Regulated in Vitro Synthesis of Escherichia coli Tryptophan Operon Messenger Ribonucleic Acid and Enzymes*

(Received for publication, August 8, 1973)

HOWARD ZALKIN,† CHARLES YANOFSKY, AND CATHERINE L. SQUIRES

From the Department of Biological Sciences, Stanford University, Stanford, California 94305

SUMMARY

DNA-dependent synthesis of tryptophan (trp) operon mRNA and enzymes was studied in vitro. With φ80 trpEDCBA template DNA trp operon transcription was initiated primarily at a phage promoter estimated to be approximately 7400 to 8000 nucleotides from trpE. Sequential transcription of trp mRNA in the correct direction proceeded at a rate of about 19 nucleotides per s. Trp mRNA resulting from read-through transcription from the phage promoter was larger than 23 S. Coupled sequential synthesis of trp operon enzymes occurred at a rate of approximately 4 amino acids per s.

Experiments were conducted to determine if ribosomes discharge from polycistronic trp mRNA following translation termination at natural chain termination codons. Kasugamycin was used to inhibit reinitiation of translation. Kasugamycin shut down of steady state translation required 0.5 min for trpE enzyme, 7 min for trpD enzyme, and 3 min for trpA enzyme. These results indicate that ribosomes which translate trpE mRNA must discharge and reattach in order to translate trpD mRNA, and that ribosomes that translate trpD mRNA must discharge and reattach in order to translate trpA mRNA. Kasugamycin inhibition of trpD enzyme and trpA enzyme synthesis under appropriate conditions of sequential transcription and coupled translation provided further evidence for obligatory discharge and reattachment of ribosomes.

Enzyme synthesis coupled to trp operon-promoted transcription from λh80 trp template DNA was repressible by tryptophan in reaction mixtures containing S-30 extract from trpR− cells. In contrast, enzyme synthesis coupled to read-through transcription from φ80 trp template DNA was essentially irrepressible. It is concluded that read-through transcription is not repressible in vitro. Trp operon mRNA transcribed from a λh80 trpEDCBA DNA template was smaller than 23 S.

The polar mutation trpC6 was expressed in vivo in phage λh80 trpEDCBA 190-9 but not in phage φ80 trpEDCBA 190. Read-through transcription of the trp operon occurs in the lattce phage but not in the former. Polarity was not expressed in vitro using template DNA from either phage. Trp operon mRNA was extremely stable in vitro; the half-life varied between 30 min and more than 2 hours.

Recent work on DNA-dependent in vitro mRNA and enzyme synthesis catalyzed by preparations from Escherichia coli has helped to clarify mechanisms for positive and negative regulation of gene expression for the lac operon (1-4), gal operon (5-7), ara operon (8-10), trp operon (11-13), and arg regulon (14). With regard to regulation of tryptophan biosynthesis, Zubay et al. (11) isolated and partially purified the protein product of trpR, the repressor gene for the tryptophan operon. Partially purified trpR protein functioned in vitro to repress β-galactosidase synthesis directed by a DNA template in which lac operon genes were fused to a portion of trpE and contiguous trp operon regulatory elements. Synthesis of trpED1 and trpBA enzymes in vitro was reported by Fouwek and Van Rotterum (15), but repression was not observed. More recent experiments (12, 13), have shown that tryptophan and partially purified trpR protein repress trp operon transcription in a partially purified system. Repression was dependent upon a functional operon and showed no requirement for tryptophanyl-tRNA or tryptophanyl-tRNA synthetase.

The present work was initiated with the objective of developing an in vitro system in which trp operon transcription coupled to enzyme synthesis could be studied biochemically, thereby providing the opportunity to examine various aspects of gene expression.

EXPERIMENTAL PROCEDURE

Enzyme Synthesis—S-30 extracts were prepared from E. coli strains derived from strain A19 (16). A tonBtrpAE deletion was transduced into isogenic trpR+ and trpR− derivatives of strain A19 used previously (17). The resulting genotypes were rns−X−ΔtonBtrpAE1 trpR− and rns−X−ΔtonBtrpAE1 trpR+ where rns

† Enzymes of the Escherichia coli trp operon are designated by gene symbols. TrpE enzyme, anthranilate synthetase Component 1, is the product of trpE; trpED enzyme is anthranilate synthetase-anthranilate 5-phosphoribosylpyrophosphate phosphoribosyltransferase; trpB enzyme is tryptophan synthetase β subunit; trpA enzyme is tryptophan synthetase α subunit. TrpR protein is the product of the trpR regulatory gene.

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is RNase 1 and X stands for an unknown growth requirement satisfied by 0.05% acid hydrolyzed casein. Bacteria were grown at 30° with vigorous shaking in 2 liter flasks containing 1 liter of medium substantially according to the procedure of Zubay et al. (2). The growth medium was modified to include 20 μg per ml of tryptophan, 0.2% acid-hydrolyzed casein, and 10 μM FeCl₃. S-30 extracts were prepared from freshly harvested and washed (2) log phase cells. Extracts were treated at 37° for 80 min (18) and then dialyzed as described by Wetekam et al. (6). S-30 extracts stored in liquid N₂ retained activity for three to four freeze-thaw cycles.

The composition of the reaction mixture was essentially as described by Zubay et al. (2). The reaction mixture was modified by decreasing the tryptophan concentration to 0.1 μM, omitting adenosine 3':5'-monophosphate, substituting calcium acetate for calcium chloride and [5,6-3H]UTP (approximately 35 Ci per mmole) were included. The optimal Mg²⁺ concentration was 12.8 μM and did not vary for various DNA or S-30 preparations. Contrary to results of earlier work (15), omission of other ingredients resulted in decreased activity. Reactions for enzyme synthesis were usually conducted in a volume of 1.0 ml, and incubations were for 30 to 60 min at 34° unless specified otherwise. Reactions were stopped by rapid chilling and 9 to 15-fold diluted into cold enzyme assay mixtures. In some experiments 50 μg per ml of chloramphenicol were used to terminate enzyme synthesis. The two methods gave comparable results. Unless specifically noted otherwise, S-30 extract was from the trp⁻ strain.

**Assay of Enzyme Activity**—The assay mixture for trpE enzyme contained 50 nm Tris-acetate (pH 8.8), 10 mM MgSO₄, 0.2 mM dithiothreitol, 50 mM (NH₄)₂SO₄, 0.3 mM chorismate, and 20% glycerol, and 0.02 to 0.1 ml of enzyme solution in a final volume of 0.5 ml.

The assay mixture for trpED enzyme contained 0.1 M potassium phosphate (pH 7.0), 5 mM glutamine, 8 mM MgSO₄, 0.3 mM chorismate, and 0.02 to 0.1 ml of enzyme solution in a final volume of 0.5 ml. For both NH₄⁺ and glutamine-dependent anthranilate synthetase assays, incubation was at 34° for 30 to 60 min. Reactions were terminated by addition of 0.05 ml of 1 N HCl and ananthranilic acid was extracted with 1.0 ml of ethyl acetate. Ananthranilic acid was determined fluorometrically (19).

The enzymatic conversion of [G-3H]indole glycero-P to [G-3H]indole in the presence of excess trpB protein was used to assay for trpA enzyme. The assay mixture contained 0.1 M potassium phosphate (pH 7.0), 0.5 M sucrose-free NH₄OH (20), 10 μg per ml of pyridoxal phosphate, 8 units of trpB protein, 0.1 mM [G-3H]indole glycero-P (30,000 cpm per nmole), and 0.09 to 0.05 ml of enzyme in a volume of 0.1 ml. Incubation was for 30 to 60 min at 34°. Reactions were terminated with 0.05 ml of 1 N NaOH. The [G-3H]indole formed was extracted with 1.0 ml of toluene and determined by liquid scintillation counting. The source of trpB protein was a crude extract of *E. coli* strain trpED102 ΔaminoBttrpA905.

**Transcription**—Reaction mixtures were identical to those used for enzyme synthesis except that the UTP concentration was reduced from 0.55 to 0.055 mM and 5 to 25 μCi of tri sodium [5.6-3H]UTP (approximately 35 Ci per nmole) were included. ¹H-labeled trp RNA was isolated and detected by hybridization to denatured DNA of λ trp phages immobilized on membrane filters exactly as described by Rose et al. (12). For some experiments hybridization of ¹H-labeled trp RNA was to the λ strand of λ trp DNA. Trp mRNA synthesized in vitro is complementary only to the λ strand of λ trp DNA.

**Phage DNA**—φ80 trp, λ trp, and λ80 trp phages were grown by infection of sensitive host strains. Phages were precipitated from lysates with 140 g per liter of polyethylene glycol 6000 plus 40 g per liter of NaCl and were then purified by twice banding in cesium chloride density gradients. Template DNA was prepared by extracting purified phage in 0.02 M Tris-PO₄ (pH 8.8)-0.01 M MgCl₂ with redistilled phenol. DNA was dialyzed against 50 to 100 volumes of 0.02 M Tris acetate (pH 7.8)-0.1 mM EDTA for 2 days with at least three changes of the buffer solution. DNA for detection of ¹H-labeled RNA by filter hybridization was extracted with phenol and dialyzed against 0.15 M NaCl plus 0.15 mM sodium citrate, pH 7.0. Separated DNA strands from λ trp phage used for detection of ¹H-labeled trp mRNA by filter hybridization were prepared by a modification (21) of the method of Itredacna and Eyzyskowski (22). DNA preparations used to direct in vitro synthesis or for detection of ¹H-labeled trp RNA by filter hybridization are described in Fig. 1. The sources of some of the phages have been given (12). Phage strains φ80 trpO2EDCB 1, λ80 trpEDCBA 190-9, λ80 trpEDCBA 190-80trpC6 and φ80 trpEDCBA 190-80trpC6 were constructed in this laboratory and will be described elsewhere.

**Phage Infection**—*E. coli* strain trpEDS82 φ80 was infected at a multiplicity of two to four phages per bacterium according to the procedure of Franklin (93). Infected cells were incubated with vigorous shaking for 30 min at 37° in minimal medium supplemented with 0.05% acid-hydrolyzed casein, 0.2 mg per ml of 5-fluorouracil, 0.2 mg per ml of uridine, and 50 μg per ml of tryptophan. Infected cells were then harvested and disrupted by sonic oscillation. Following centrifugation at 27,000 × g for 30 min, extracts were assayed for enzymes of tryptophan biosynthesis.

**Materials**—The following reagents were purchased from the companies indicated in parentheses: L-amino acids (Calbiochem), nucleoside triphosphates (Sigma), trisodium phosphoenolpyruvate (Sigma), calcium leucovorin (Lederle), *E. coli* strain K-12 tRNA (General Biochemicals), chloramphenicol (Sigma), aminotricarboxylate (Eastman Organic Chemicals), tetra sodium [5.6-3H]UTP (New England Nuclear), [5-6-3H]anthranilate (Amer- sham-Searle), and electrophoretically purified DNase (Worthington Biochemical Corporation).

The following were gifts: rifampin (Gruppo Lepetit, Milan,
Italians, streptolydigan (The Upjohn Company, Kalamazoo, Mich.), and kasugamycin (Bristol Laboratories, Syracuse, N.Y.).

Chorismic acid was made by the method of Gibson (24). [G-3H]Indole glycerol-P was made as described by Creighton and Yanofsky (35).

RESULTS

Properties of in Vitro Enzyme Synthesis—E. coli trpED enzyme is an oligomer apparently (26) containing two subunits of trpE protein and two subunits of trpD protein; each subunit has a molecular weight of approximately 60,000 to 65,000. TrpE enzyme catalyzes NH₃-dependent anthranilate formation and carries the feedback site for tryptophan. TrpD enzyme confers glutamine reactivity to trpE enzyme and also catalyzes conversion of anthranilate plus 5-phosphoribosyl-1-pyrophosphate to phosphoribosyl anthranilate. TrpED enzyme synthesized in vitro catalyzes chorismate- and glutamine-dependent anthranilate synthetase activity of anthranilate synthetase (trpED) aggregate. Assay was for 30 min. The concentration of tryptophan due to carry over from enzyme synthesis was 14 μM.

| Assay conditions | trpED enzyme activity (μmol/30 min/0.1 ml) |
|------------------|-------------------------------------------|
| Complete         | 1.66                                      |
| Complete — chorismate | 0.05                                    |
| Complete — glutamine | 0.16                                    |
| Complete + 0.5 mm tryptophan | 0.41                                    |
| Complete — template DNA during synthesis | <0.01 |

Enzyme synthesis using various DNA templates

Reaction mixtures for enzyme synthesis were incubated for 50 to 60 min. Assays for enzyme activity were for 30 to 60 min. Data with different DNA templates were collected from experiments performed over a period of 9 months.

| DNA template      | DNA concentration | Enzyme activity (μmol/30 min/0.1 ml) |
|-------------------|-------------------|--------------------------------------|
| φ80 trpEDCBA 190  | μg/0.1 ml         | trpE trpED trpA                      |
| 1.8               | 5.94              | 2.35                                 |
| φ80 trpEDCB 1     | 2.0               | 10.9                                 |
| 7.75              | 1.10              |
| λ80 trpEDCBA 190.9 | 3.7                | 0.42                                 |
| λ80 trpED 37      | 0.9               | 13.3                                 |
| φ80 trpCBA 77     | 2.0               | <0.02                                |
| λ80 trpE          | 0.9               | <0.02                                |

Enzyme Synthesis

Enzymes were obtained from a crude extract of E. coli strain trpE-W3110 and a reaction mixture from in vitro synthesis. For in vitro synthesis incubation was at 37° for 50 min using 3.5 μg/0.1 ml of φ80 trpEDCBA 190 template DNA. All enzyme assays were conducted at 37° for 30 min.

| Enzyme synthesis | Enzyme activity (μmol/30 min/0.1 ml) |
|------------------|--------------------------------------|
| In vitro         | 21.8                                 |
| In vitro         | 2.58                                 |

The dependence on DNA for synthesis of trpED and trpA enzymes is shown in Fig. 2. Nonlinearity of trpED enzyme synthesis at low DNA concentrations may reflect inefficient subunit aggregation at low subunit concentrations. At higher subunit concentrations aggregation occurred rapidly since the activity obtained immediately after synthesis was not further increased by incubation at 0° for several hours.

An estimate of the amount of enzyme synthesis obtained in vitro is shown in Table III. The specific activities of trpED and trpA enzymes resulting from in vitro synthesis were approximately 1.7 to 3.2% of those obtained from trpK cells. This comparison does not take into account differences in DNA concentration between in vivo and in vitro syntheses and only serves to indicate that significant enzyme synthesis is obtained in vitro. The specific activity of trpED enzyme relative to trpA enzyme from in vitro synthesis is within 2-fold of that obtained by in vivo synthesis (see also Table VII).

Enzyme synthesis was inhibited by drugs such as rifampicin and streptolydigan, which affect transcription, and by chlor-

![Fig. 2. Dependence of enzyme synthesis on DNA concentration](http://www.jbc.org/)
Fig. 3. Time course for synthesis of trp mRNA and enzymes with φ80 trpEDCBA 190 template. Samples were removed at the indicated times from similar but separate reactions and assayed for trp mRNA and enzymes. Enzyme activity was assayed for 60 min. The 100% values are: trpED mRNA, 3620 cpm; trpCB mRNA, 2174 cpm; trpE enzyme, 5.34 nmoles of anthranilate; trpA enzyme, 0.33 nmoles of [GJH]indole. For detection of trp mRNA hybridization was to double stranded λ Irp DNA. Data are corrected for nonspecific hybridization of 3H-labeled RNA to λ DNA (30 to 77 cpm).

Sequential Transcription and Enzyme Synthesis—In vivo experiments (23) have shown that in trp transducing phage transcription of the trp operon is initiated at either or both of the following sites, the trp operon promoter and the phage N operon promoter. Rates of read-through transcription from the phage promoter into the trp operon frequently exceed rates of trp-operon promoted transcription. Sequential transcription and translation of trp genes in the order trpE to trpA should result from initiation at either the phage or trp promoter. Data in Fig. 3 show the time course for transcription and enzyme synthesis from φ80 trpEDCBA 190 template DNA. Transcription under the conditions employed gave greater than 90% correct 1 strand trp mRNA. Transcription and enzyme synthesis were assayed in similar reaction mixtures which differed only in the concentration of UTP. Extrapolation from the initial linear rates indicates that trpED mRNA begins to appear at 8 min while trpCB mRNA first appears at 11 min. In another experiment, initial appearance occurred at 7 and 10 min for trpED and trpCB mRNA, respectively. Transcription of trpED (approximately 3400 nucleotides, Fig. 1) in 3 min indicates an RNA chain elongation rate of approximately 19 nucleotides per s. At this rate of transcription, the lag in appearance of trpED mRNA could result from transcription initiation at a phage promoter approximately 8,000 nucleotides from trpE. This interpretation would suggest that, with the template employed, transcription initiations at the trp operon promoter are relatively infrequent. TrpE and trpA enzymes routinely appeared following a longer lag. For the experiment shown in Fig. 3, the extrapolated initial appearance was at 17 min for trpE enzyme and 24 min for trpA enzyme. For an estimated operon segment of 5030 nucleotides for trpD through trpA, a translation rate of approximately four amino acids (or 12 nucleotides) per s is calculated from the 7-min delay between the appearance of trpE enzyme activity and trpA enzyme.

The initial lag of approximately 7 to 8 min in appearance of trpED mRNA appears to reflect mainly the time for read-through transcription from the phage N operon promoter. This is shown by the data in Fig. 4. The capacity for synthesis of trpED mRNA was rifampicin-sensitive within 1 min, yet as shown in Fig. 3 trpED mRNA did not appear for 7 to 8 min. This indicates that the site of transcription initiation for synthesis of trpED mRNA must precede trpE in the phage template by a relatively large region. The linear initial increase of trpED mRNA appearance is consistent with this interpretation as is the observation (see “Repression of Enzyme Synthesis”) that read-through transcription is not subject to inhibition by the repressor of the trp operon.

RNA Polymerase “Run-off”—An alternative method was employed to estimate the location of the major trp operon transcription initiation site of φ80 trpEDCBA 190 template DNA. During steady state transcription, rifampicin was added to block initiation, and the time course for shut down of trpED mRNA synthesis was measured. Since transcription of an operon region ceases following run-off of all RNA polymerase molecules, the run-off time is a measure of the distance from the promoter to the end of the operon region being assayed. Such an experiment using φ80 trpEDCBA 190 template DNA is shown in Fig. 5. Following addition of rifampicin at 15 min, synthesis of trpED mRNA proceeds similarly to the untreated control for several minutes and then stops. Extrapolation yields an RNA polymerase run-off time for trpED transcription of approximately 0.5 min. At a transcription elongation rate of 19 nucleotides per s, approximately 10,800 nucleotides should be transcribed in the 9.5 min. This suggests that transcription initiation occurred at

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1 Catherine L. Squires, unpublished experiments.
Aurintricarbosylate (28-30) and kasugamycin (31-33) have been reported to act as translation inhibitors that specifically inhibit ribosome initiation. Because of the recently reported greater selectivity of kasugamycin (33) and our finding of partial inhibition of ribosome run-off time to approximately 4 min. Conceivably rho factor acts to inhibit read-through transcription.

Data in Fig. 5 also show virtually instantaneous arrest of transcription by streptolydigan. Hybridizable trpED mRNA is seen to be extremely stable, having a half-life of more than 2 hours. Although not shown in Fig. 5, the translation inhibitors kasugamycin and chloramphenicol were entirely without effect on transcription under these conditions.

Ribosome Discharge—Experiments were conducted to determine if ribosomes discharge from segments of trp mRNA following transcription termination at natural chain termination codons. Aurintricarbosylate (28-30) and kasugamycin (31-33) have been reported to act as translation inhibitors that specifically inhibit ribosome initiation. Because of the recently reported greater selectivity of kasugamycin (33) and our finding of partial inhibition of transcription by 70 μM aurintricarbosylate, kasugamycin was used to block transcription initiation. Data in Fig. 6 show that approximately 0.2 mM kasugamycin was required for 90% inhibition of trpE enzyme synthesis. The data in Fig. 7 show the kinetics of synthesis of trpE and trpA enzymes following addition of kasugamycin. Note that enzyme synthesis is unaffected for several minutes following addition of the drug. It appears from this and other similar experiments that the rate of polypeptide elongation is not detectably changed by kasugamycin. In vitro polypeptide elongation on E. coli polysomes was also unaffected by kasugamycin (33). At the concentrations used in these experiments, kasugamycin was without effect on transcription of trp mRNA. The abrupt inhibition of enzyme synthesis shown in Fig. 7 is attributed to inability of ribosomes to reinitiate translation following discharge from mRNA at putative intercistronic termination sites. The secondary slope of enzyme appearance presumably reflects kasugamycin-resistant synthesis (see Fig. 6).

The times for shut down of translation due to discharge of ribosomes from segments of trp mRNA were approximately 7,340 nucleotides from the start of trpE. Thus, the data in Figs. 3 and 5 indicate that with φ80 trpEDCBA 190 DNA as template, transcription is initiated at a phage promoter approximately 7,400 to 8,000 nucleotides from trpE and that transcription continues into the trp operon. Preliminary experiments with rho factor (27) show a reduction in the RNA polymerase run-off time to approximately 4 min. Conceivably rho factor acts to inhibit read-through transcription.

Data also show inhibition by 0.15 mM streptolydigan (strepto) added at 14 min. Samples were removed at various times, and 3H-labeled +30 trpEDCB.4 190 DNA as template, transcription is initiated at a phage promoter approximately 7,400 to 8,000 nucleotides from trpE. Thus, the data in Figs. 3 and 5 indicate that with φ80 trpEDCBA 190 DNA as template, transcription is initiated at a phage promoter approximately 7,400 to 8,000 nucleotides from trpE and that transcription continues into the trp operon. Preliminary experiments with rho factor (27) show a reduction in the RNA polymerase run-off time to approximately 4 min. Conceivably rho factor acts to inhibit read-through transcription.

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A length of 10,800 nucleotides minus 3460 nucleotides (the approximate length of trpED).

**Fig. 5** (left). RNA polymerase run-off following addition of rifampicin. Template DNA was φ80 trpEDCBA 190 (1.3 μg/0.1 ml). At 15 min 10 μg per ml of rifampicin (rif) was added to a portion of the reaction mixture. Data also show inhibition by 0.16 mM streptolydigan (strepto) added at 14 min. Samples were removed at various times, and 3H-labeled trp RNA was detected by hybridization to a trpEDCBA 190 template DNA. Data are corrected for 25 to 65 cpm of nonspecific hybridization of 3H-labeled RNA to a DNA.

**Fig. 6** (center). Inhibition of trp enzyme synthesis by kasugamycin. Synthesis of trpE enzyme was for 60 min with varied concentrations of kasugamycin added prior to the start of incubation. DNA template was φ80 trpEDCBA 190.

**Fig. 7** (right). Kinetics for kasugamycin shut down of trpE and trpA enzyme synthesis. Synthesis of trpE and trpA enzymes was determined in a single reaction using φ80 trpEDCBA 190 template DNA. Samples for enzyme assay were removed at various times. At 25 min (△) 0.44 mM kasugamycin was added to a portion of the reaction mixture and sampling was continued. Enzyme synthesis was terminated with 50 μg per ml of chloramphenicol. ●——●, control; ○——○, kasugamycin.
**Table IV**

Selective inhibition of the translation of operator distal mRNA by kasugamycin

| Kasugamycin added at | Enzyme activity | Enzyme synthesis corrected for drug-resistant activity |
|----------------------|-----------------|------------------------------------------------------|
|                      | trpE | trpA | trpE:trpA | trpE | trpA | trpE:trpA | trpE:trpA |
|----------------------|------|------|-----------|------|------|-----------|-----------|
| 0                    | 0.83 | 0.12 | 0.9       | 0    | 0    | 1.0       | 1.0       |
| 1                    | 0.61 | 0.00 | 0.6       | 0    | 0    | 1.0       | 1.0       |
| 8                    | 0.83 | 0.12 | 0.9       | 0    | 0    | 1.0       | 1.0       |
| 12                   | 2.02 | 0.13 | 15.5      | 1.19 | 0.01 | 10        | 10        |
| 16                   | 3.58 | 0.19 | 18.8      | 2.75 | 0.07 | 10        | 10        |
| 20                   | 5.45 | 0.33 | 16.5      | 4.62 | 0.21 | 22        | 22        |
| 60                   | 10.4 | 1.33 | 7.8       | 0.57 | 1.21 | 8.5       | 8.5       |

This amount is less than 10% of the control as shown by comparison of the first three lines in Table IV with the last line. More importantly, addition of kasugamycin at 12 min allows completion of 1.19 units of drug-sensitive trpE enzyme, but essentially no drug-sensitive trpA enzyme. Addition of kasugamycin at 16 and 20 min also shows strong selective inhibition of trpE enzyme synthesis.

The preceding experiments indicate that ribosomes discharge at a site following trpE mRNA and reattach at a site preceding trpA mRNA. Similar experiments were conducted to determine if ribosomes discharge at the end of trpE mRNA, or continue moving on the messenger to translate trpD mRNA. Following addition of kasugamycin, translation shut down, and reattachment of RNA was observed. This was accomplished by inhibition of new mRNA synthesis. This was accomplished by interrupting transcription with rifampicin or streptolydigan. As shown in Fig. 9, the two drugs were added at times chosen so that approximately equal amounts of trpED mRNA were synthesized in reactions containing either of the two drugs. Rifampicin allowed completion of transcription in progress as shown by the appearance of trpCB mRNA, while streptolydigan blocked transcription in progress as indicated by the absence of trpCB mRNA.

Since streptolydigan blocks all transcription instantaneously, some mRNA chains will contain incomplete gene transcripts and therefore will not yield functional enzyme. The fraction of such incomplete chains will decrease with time, as the period allowed for trp mRNA synthesis increases before addition of streptolydigan. In Fig. 9 it is seen that although approximately the mRNA template DNA was from phage φ80 trpEDCB 1. Kasugamycin (0.5 mM) and rifampicin (10 μg per ml) were added simultaneously. Synthesis was for a total of 40 min. Enzyme synthesis was terminated with 50 μg per ml of chloramphenicol and the tubes were then incubated for 3 hours at 37°C to insure subunit aggregation (15). Samples of 0.05 ml were used for assay of trpE and trpED enzymes at 34°C for 30 min.

**Table V**

Kasugamycin inhibition of trpD mRNA translation

| Kasugamycin added at | Enzyme synthesis |
|----------------------|------------------|
|                      | trpE | trpED |
|----------------------|------|------|
| 0                    | 0    | 0    |
| 5                    | 0.24 | 0    |
| 10                   | 0.36 | 0.05 |
| 12                   | 0.53 | 0.11 |
| 14                   | 0.95 | 0.33 |
| 16                   | 1.14 | 0.43 |
| 20                   | 1.52 | 0.60 |
| 40                   | 3.78 | 1.50 |
|                      | 7.55 | 3.97 |

FIG. 8. A, kinetics for kasugamycin shut down of trpE and trpED enzyme synthesis. The experiment was similar to that shown in Fig. 7 except that φ80 trpEDCB 1 template DNA was used and trpE and trpED enzyme activities were monitored. Kasugamycin (0.44 mM) was added at 16 min (II) as indicated. Enzyme activities are plotted relative to that obtained at 50 min in the absence of kasugamycin (4.50 nmoles/0.05 ml for trpE enzyme and 0.00 nmoles/0.05 ml for trpED enzyme). B, kinetics for kasugamycin shut down of trpE enzyme synthesis. The DNA template was λ80 trpE. Kasugamycin (0.39 mM) was added at 18 min (I). Enzyme activities are expressed relative to that obtained at 50 min in the absence of kasugamycin (4.97 nmoles/0.04 ml).
Enzyme synthesis in the rifampicin uncoupled mixture was lower, as expected, and translation efficiency in the streptomycin-treated mixture was approximately 64%, as efficient as coupled translation (control). Similar comparisons of trp enzyme synthesis with several DNA templates are shown in Table VII. A marginal and variable repression of enzyme synthesis was obtained with ϕ80 trpEDCBA 190 and ϕ80 trpEDCB 1 DNA templates. On the other hand, 80 to 90% repression of trpE enzyme synthesis was routinely obtained when the DNA template was from certain ϕ80 trpEDCB 1 carrying an 0 mutation. In this experiment enzyme synthetic capacity for the two S-30 extracts was identical and a correction was not required.
Repression of enzyme synthesis by S-100 extracts from trpR+ strains

Synthesis of trpE enzyme was measured using 5.9 μg/0.1 ml of λ80 trpED 37 DNA, 1.3 μg/0.1 ml of φ80 trpEDCBA 190 DNA, and 1.3 μg/0.1 ml of φ80 trpEDCBA 1 DNA and S-100 extract from trpR+ or trpR- strains. CORRECTIONS FOR NONSPECIFIC INHIBITION OF PROTEIN SYNTHESIS BY S-100 EXTRACTS were made using φ80 trpEDCBA 0-8 DNA template. For reactions containing S-100 extract uncorrected enzyme syntheses are shown in parentheses.

| DNA template       | S-100 | trpR+ | trpE enzyme activity | Repression |
|--------------------|-------|-------|----------------------|------------|
| λ80 trpED 37       |       | ng    | nmoles/0.1 ml        | %          |
| 0                  | 0     | 2.69  |                      |            |
| 0.13 R+            |       | 1.95  | (1.84)               | 27         |
| 0.27 R+            |       | 1.26  | (1.07)               | 53         |
| 0.54 R+            |       | 1.09  | (0.71)               | 50         |
| 0.14 R+            |       | 1.23  | (0.29)               | 64         |
| 0.36 R-            |       | 2.65  | (0.95)               | 5          |
| 0                  | 0     | 6.74  |                      |            |
| 0.27 R+            |       | 7.15  | (5.74)               | 0          |
| 0.81 R+            |       | 6.48  | (3.64)               | 4          |
| 0                  | 0     | 8.74  |                      |            |
| 0.27 R+            |       | 9.40  | (7.54)               | 0          |
| 0.81 R+            |       | 7.73  | (4.34)               | 11         |

Table VIII

Comparison of repression of transcription and enzyme synthesis

Transcription and enzyme synthesis were conducted for 60 min using 3.7 μg/0.1 ml of λ80 trpEDCBA 190-9 and 2.0 μg/0.1 ml of φ80 trpEDCBA 0-8 DNA templates and S-30 extracts from trpR+ and trpR- cells. The trpR+ DNA was used to correct for different synthetic capacities of the two S-30 extracts. The uncorrected value for trpE enzyme is shown in parentheses. Assay of trpE enzyme was for 60 min. For detection of trp RNA, hybridization was first to filters containing 20 μg of λ DNA for 16 hours followed by hybridization for 16 hours to filters containing 2 μg of λ trpCB I strand, λ trpCB r strand, and λ DNA. Transcription was similar for trpR+ and trpR- extracts using the 0 strand and no correction was applied to transcription data.

| S-30 extract | Corrected trpE enzyme | Repression | trpCB | Repression |
|--------------|-----------------------|------------|-------|------------|
|               | nmoles/0.1 ml         | %          | nmoles | %          |
| trpR+        | 6.90                  | 71         | 914   | 1827       |
| trpR-        | 1.98 (0.52)           |            | 439   | 1969       | 52         |

trpR+ strains. Data in Table VIII show repression of λ80 trpED 37 DNA-directed trpE enzyme synthesis when S-100 protein from trpR+ strains was added to S-30 extract from a trpR- strain. It appears that S-100 extract from a strain diploid for trpR contains twice as much trp repressor as a haploid strain. Little or no repression of enzyme synthesis was obtained with φ80 trpEDCBA 190 and φ80 trpEDCBA 1 DNA templates. S-100 prepared from a trpR- strain has little or no repressor activity.

A comparison of repression of transcription and enzyme synthesis is presented in Table IX. These data show somewhat greater repression of trpE enzyme synthesis than trp mRNA transcription but more extensive studies are required to determine the significance of this difference.

Repression of Transcription—The kinetics of transcription of trp mRNA catalyzed by trpR+ S-30 extracts in the presence and absence of tryptophan are shown in Fig. 11. Transcription from φ80 trpEDCBA 190 DNA template exhibited biphasic kinetics (Fig. 11A). A slow initial rate of trpCB mRNA synthesis (33 cpm per min) was detected prior to the expected read-through transcription, which was at a rate of 430 cpm per min. Presumably the trpCB mRNA observed early was initiated at the trp operon promoter. TrpR+ promoted transcription was repressed by tryptophan whereas read-through transcription was not.

The kinetics of transcription of trp mRNA from repressible λ80 trp DNA templates was not obtained due to difficulties with the detection of the small amounts of trp mRNA transcribed from λ80 trp hybrid phage. However, transcription kinetics were determined with template DNA from phage φ80 trpEDCBA 190-10 (Fig. 11B). The extrapolated time of appearance of trpCB mRNA at about 4 min suggests that the initial transcription was initiated at the trp operon promoter. The trpR+ promoted transcription by trpR+ S-30 extract was 94% repressed by tryptophan. Read-through transcription of trpCB mRNA appeared at approximately 15 min and accounts for the decreased repression. Under these conditions the trpR- promoted transcription on φ80 trpEDCBA 190-9 templates was at a rate approximately 13 times that on φ80 trpEDCBA 190 DNA. E. coli strain trpED 102 has a 4- to 6-fold increased rate of in vivo trpCB mRNA synthesis compared to control strains (94).

Size of Trp mRNA Synthesized in Vitro—Since trp operon transcription is initiated at a phage promoter approximately 7100 to 8000 nucleotides from the start of trpE with φ80 trpEDCBA 190 DNA as template, trp operon mRNA from this template should be approximately twice the size of that initiated at the trp operon promoter, provided that the transcription termination site at the end of the trp operon is functioning. Profiles of trp mRNA made in vitro from φ80 trpEDCBA 190 and λ80 trpEDCBA 190-9 templates are shown in Fig. 12. TrpEDCBA mRNA transcribed from the φ80 trp operon template DNA was larger than 23 S whereas trpEDCBA mRNA transcribed from the λ80 trp operon template DNA was smaller than 23 S. Franklin has deduced that essentially all in vivo trp transcription is initiated at the trp promoter in phage λ80 trpEDCBA 190-9.4 These results demonstrate the relatively large size of trp mRNA resulting from read-through transcription.

Polarity—In order to examine polarity in vitro the polar chain termination mutation trpC6 (35) was crossed into φ80 trp and λ80 trp phage. The data in Table X show the polar effect of trpC6 in vivo. A 7-fold reduction of trpA enzyme activity was obtained for trpC6 relative to the parental bacterial strain. In vivo mutation trpC6 exhibited polarity of the λ80 trp phage but not in the φ80 trp phage. Polarity was not obtained in vitro using template DNA from either the λ80 trp or φ80 trp phages (Table XI).

Discussion

Enzymes of the trp operon have been synthesized by in vitro DNA-directed transcription and coupled translation. We have detected and measured synthesis of mRNA corresponding to the first four genes, and enzyme subunits corresponding to the first, second and fifth genes, of the E. coli trp operon. We have studied expression of the trp operon in vitro using two types of DNA templates: (a) templates from φ80 trp phage exhibiting mostly read-through expression in vivo and in vitro; (b) templates prepared from certain λ80 trp phage exhibiting trp operon expres-

4 Naomi C. Franklin, unpublished data.
FIG. 11 (left and center). Effect of tryptophan on transcription.

Standard reaction mixtures contained S-30 extract from trpB+ cells and (A) 53 pg per ml of $\phi 80$ trpEDCBA 190 DNA or (B) 52 pg per ml of $\phi 80$ trpED102 trpCB DNA. Reaction mixtures were otherwise similar except for the absence (•••••) or presence (O---O) of 0.1 mM tryptophan. $^{35}$S RNA was isolated from samples removed at various times. TrpCD mRNA was detected by hybridization to $\lambda$ trpCB 1 strand DNA. - - - - , $\sim 70$% repression.

FIG. 12 (right). Sucrose gradient centrifugation of trp mRNA synthesized in vitro. Reaction mixtures (0.2 ml) containing 7.0 pg of $\phi 80$ trpEDCBA 190 (O---O) and 7.4 pg of $\lambda$h80 trpEDCBA 190-9 (O--C) template DNA were incubated for 15 min, at which time 50 &mu of [3H]UTP (36.8 Ci per mmole) were added. Incubation was continued for an additional 10 min. $^{35}$S-labeled RNA was isolated and 0.15-ml portions were layered on 4% ml 5 to 20%, sucrose gradients containing 0.15 M NaCl and 0.15 M sodium citrate, pH 7.0. Centrifugation was for 2 hours at 50,000 rpm in a Spinco SW65 rotor. Fractions of 10 drops were collected. Peaks corresponding to 23 S and 16 S endogenous ribosomal RNA were detected by measurement of absorbancy at 260 nm. Trp RNA from $\phi 80$ trpEDCBA 190 template was detected by hybridization of 0.1-ml portions to $\lambda$ trpCB 1 strand DNA and corrected for radioactivity hybridizing to $\lambda$DNA (73 to 104 cpm). Trp RNA from $\lambda$h80 trpEDCBA 190-9 template was detected by first removing the bulk of $^{35}$S-labeled $\phi 80$ RNA by hybridization to 20 pg of filter-bound $\phi 30$ DNA for 26 hours at 66°C, followed by hybridization to 2 pg of filter-bound $\phi 80$ trpEDCBA 1 strand DNA for the standard 16 hours at 66°C. Data were corrected for radioactivity hybridizing to $\phi 80$ DNA (27 to 218 cpm). Maximum $^{35}$S-labeled trp RNA was 4327 cpm for $\phi 80$ trpEDCBA 190 and 372 cpm for $\lambda$h80 trpEDCBA 190-9.

TABLE X

| Strain          | Enzyme activity | trpED:trpA | Polarity |
|-----------------|-----------------|------------|---------|
|                 | trpED | trpA |
| W3110 (parent) | 21.5  | 2.6  | 8.4    |
| trpC6           | 36.9  | 0.46 | 85.5   | 7.0    |

Experiment 1 (bacteria)

TABLE XI

| Template DNA | Enzyme activity | $^{35}$S trpA | Polarity |
|--------------|-----------------|---------------|---------|
|              | trpED:trpA      | trpED:trpA    |         |
| $\phi 80$ trp 190 | 29.8  | 8.5  | 3.5    |
| $\phi 80$ trp 190 C6 | 53.2  | 12.0 | 4.4    | (1.1)$^a$ |
| $\lambda$h80 trp 190-9 | 11.0  | 1.58 | 7.0    |
| $\lambda$h80 trp 190-9 C6 | 13.6  | 3.40 | 4.0    | 0.57 (0.68) |

$^a$ Numbers in parentheses are averages for assays at three different concentrations of template DNA.

direction, trpE through trpA (Fig. 3). From the time required for completion of synthesis of trpED mRNA (Fig. 3) and the approximate length of this operon segment, the mRNA chain elongation rate was estimated as 19 nucleotides per s at 34°C. This compares with estimates of 25 to 28 nucleotides per s at 30°C and 37 to 45 nucleotides per s at 37°C for in vivo trp operon transcription (36). Information is not available to indicate whether or not in vitro transcription terminates at the normal site following trpA. The size of the polycistronic mRNA resulting from phage-promoted read-through transcription into the trp operon is estimated to be approximately 14,400 nucleotides in length (approximately 7,700 nucleotides transcribed from phage genes

TABLE XII

| Strain          | Enzyme activity | trpED:trpA |
|-----------------|-----------------|------------|
|                 | trpED | trpA |
| Experiment 1 (bacteria) | 29.8  | 8.5  |
| W3110 (parent) | 21.5  | 2.6  |
| trpC6           | 36.9  | 0.46 |

Enzyme assays were conducted as described under "Experimental Procedure." Lack of polarity was independent of template DNA concentration.

TABLE XIII

| Polarity of trpC6 in vivo |
|--------------------------|
| To examine the effect of trpC6 in vivo, enzyme assays were performed with extracts from W3110 trpR- strains containing the nonsense mutation trpC6 in the bacterial genome (Experiment 1) or in strains infected with phage carrying trpC6 (Experiment 2) as described under "Experimental Procedure." Polarity is expressed as the ratio of enzyme activity for trpED:trpA for trpC6 relative to the wild type trpC.

Enzyme assays were performed with extracts from W3110 trp- strains containing the nonsense mutation trpC6 in the bacterial genome (Experiment 1) or in strains infected with phage carrying trpC6 (Experiment 2) as described under "Experimental Procedure." Polarity is expressed as the ratio of enzyme activity for trpED:trpA for trpC6 relative to the wild type trpC.

## Table X

| Strain          | Enzyme activity | trpED:trpA | Polarity |
|-----------------|-----------------|------------|---------|
|                 | trpED | trpA |
| W3110 (parent) | 21.5  | 2.6  | 8.4    |
| trpC6           | 36.9  | 0.46 | 85.5   | 7.0    |

Experiment 1 (bacteria)

## Table XI

| Template DNA | Enzyme activity | $^{35}$S trpA | Polarity |
|--------------|-----------------|---------------|---------|
|              | trpED:trpA      | trpED:trpA    |         |
| $\phi 80$ trp 190 | 29.8  | 8.5  | 3.5    |
| $\phi 80$ trp 190 C6 | 53.2  | 12.0 | 4.4    | (1.1)$^a$ |
| $\lambda$h80 trp 190-9 | 11.0  | 1.58 | 7.0    |
| $\lambda$h80 trp 190-9 C6 | 13.6  | 3.40 | 4.0    | 0.57 (0.68) |

$^a$ Numbers in parentheses are averages for assays at three different concentrations of template DNA.

## Table XII

| Strain          | Enzyme activity | trpED:trpA |
|-----------------|-----------------|------------|
|                 | trpED | trpA |
| Experiment 1 (bacteria) | 29.8  | 8.5  |
| W3110 (parent) | 21.5  | 2.6  |
| trpC6           | 36.9  | 0.46 |

Enzyme assays were performed with extracts from W3110 trpR- strains containing the nonsense mutation trpC6 in the bacterial genome (Experiment 1) or in strains infected with phage carrying trpC6 (Experiment 2) as described under "Experimental Procedure." Polarity is expressed as the ratio of enzyme activity for trpED:trpA for trpC6 relative to the wild type trpC.

## Table XIII

| Polarity of trpC6 in vivo |
|--------------------------|
| To examine the effect of trpC6 in vivo, enzyme assays were performed with extracts from W3110 trpR- strains containing the nonsense mutation trpC6 in the bacterial genome (Experiment 1) or in strains infected with phage carrying trpC6 (Experiment 2) as described under "Experimental Procedure." Polarity is expressed as the ratio of enzyme activity for trpED:trpA for trpC6 relative to the wild type trpC.
and 6,760 from \( trp \) genes). Analysis by sucrose gradient centrifugation indicates that \( trp \) mRNA transcribed from \( \phi 80 \) \( trpEDCBA \) 190 template DNA is considerably larger than \( trp \) mRNA that appears to have been initiated at the \( trp \) promoter (Fig. 12).

Coupled translation of \( \phi 80 \) \( trpEDCBA \) 190 transcripts starts at the \( trpE \) end of the message and proceeds sequentially in the correct direction, \( trpE \) through \( trpA \). Indications of an assembly of subunits were noted only at low levels of enzyme synthesis. A translation elongation rate of four amino acids per s was estimated from the time required to complete synthesis of \( trpA \) enzyme following completion of \( trpE \) enzyme and the length of the operon segment \( trpD \) through \( trpA \) (Fig. 1). A rate of four amino acids per s at 34° may be compared with an estimate of in vivo polypeptide elongation for \( E. coli \) β-galactosidase of 15 amino acids per s at 37° (37) or 14 to 17 amino acids per s for in vivo peptide elongation in \( S. typhimurium \) at 37° (38). These comparisons suggest that in vitro synthesis occurs at a rate approximately 25% that occurring in vivo.

Using this system for in vitro transcription and coupled translation we have examined the question of whether ribosomes discharge from \( trp \) mRNA upon translating normal chain terminating codons. From measurements of kasugamycin shut down of translation (Fig. 7), we conclude that ribosomes which initiate translation of \( trpE \) mRNA dissociate and reattach prior to initiating translation of either \( trpD \) or \( trpA \) mRNA. Addition of kasugamycin at times when ribosomes have attached to newly synthesized \( trpE \) mRNA but prior to synthesis of more distal \( trp \) mRNA results in \( trpE \) enzyme synthesis with little or no sequential synthesis of \( trpD \) or \( trpA \) enzymes (Tables IV and V). These results provide direct evidence for a model of ribosome discharge and reattachment at intercistronic termination and initiation sites.

Kasugamycin translation shut down times of approximately 0.5 min for \( trpE \) mRNA, 7 min for \( trpD \) mRNA, and 3 min for \( trpA \) mRNA, are proportional to the respective mRNA lengths (Fig. 1). This result is in accord with the conclusion that shut down results from discharge of ribosomes following recognition of the chain termination codon for each polypeptide.

Because steady state enzyme synthesis was not completely shut down by kasugamycin (Figs. 7 and 8) it is possible that up to 30% of the translating ribosomes escape discharge from \( trp \) mRNA and continue through regions of intercistronic punctuation. We considered this possibility unlikely in view of the complete inhibition of \( trpD \) enzyme synthesis obtained when kasugamycin and rifampicin are added together prior to synthesis of \( trpD \) mRNA (Table V). Somerville and Yanofsky (39) and Morse et al. (40) have shown continued in vivo synthesis of \( trpA \) enzyme, which lacks tryptophan, when \( trpEDCB \) enzyme synthesis is inhibited by starvation for tryptophan. This result suggests that free ribosomes attach to the \( trpA \) initiator codon in vivo.

Earlier work of Webster and Zinder (41) has shown rapid release of a single translating ribosome from phage 12 RNA upon reading amber codons as chain terminators. However, only about 40% dissociation of the ribosome-12 RNA message complex occurred upon chain termination at the end of the coat protein cistron. It was suggested that translation by the same ribosome may continue if there is another initiation signal in close proximity to a termination signal.

Addition of rifampicin and streptolydigen allowed translation uncoupled from transcription. Experiments with both drugs showed that accumulated \( trpED \) mRNA chains were translated at about 60% efficiency compared to coupled translation. Efficient translation of accumulated completed \( trp \) mRNA is consonant with the stability of mRNA obtained under these in vitro conditions. The data in Figs. 5 and 9 show essentially no degradation of \( trp \) mRNA as measured by RNA-DNA hybridization.

In these experiments \( trp \) mRNA half-lives were in excess of 2 hours. Although not studied in detail, it was observed that when transcription reaching 50% or more of completion, was inhibited with rifampicin or streptolydigen, the half-life of \( trp \) mRNA decreased to 35 to 40 min. Uncoupled translation of \( gal \) operon mRNA has been recently reported by Wietekam and Ehring (7). In this case the functional half-life of \( gal \) mRNA was 6 to 7 min. The unusual stability of \( trp \) mRNA in vitro suggests that our S-30 preparations lack the nucleases activities involved in \( trp \) mRNA degradation, affording the opportunity to study mRNA degradation.

The mutation \( trpC6 \) of the bacterial \( trp \) operon has a polar effect on \( trpA \) enzyme synthesis in vivo. This polar effect was maintained in vivo for \( trpC6 \) in \( \lambda h80 \) \( trpEDCBA \) 190-9 phage but not in phage \( \phi 80 \) \( trpEDCBA \) 190. The main differences between these phages with respect to the \( trp \) operon are that the \( \lambda h80 \) \( trp \) strain is \( N^+ \) and appears to have a transcription termination site prior to the \( trp \) operon which prevents read-through transcription from the phage promoter into the \( trp \) operon (23). It is not known if either of these factors is responsible for the expression of polarity. Since polarity is not obtained in vitro with template DNA from either of these phage, it appears that one or more factors essential for polarity are missing in the S-30 extract. It is tempting to speculate that at least one of the missing components is a nuclease involved in mRNA degradation. Wietekam and Ehring (7) have recently isolated a protein fraction from \( E. coli \) that restores polarity to in vitro gal operon enzyme synthesis.

An important distinguishing feature between template DNA from \( \phi 80 \) \( trp \) phages and \( \lambda h80 \) \( trp \) phage was detected in in vitro repression experiments. \( Trp \) operon enzyme synthesis directed by \( \lambda h80 \) \( trp \) template DNA was strongly repressed by tryptophan with \( trpR^- \) S-30 extracts whereas enzyme synthesis from \( \phi 80 \) \( trpEDCBA \) 190 and \( \phi 80 \) \( trpEDCB \) 1 template DNA was essentially not repressible. Our results indicate that more than 90% of \( trp \) mRNA transcribed from \( \phi 80 \) \( trpEDCBA \) 190 template DNA was synthesized by read-through from a phage promoter. Read-through \( trp \) transcription was not repressible in vivo just as it is not repressible in vitro (23). Negligible read-through for in vivo \( trp \) operon expression was obtained with the \( \lambda h80 \) \( trp \) phage used in this work. In preliminary experiments enzyme synthesis from \( \phi 80 \) \( trpEDCBA \) 190 template DNA was made 80% repressible by rho factor. Rose et al. (12) obtained repression of in vitro transcription using template DNA from \( \phi 80 \) \( trp \) phage, highly purified RNA polymerase and partially purified \( trpR \) protein. In this case essentially all \( trp \) transcription was initiated at the \( trp \) promoter since read-through transcription initiated at a phage promoter was excluded by the low rate of RNA chain elongation.

Note Added in Proof—Dr. Fumio Imamoto has informed us of his recent experiments which indicate no polarity in vivo for \( MrpE8829 \) (amber) or \( MrpE9763 \) (ochre). These phages exhibit read-through expression of \( trp \) genes from transcription initiated at the phage promoter \( Pr_L \). Dr. Naomi Franklin of our laboratory has also shown that polarity in the \( trp \) operon is largely relieved in vivo in \( \lambda trp \) phage when transcription of the
trp operon is initiated at the phage promoter P_L in the presence of the N protein of λ.

Acknowledgments—The authors are indebted to Naomi Franklin for her interest and expert advice and for the preparation of some of the phages employed in this investigation. We are also indebted to Miriam Bonner, Virginia Horn, and Joan Hanlon for constructing bacterial and phage strains and preparing large supplies of purified phage. H. Z. is particularly grateful to John Rose for instruction, criticism, and materials.

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