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Nucleotide Sequences from Tryptophan Messenger RNA of *Escherichia coli*: The Sequence Corresponding to the Amino-Terminal Region of the First Polypeptide Specified by the Operon

(anthranilate synthetase component I/tryptophan operon)

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ABSTRACT **Mutants with internal deletions that terminate near the operator end of the tryptophan operon of *E. coli* were used in studies on the nucleotide sequence at the 5′ end of the messenger RNA transcribed on this operon. The findings obtained permitted identification of a sequence of 43 nucleotides corresponding to the amino-terminal 11 amino acids of anthranilate synthetase component I, the polypeptide specified by the operator-proximal structural gene of the operon. It was also shown that the translation initiation codon for this polypeptide is preceded on the messenger by a "leader" sequence of unknown function, at least 150 nucleotides in length.**

Although nucleotide sequences have been reported for various viral and bacterial RNA species (1–8), few sequences have been described for messenger RNAs (9–11). In particular, there is no nucleotide sequence data for any messenger RNA region that specifies the amino-acid sequence of a protein of an organism with a DNA genome. In addition, there is no information on the structure or function of nonamino-acid specifying regions of such messengers. In view of the importance of knowledge concerning messenger sequences and their functions we have begun an analysis of the mRNA transcribed on the tryptophan (*trp*) operon of *Escherichia coli*.

In this communication we present the RNA nucleotide sequence that corresponds to the amino-terminal amino-acid sequence of the polypeptide specified by the operator-proximal structural gene of the *trp* operon. In addition, we describe findings that indicate that the initiation codon for this polypeptide is preceded in *trp* mRNA by a "leader" sequence of about 150 nucleotides.

MATERIALS AND METHODS

The deletion mutants used to produce operator-proximal *trp* mRNA are derivatives of *E. coli* strain W3110 (12). Each deletion lies entirely within the *trp* operon and removes most or all of *trpE* and most of *trpD*, as determined by recombination tests with various point mutants (12) (Fig. 1). All the deletion strains are repressible by tryptophan, indicating that the *trp* operator is intact and functional. Merodiploid strains, possessing two copies of the *trp* operon, were used to increase the yield of [^{32}P]phosphate-labeled *trp* mRNA. The merodiploids were constructed by transducing each deletion into the Fredericq *colVB cysB trp* episome and then transferring the episome into a strain containing the original deletion on its chromosome. The phage used as sources of DNA for hybridization have been described (11, 13).

Materials and methods for labeling cells with [^{32}P]phosphate, and for purification, hybridization, and sequencing of *trp* mRNA, are essentially as described by Cohen *et al.* (11). A single liquid hybridization was used in purification of *trp* mRNA (14) unless otherwise stated. In some experiments the temperature and time of annealing were altered to maximize the extent of hybridization. RNA–DNA hybrids were collected on nitrocellulose membrane filters, and nonbase-paired RNA "tails" as well as nonspecific RNA contamination were removed by treatment of the filters with RNase. The filters were subsequently treated with iodoacetate to inactivate the RNase (14). RNA was eluted from the filters in aqueous medium of low ionic strength at 92°. All fingerprints used the conventional pH 3.5 cellulose acetate system for the first dimension followed by homochromatography (15) on commercially available 20 × 20-cm DEAE–cellulose thin-layer sheets (Macherey–Nagel). Pancreatic ribonuclease A ε-carboxymethylated at the lysine in position 41 (CM-RNase) was prepared as described by Heinrikson (16) and was used to generate partial digestion products of mRNA as described by Contreras and Fiers (4).

RESULTS

Fingerprint Patterns of trp mRNA Isolated from Various Deletion Mutants. The internal deletion mutants *trpΔED102*, *trpΔED2*, and *trpΔED24* (Fig. 1) were used as sources of ^{32}P-labeled *trp* mRNA. The *trp* mRNA transcribed from the operator-proximal region of the *trp* operon of each of these mutants was isolated by hybridization to the DNA of phage λ*φ80trpE*. Since each of the mutants has a deletion terminus in *trpD* (Fig. 1), the only *trp* mRNA with sequences homologous to λ*φ80trpE* DNA must correspond to the initially transcribed region of the operon. The length of this region differs in the three deletion mutants. Therefore, the number of oligonucleotides found on fingerprints obtained with digests of mRNA purified from these strains would be expected to increase as the length of the transcribed region increases. The T1 RNase fingerprint of ^{32}P-labeled *trp* mRNA isolated from the deletion mutants increases in complexity in the order *trpΔED102* < *trpΔED2* < *trpΔED24* (Fig. 2). The T1 RNase fingerprint of labeled *trp* mRNA of deletion mutant *trpΔED11* (see Fig. 1), presented in a previous publication (11), has many more oligonucleotides than that of any of the above three internal deletion strains. A similar increase in complexity is noted in RNase A fingerprints of the same *trp* mRNA preparations. These data are in qualitative agreement

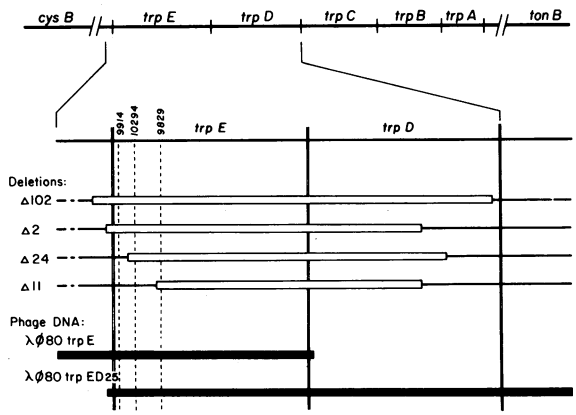


FIG. 1. Internal deletions of the tryptophan operon and *trp* operon regions in *trp* transducing phage. *Open bars* denote the extent of deletions (12). DNA at either side of the deleted portion is represented by a *line*. *Solid lines* indicate known transcribed portions of *trp* operon DNA. The scale of the region to the left of *trpE* has been expanded to indicate the relative positions of the deletion end points in *trpΔED102* and *trpΔED2*. *Solid bars* indicate the extent of bacterial DNA carried by the nondefective transducing phage, *λφ80trpE* (*i*$^{\lambda}h^{+}$*φ80trpE*) and *λφ80trpED25* (*i*$^{\lambda}h^{-}$*φ80trpED25*). These phage have been designated *ptE* and *ptED25* in previous publications. The right-hand end point of the bacterial DNA carried by *λφ80trpED25* lies in the middle of *trpC* (not shown).

with genetic mapping of the deletion termini in these strains (Fig. 1) (12) and with estimates of *trp* mRNA length based on measurements of the relative amounts of deletion mutant *trp* mRNA hybridizable to *λφ80trpE* DNA, as determined by pulse labeling of RNA with [^{3}H]uridine.

The oligonucleotides in total T1 RNase and RNase A digests of *trp* mRNA of the deletion mutants were isolated and their sequences were determined (Table 1). These data permit allocation of the oligonucleotides observed on the fingerprints into three groups (Table 1): The oligonucleotides of *trpΔED102* mRNA are in Group I, those of *trpΔED2* mRNA are in Groups I and II, and those of *trpΔED24* mRNA are in all three groups. These data permit the following estimates of the minimum number of nucleotides comprising the operator-proximal *trp* mRNA segment of each of the deletion strains: 18 for *trpΔED102*, 43 for *trpΔED2*, and 162 for *trpΔED24*. These estimates ignore the likelihood that there are multiple copies of some of the small oligonucleotides.

Since transcription of the operon begins at the *trpE* end of the operon and proceeds sequentially to the *trpA* end (17), Group III oligonucleotides must be present in the 3′ portion of the *trpΔED24* mRNA analyzed. Further evidence that this is the case comes from an experiment using the DNA of phage *λφ80trpED25*. This phage lacks the *trp* operator and the initially transcribed region of the operon (13) (Fig. 1). Operator-proximal *trp* mRNA produced by *trpΔED24* was annealed to *λφ80trpE* DNA and then eluted and rehybridized to *λφ80trpED25* DNA. The hybrids were treated with T1 RNase to remove single-stranded ends, and the remaining *trp* mRNA was eluted, digested with T1 RNase, and fingerprinted. All the T1 RNase oligonucleotides of Group III were observed, whereas all the Group I and Group II oligonucleotides were absent from the fingerprint. Thus, on the basis of the characteristics of both phage *λφ80trpED25* and the internal deletion mutants, Group III oligonucleotides are placed on the 3′ side of Group II oligonucleotides.

Identification of the RNA Nucleotide Sequence Corresponding to the First 11 Amino Acids in the trpE Polypeptide. The amino-acid sequence of the first 25 amino acids at the amino-terminal end of the *trpE* polypeptide (anthranilate synthetase component I) is known from other studies (S. L. Li, J. Hanlon & C. Yanofsky, unpublished). We have deduced a continuous

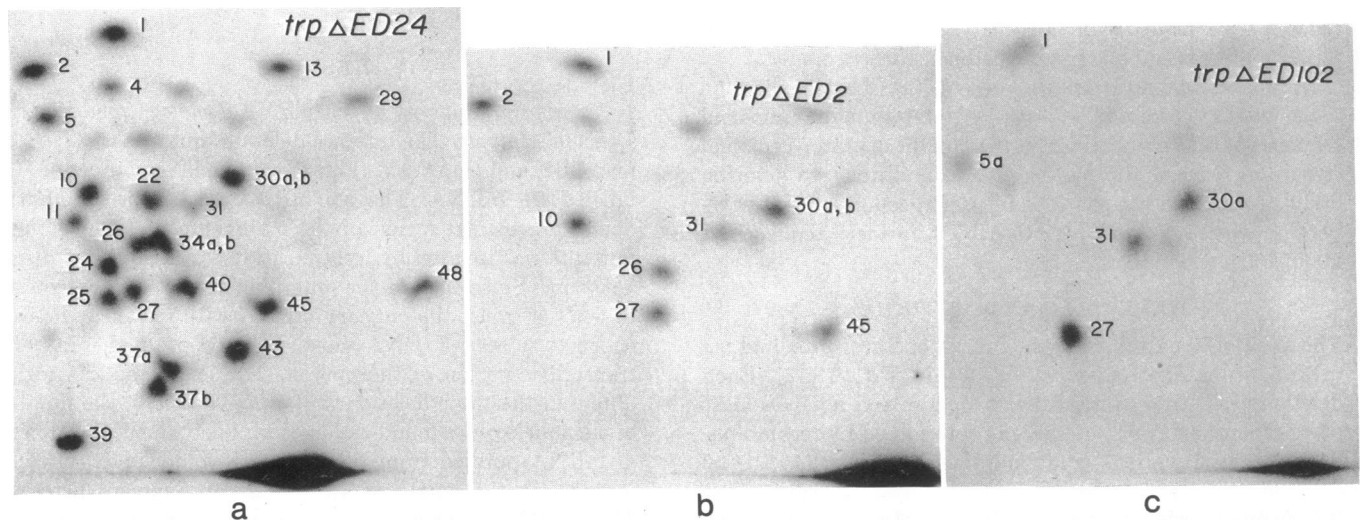


FIG. 2. T1 RNase fingerprints of operator-proximal mRNA from deletion strains. Liquid hybridizations of *trp* deletion mRNA to *λφ80trpE* DNA were performed as follows to maximize the yield of hybrids: (*a*) *trpΔED24* mRNA 4 hr at 65°; (*b*) *trpΔED2* mRNA, 8 hr at 55°; (*c*) *trpΔED102* mRNA, 16 hr at 45°. T1 RNase was used to digest nonhybridized "tails." Fingerprints were prepared by electrophoresis on cellulose acetate at pH 3.5 (left to right). The region on the cellulose acetate strip between the xylene cyanol FF marker and the methyl orange marker was transferred to a DEAE–cellulose thin-layer sheet, followed by homochromatography (bottom to top) with a 3% RNA solution that had been digested with 1 N KOH for 15 min at 27° (Homomix C-15).

T1 RNase oligonucleotide t21a (AACUG) has not been definitely located on the fingerprint of *trpΔED24* mRNA, but probably coincides with t22 (UAAAG). t21a has been characterized as a segment of several CM-RNase partial digestion oligonucleotides. CCCG (t6) lies somewhat to the left of the cyanol marker and was not transferred from the first dimension of the *trpΔED24* mRNA fingerprint.

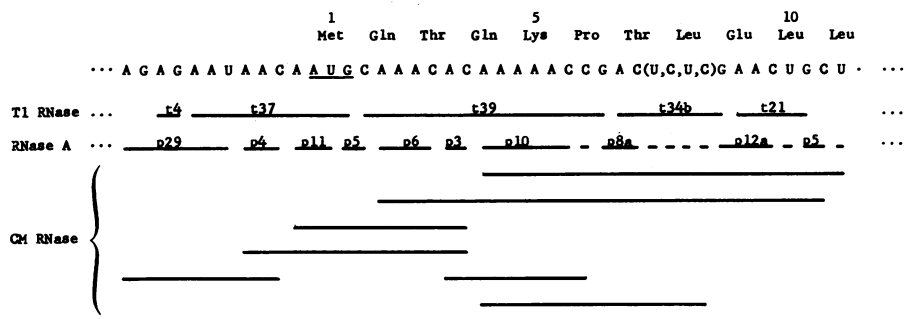
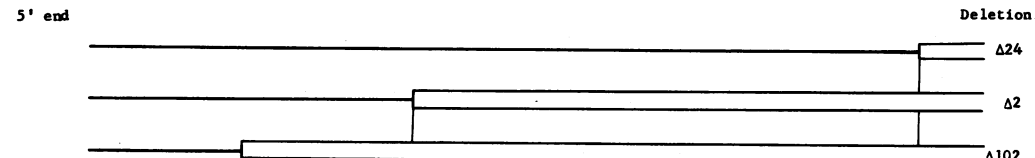


FIG. 3. Nucleotide sequence corresponding to the amino-terminal region of the *trpE* polypeptide. *Horizontal lines* denote T1 RNase, RNase A, and CM-RNase oligonucleotides. The latter nuclease has the same specificity as RNase A; however, under the conditions used (4) it is quite inactive towards pyrimidine–pyrimidine linkages but cleaves pyrimidine–purine linkages slowly. The CM-RNase partial digestion products shown have been used to overlap the T1 RNase and RNase A oligonucleotides. *Parentheses* indicate that the absolute sequence of the enclosed nucleotides is not known.

nucleotide sequence of 43 bases from sequence analyses of the oligonucleotides obtained in carboxymethyl (CM)-RNase partial digests and T1 RNase and RNase A digests of *trp-ΔED24* mRNA (Fig. 3). This 43-base sequence matches the initiation codon as well as codons corresponding to the next 10 amino acids of the *trpE* polypeptide (Fig. 3). The T1 RNase and RNase A oligonucleotides that comprise this sequence *are not* observed in fingerprints of the *trp* mRNA of *trpΔED102* and *trpΔED2*. This result indicates that the *trp* mRNA isolated from *trpΔED102* and *trpΔED2* must lie on the 5′ side of the identified initiation codon.

Orientation of the trpE Initiation Codon Relative to the Origin of Transcription. The evidence presented places the *trpE* initiation codon (and the adjacent 40-nucleotide sequence of Fig. 3) at some position within the estimated 119 nucleotides of Group III. Two observations indicate that the sequence presented in Fig. 3 occupies the extreme 3′ end of the *trp* mRNA fragment from *trpΔED24*. First, the remaining Group III oligonucleotides (76 nucleotides) that are *not* found in the sequence given in Fig. 3 *do not* match the possible codon assignments for amino-acid residues 12–25 of the *trpE* polypeptide. Secondly, as shown below, a discrete RNA fragment

TABLE 1. *Oligonucleotides from the 5′ end of tryptophan messenger RNA*



	Group I	Group II	Group III		
T1 RNase	t1 G	t1 G	t1 G	t21a AACUG	t37a AAUAACAAUG
	t27 UAAAAG	t2 CG	t2 CG	t22 UAAAG	t37b (AAAAU,U,AAC)AG
	t30a UAUCG	t10 AAAG	t4 AG	t24 CAAUCAG	t39 CAAACACAAAAACCG
	t31 UUCACG	t26 ACAAUG	t5 CAG	t25 CCCAUAG	t40 CACUUUG
		t30b UACUG	t6 CCCG	t29 UUG	t43 (CCAC,UUUC)AUG
		t45 CAAUUUUG	t11 AAACG	t34a (C,U,AAU)G	t48 CUUUUG
			t13 UG	t34b AC(C,C,U,U)G	
RNase A	p1 U	p1 U	p1 U	p10 AAAAAC	p17b GAU
	p2 C	p3 AC	p2 C	p11 AAU	p19 GAGC
	p3 AC	p5 GC	p3 AC	p12a GAAC	p20 AGAU
	p7 AU	p11 AAU	p4 AAC	p12b AGAC	p21 AAAAU
	p8a GAC	p15 GU	p5 GC	p14a AAAGC	p22 GGU
	p15 GU	p25 GAAAGC	p6 AAAC	p14b GAAAC	p23 GAGU
	p33 AAAAGGU		p7 AU	p15 GU	p24 GGGC
			p8a GAC	p16 GGC	p29 AGAGAAU
			p8b AGC	p17a AGU	p32 GGAAAGU

Oligonucleotides present in operator-proximal *trp* mRNA. The sequence of each oligonucleotide follows its numerical designation. Group I oligonucleotides are those from *trpΔED102* mRNA. The oligonucleotides of *trpΔED2* mRNA are found in Groups I and II, while *trp-ΔED24* mRNA oligonucleotides are found in Groups I, II, and III. Several small oligonucleotides occur more than once and are assigned to more than one group on the basis of sequence data obtained with CM-RNase partial digestion products. Oligonucleotides unique to *trpΔED102* and *trpΔED2* mRNA can be assigned to the region where each deletion has fused the operator proximal region of the operon to the end of *trpD*. ACG (t5a) is the unique *trpΔED102* fusion oligonucleotide (shown in Fig. 2*c*), while (G,G,AAAG)C (p 34) is a fusion oligonucleotide found only in RNase A fingerprints of *trpΔED2* mRNA (not shown).

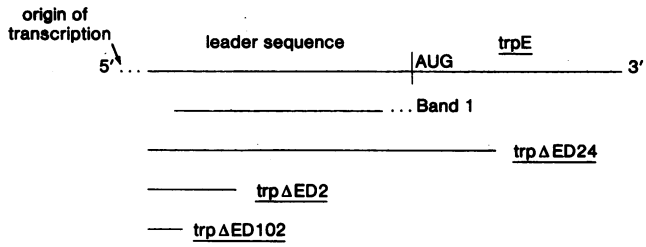


FIG. 4. Relative location of the *trp* mRNA sequence of polyacrylamide band 1. *Horizontal lines* represent operator-proximal mRNA.

containing about 120 nucleotides from Groups I, II, and III must be located on the 5′ side of the *trpE* polypeptide initiation codon.

Cohen *et al.* (11) observed patterns of discrete RNA fragments (bands) after polyacrylamide gel electrophoresis of *trp* mRNA from *trpΔED11*. Identical band patterns are obtained with RNA fragments from *trpΔED24*. The largest fragment, band 1, consists of an unbroken sequence of about 120 nucleotides and includes all of the oligonucleotides observed in the smaller fragments (11). The T1 RNase oligonucleotide composition of band 1 is derived from all three groups in Table 1. All of the oligonucleotides of Group II, one oligonucleotide from Group I, and 10 oligonucleotides from Group III are represented. None of the T1 RNase oligonucleotides obtained from band 1 corresponds to the amino-terminal amino-acid sequence of the *trpE* polypeptide (Fig. 3). Thus, the 5′ position of band 1 relative to the initiation codon is established. The orientation of band 1 with respect to the deletion termini and the initiation codon is represented in Fig. 4. The oligonucleotides comprising band 1 plus the oligonucleotides in the sequence containing the initiation codon (Fig. 3) account for all but two of the T1 RNase oligonucleotides (t48 and t37b) located in Group III. Since neither of these oligonucleotides has a sequence that corresponds to *trpE* polypeptide amino-acid residues 12–25, it is likely that they lie between the 3′ end of band 1 and the 5′ end of the sequence containing the initiation codon (Fig. 3).

The minimum length of the "leader" sequence that precedes the *trpE* polypeptide initiation codon is about 150 nucleotides. Our estimate is based on the 120 nucleotides present in band 1 plus the additional nucleotides found in oligonucleotides adjacent to band 1. This value is in agreement with the observation of Hiraga and Yanofsky (13) that as many as 200 nucleotides may be transcribed before the initiation codon for the *trpE* polypeptide. Rose and Yanofsky (18) have concluded, on the basis of studies of the time required to transcribe *trp* operon regions of known genetic length, that such a "leader" sequence, if it exists, must be less than 160 nucleotides long. A precise determination of this value must await identification of the 5′ end of the messenger and elucidation of the complete nucleotide sequence of the "leader" mRNA.

DISCUSSION

A set of mutants that contains overlapping deletions terminating near the operator end of the *trp* operon has been used for the *in vivo* synthesis of [^{32}P]phosphate-labeled *trp* mRNA. Sequence analyses of this RNA have resulted in identification of the nucleotide sequence corresponding to the amino-terminal 11 amino acids in the polypeptide specified by the operator-proximal structural gene in the operon, *trpE*. In addition, these studies permit the conclusion that at least 150 nucleotides precede the translation initiation codon for the *trpE* polypeptide.

Confirmation that the nucleotide sequence presented in Fig. 3 corresponds to the amino-acid sequence of the *trpE* polypeptide was obtained from an analysis of T1 RNase fingerprints of *trp* mRNA of strain *trpE9914am*, an amber mutant altered at the extreme operator-proximal end of *trpE* (Fig. 1). Mutant *trpE9914am* was derived from *trpE9914oc*, an ochre mutant isolated from the wild-type strain. The T1 RNase fingerprints of *trpE9914am trp* mRNA reveal two unique oligonucleotides, t53 and t14. The RNase A digestion products of these oligonucleotides suggest the following sequences: t53: (AC, C_2, U_3)AG, and t14: (C,U)G. These sequences are consistent with a two-step conversion of the GAA (Glu) codon for amino-acid residue 9 in the wild-type *trpE* polypeptide to a UAG (amber) codon (Fig. 5). Thus, fingerprint analyses of *trp* mRNA from mutant *trpE9914am* corroborate the conclusion that a segment of *trp* mRNA isolated from *trpΔED24* and not present in *trpΔED102* and *trpΔED2* (strains deleted for the mutational site of *trpE9914*) does in fact correspond to the *trpE* polypeptide.

The observation that a "leader" sequence is transcribed before the first translation initiation codon of *trp* mRNA raises the question as to its function. In studies with RNA phage, there is strong selective pressure to maintain an untranslated "leader" sequence (5, 6). It is likely that specific nucleotide sequences adjacent to translation initiation codons are necessary for attachment of ribosomes (2). "Leader" sequences have also been postulated to play a role in phage replication by providing a recognition site for RNA synthetase (5, 6). It has been further speculated that the "leader" sequence protects phage RNA against nucleases that degrade cellular RNA (7) and that it binds the phage-specified maturation protein (7). Clearly, most of these postulated functions do not apply to a "leader" sequence of bacterial mRNA.

RNA phage genomes possess extensive secondary structure in their "leader," intercistronic, and translated sequences (3, 5, 6). The initiation codon of the coat-protein cistron of MS-2 (and R-17) occupies a central position in the loop of a stalk in which the intercistronic sequence is base-paired with the sequence specifying the amino terminus of the coat pro-

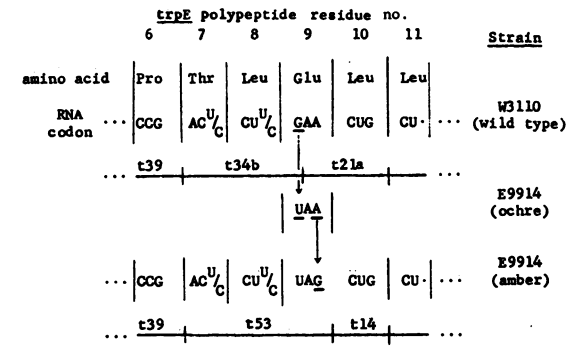


FIG. 5. Presumed base changes giving rise to the ochre and amber mutant codons of strains *trpE9914oc* and *trpE9914am*. The nucleotides present before and after the mutational change are underlined.

tein (2, 8). In addition, the DNA sequence corresponding to a a coat protein of ϕX174 can be drawn as a hydrogen-bonded structure with the initiation codon ATG at the top of a hairpin loop (19–21). No similar secondary structure is observed in the sequence containing the initiation condon for the *trpE* polypeptide. However, our preliminary attempts to order the nucleotides in the "leader" sequence do suggest the existence of extensive secondary structure in this region of *trp* mRNA (Squires, Bronson & Yanofsky, unpublished).

A possible function for the "leader" sequence of *trp* mRNA is suggested by the observation of Jackson and Yanofsky (22) that deletion mutants *trpΔED102* and *trpΔED2* have increased cellular amounts of *trp* mRNA and polypeptide products of the *trp* operon. In addition, an increased amount of *trp* mRNA is synthesized *in vitro* from the DNA of *trp* transducing phage containing the *trpΔED102* deletion (23). Under both *in vivo* and *in vitro* conditions, repression of *trpΔED102* mRNA synthesis is normal, indicating that this deletion does not affect operator function (22, 23). *In vivo* repression of *trp* mRNA synthesis is also normal in strains containing the *trpΔED2* deletion (22). As noted in this paper, the *trpΔED102* and *trpΔED2* deletions terminate between the transcription initiation site and the region corresponding to the first translation initiation codon of the operon. Apparently these deletions remove or alter some regulatory region that affects the level of operon expression. The *trp* mRNA bands observed on polyacrylamide gels (11) may be formed as a consequence of the postulated regulatory function. Their presence is suggestive of a structure and/or a mechanism of degradation that differs from that of the *trp* mRNA that corresponds to the structural genes.

Of interest also is the location of the DNA region specifying the "leader" sequence relative to the *trp* operator and promoter elements. Transcription of the operator would be expected if it were located between the *trp* promoter and *trp* structural genes and if transcription originated at the promoter. In such a case, an operator-constitutive mutation might alter one or more oligonucleotides on an appropriate *trp* mRNA fingerprint. Alternatively, as in phage λ, the promoter–operator region may precede the actual site of transcription initiation (10).

In this communication, we have presented the first example of a transcribed nucleotide sequence of mRNA that corresponds to a known polypeptide amino-acid sequence. A nucleotide sequence containing an amber codon has also been tentatively correlated with this amino-acid sequence. In addition, new information concerning the structure of bacterial mRNA is presented; a "leader" sequence at least 150 nucleotides in length precedes the first known translation initiation codon in *trp* mRNA. Whether the properties of the bacterial mRNA we have studied are characteristic of other mRNAs or are unique to *trp* operon mRNA of *E. coli* remains to be determined.

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