reflect the imposition of the consequences of mutational change on the true properties of the polypeptide.

It appears from the present and previous studies (SMITH 1967; CREIGHTON 1970) that there is regional control over the two enzymatic reactions catalyzed by the *trpC* polypeptide, since missense mutants invariably have full activity in either of the reactions. The analyses with chain termination and terminal deletion mutants suggest, however, that a near-intact polypeptide chain may be required for either activity to be expressed fully. It is interesting that the regional control which can be assigned, the operator-proximal segment of *trpC* over catalysis of the InGP synthetase reaction and the operator-distal segment over catalysis of the PRA isomerase reaction, is out of order with respect to the biochemical sequence, in which the isomerase reaction precedes the synthetase reaction.

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SUMMARY

Polarity gradients were determined for the first three genes of the *trp* operon

of *E. coli*. Strong polar mutants were encountered in each of the genes; however, the shape of the gradient and the severity of polarity varied. Non-polar chain-termination mutants were found for two of the genes. Polar mutants of the first gene of the operon exhibited non-equivalent reduction of polypeptides specified by distal genes of the operon. Antipolarity was not appreciable in strong polar mutants of *trpD*, *C* or *B*.—The anthranilate sensitivity of strong polar mutants of *trpE* appears to be due to anthranilate inhibition of the low levels of *trpD* and *trpC* polypeptides. The first quarter of the *trpD* polypeptide is sufficient for formation (with the *trpE* polypeptide) of a functional glutamine-utilizing anthranilate synthetase complex. Several chain-termination mutants altered early in *trpD* are leaky, suggesting that a reinitiation fragment with PRA transferase activity is produced. The properties of *trpC* and *trpD* mutants indicate that different regions of the respective polypeptides have some degree of autonomy over the different catalytic functions performed by these polypeptides.

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