*TRANSLATION OF THE TRYPTOPHAN MESSENGER RNA OF ESCHERICHIA COLI**

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Communicated June 18, 1968

Translation of the messenger RNA (mRNA) transcribed from the tryptophan operon of *Escherichia coli* has been examined, with the use of the recently reported[1] procedures for synchronous and controlled transcription of the operon. The experiments to be described permit estimation of the number of ribosomes that translate each molecule of *trp*-mRNA, and of their spacing and rate of travel along the messenger. Several features of the temporal relationship between transcription and translation are elucidated.

The tryptophan operon of *E. coli* consists of five contiguous genes[2] and an operator control element,[3] as illustrated in Figure 1. Repression of the operon

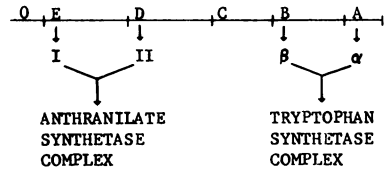

FIG. 1.—*E. coli* tryptophan operon (approximately to scale) and the gene products studied in this work.

by tryptophan is mediated by a repressor molecule that is the product of an unlinked (*trpR*) gene.[4] The addition of indole-3-propionic acid (IP) to growing bacteria causes derepression of the operon[5] and, under appropriate conditions, leads to synchronous initiation of synthesis of the polycistronic *trp*-mRNA.[1] Under these conditions, the subsequent addition of high concentrations of tryptophan prevents further transcription initiations, while allowing completion of those mRNA chains which had already begun to be synthesized. In these studies, it was discovered that rounds of synthesis of *trp* mRNA molecules in synchronously derepressed cells occurred with a regular periodicity of about 2.5 minutes; a similar observation was made by Imamoto[6] who used tryptophan starvation to achieve derepression. In our experiments, we have used IP addition and subsequent repression by tryptophan to obtain synchronous and limited transcription, and have studied the total yield of the protein products of the *trp E* and *D* genes (anthranilate synthetase complex) and the *trp A* gene (tryptophan synthetase α) per *trp*-mRNA molecule. In other experiments, we have examined the kinetics of appearance of these enzymes following derepression.

Methods.—Bacterial cultures: (1) *Repression:* Repressed cultures of *E. coli*, strain W3110, in the logarithmic phase of growth were obtained by adding fresh inocula (grown to stationary phase in L broth) to minimal medium[7] supplemented with 1% glucose and 50 μg/ml *L*-tryptophan. After shaking for 4–6 hr at 37°C, these cultures (0.7–1.0 $\times$ 10^9

cells/ml) were harvested by centrifugation at 0°C and resuspended at 0°C in minimal medium containing 1% glucose and 10 μg/ml tryptophan to yield 0.9–1.0 × 10^9 cells/ml. Such cultures (100–300 ml) were promptly placed in a 37°C water bath and incubated with vigorous shaking; cell growth was monitored with a Klett-Summerson colorimeter (660 filter).

(2) *Derepression:* Ten min after transfer of the culture from 0° to 37°C, IP was added to a final concentration of 30 μg/ml.

(3) *Sampling:* In experiments examining the kinetics of translation of the newly synthesized *trp*-mRNA, samples (10 ml) were withdrawn at regular intervals before and after the addition of IP. These were rapidly mixed with 1 ml of a solution containing chloramphenicol (4 mg/ml) and NaN_3 (0.2 *M*) and then chilled in ice. Prior to the addition of IP, an aliquot of the culture was transferred to a second flask and incubated as a control culture that had not received IP.

In experiments examining the total translational capacity of each *trp*-mRNA molecule, aliquots of the culture were removed at regular intervals and incubated in the presence of additional tryptophan at a sufficiently high concentration (100 μg/ml) to establish immediate repression of further transcription initiations.[1] All cultures were incubated at 37°C with vigorous shaking for 30 min after the addition of IP and were then treated with chloramphenicol and NaN_3 as described above. A control culture that did not receive IP was incubated as described above.

Enzyme assays: Cells were collected by centrifugation, washed in 0.85% NaCl, and centrifuged again. Extracts were prepared by suspending the cells in 0.5 ml of Tris-Cl (0.1 *M*, pH 7.8, 0°C) and disrupting them by ultrasonic oscillation with a Branson sonifier (equipped with a microprobe) at 0°C; cell debris was removed by centrifugation (2000 × *g*, 30 min, at 0°C). Enzymatic activities of cell-free extracts were measured as described previously;[8] protein concentrations were determined by the method of Lowry *et al.*[9] Alternatively, tryptophan synthetase activity was measured in washed, toluene-treated cells, with the use of a modification of the technique reported by Herschman and Helinski.[10] The number of molecules of each enzyme per cell was calculated from the specific activity and yield of soluble protein per cell on the basis of the specific activity and molecular weight of the purified protein.[11]

Results.—A fixed number of ribosomes translates each messenger: An experiment measuring the total yield of tryptophan synthetase resulting from each round of transcription is shown in Figure 2. Cells were derepressed by exposure to IP for increasing lengths of time prior to repression by excess tryptophan, and then translation of new mRNA molecules was permitted for sufficient time to allow complete expression of the increased capacity for protein synthesis. The

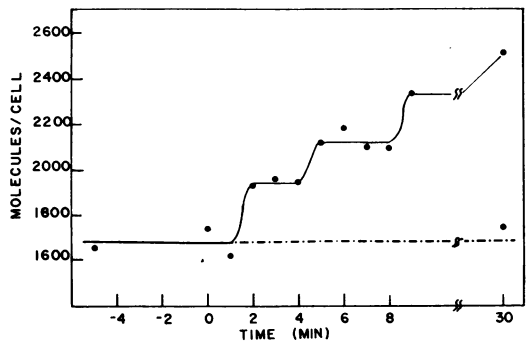

FIG. 2.—The fixed translational capacity of each messenger. Incubation of cells (in 10 μg/ml tryptophan) was begun at −10 min, and IP was added at zero time; the dotted line indicates a control that received no IP. Tryptophan (100 μg/ml) was added to portions of the culture at the indicated times, and translation was allowed to proceed for 30 min after the addition of IP; samples were then analyzed for tryptophan synthetase (toluenized cell assay). It should be noted that the starting culture had not reached the basal level of enzyme found in maximally repressed cells.

control value (no IP) indicates that derepression was dependent upon the addition of IP. It is evident from the figure that the enzyme level (molecules/cell) increases discontinuously as a function of the length of time during which the cells remained derepressed. The pattern is strikingly similar to that of *trp*-mRNA production observed under these conditions.[1]

The fact that the capacity for enzyme synthesis increases to a new plateau within a few minutes after the start of derepression reflects the rapid initiation of synthesis of the first round of *trp*-mRNA; the second round of enzyme synthesis is not initiated unless repression by tryptophan is delayed until a second round of transcription has started. The limited extent of the increase in capacity to synthesize this protein as a result of the synthesis of one copy of mRNA indicates that only a fixed number of ribosomes translates this message. When derepression extends for progressively longer periods, two succeeding increases in the capacity for enzyme synthesis are observed; these correspond approximately to the 2.5-minute periodicity reported[1] for the initiation of succeeding rounds of *trp*-mRNA synthesis.[12]

The approximate constancy in the number of enzyme molecules synthesized by translation of each of the three successive copies of *trp*-mRNA (Table 1)

TABLE 1. *Fixed translational capacity of each messenger.*

	Tryptophan synthetase increment (molecules/cell)
First round	215
Second round	165
Third round	160
Average/round	180
Average/mRNA molecule	90

Calculated from the data in Fig. 2, after correction of the plateau values for the dilution resulting from cell growth between the time of synthesis and the termination of the experiment.

indicates that approximately the same number of ribosomes translates each messenger molecule. Since all samples were allowed to synthesize enzyme for 30 minutes following the IP addition, it is clear that the limitation in the expression observed at the three plateaus reflects the limitation in the number of mRNA molecules produced. On the assumptions that the population contains an average of two genomes per cell during logarithmic growth in minimal medium[13] and that all of the genomes have been transcribed, we may calculate the average number of enzyme molecules produced, and hence the average number of ribosomes, as 90 per *trp*-mRNA molecule (Table 1).

In a similar experiment (Fig. 3), the yields of anthranilate synthetase and tryptophan synthetase α were compared; the results indicate averages of 105 and 96 molecules made per *trp*-mRNA molecule, respectively. This demonstrates that approximately equal numbers of molecules of the two enzymes are formed upon translation of each mRNA molecule, in agreement with the finding[14] that constitutive synthesis results in equimolar production of these enzymes. The initial discrepancy between the intracellular contents of the two enzymes reflects the presence of a low-efficiency promoter region located in the vicinity of

the *D* gene-*C* gene boundary; this promoter apparently is the site of initiation for mRNA chains carrying information only for the distal three genes in the operon.[15] Since these initiations occur with a frequency that is only about 2 per cent of the frequency of initiations at the operator region during derepression, they do not contribute a significant error to these estimations. An analogous promoter was previously identified in this region of the *trp* operon of *Salmonella typhimurium* by Bauerle and Margolin.[16]

Kinetics of translation: Results of an experiment designed to measure the translation of the *E*, *D*, and *A* genes as a function of the time following derepression are shown in Figure 4. Anthranilate synthetase, the enzyme complex composed of the products of the operator-proximal *E* and *D* genes, starts to increase within a few minutes after addition of IP. Translation of the terminal *A* gene, monitored by the level of tryptophan synthetase α, commences a few minutes after the start of translation of *E* and *D*. Having observed that translation of a single round of mRNA synthesis contributes approximately 200 molecules of these gene products per cell, we can estimate the time at which the *first* molecule of *trp*-mRNA has been completely translated as that time at which an increment of 200 molecules/cell has been added to the intracellular enzyme level.

Essentially the same results have been obtained in kinetic measurements of enzyme appearance following synthesis of a single copy of *trp*-mRNA. In these experiments, tryptophan (100 μg/ml) was added two minutes after derepression with IP to prevent synthesis of more than a single round of *trp*-mRNA.

The incorporation of C^{14}-leucine into protein was measured in a control experiment under the conditions described above. It was found that addition of cells to chloramphenicol and NaN_3 caused virtually immediate and complete

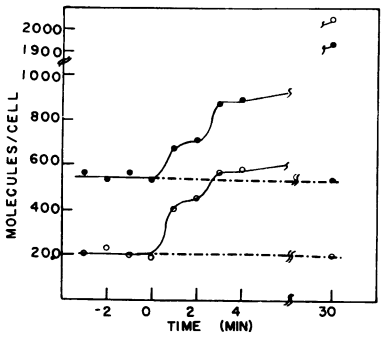

FIG. 3.—Coordinacy of translational capacities. Cultures were treated as illustrated in Fig. 2 and described in *Methods*, and extracts then analyzed for anthranilate synthetase complex (O) and tryptophan synthetase α(●). The dotted lines indicate values for a control that received no IP.

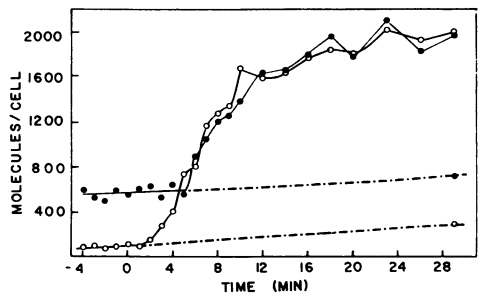

FIG. 4.—Kinetics of translation. As described in *Methods*, cells were derepressed by the addition of IP at zero time, and analyzed for anthranilate synthetase complex (O) and tryptophan synthetase α (●) as a function of time. The dotted lines indicate values for a control that received no IP.

cessation of protein synthesis. Thus we can presume that the data obtained under these conditions truly reflect the kinetics of translation.

Discussion.—The fixed number of ribosomes translating each messenger: From the data in Figures 2 and 3 and Table 1, it is seen that a fixed, approximately constant number of ribosomes translates the entire length of each *trp*-mRNA molecule. The estimate of the absolute number of ribosomes per messenger is calculated from the specific activities and molecular weights of the purified proteins, assuming the turnover numbers of the crude and purified enzymes to be equivalent. The uncertainties associated with the determination of each of these parameters and of the yield of soluble protein per cell limits the accuracy of the final estimate to about ± 50 per cent.

The fact that each mRNA molecule has a precisely limited capacity for translation suggests that some regulatory signal occurs at a precise time and prevents further ribosome attachment to the 5′-end of the mRNA. From the results of the kinetic experiments discussed below, it is clear that this signal has occurred prior to completion of transcription and release of the *trp*-mRNA from its DNA template. The kinetic experiments allow us to estimate that loading of ribosomes is completed within a few minutes after the initiation of transcription; further data are needed to decide the temporal relationship between this regulatory event terminating ribosome loading and the signal governing the initiation of a new round of mRNA synthesis at about every 2.5 minutes. This event cannot be the *gross* degradation of mRNA chains from the 5′-end, since *trp*-mRNA degradation has recently been found to occur exonucleolytically from the 3′-end.[17] The possibility that this "signal" might be reversible would help explain the regulation of translation of stable mRNA molecules observed in higher organisms.[18]

From the finding that fully repressed cultures contain approximately 50 molecules of anthranilate synthetase per cell[15] and the present finding that each messenger contributes about 100 molecules/cell, it would appear that cultures synthesize an average of about 0.25 molecule of full-length *trp*-mRNA/genome per generation when maximally repressed; it is unlikely, therefore, that this represents transcription accompanying replication of the genome.[19]

The kinetics of translation with respect to transcription: The estimated times for the commencement of appearance of the two enzymes presumably correspond to the times at which the first ribosome has passed the end of the D region (2.5 min), and then the end of the A region (5.5 min) of the messenger. From the molecular weights of the polypeptide chains encoded by all five genes[20] and a coding ratio of three nucleotides per amino acid, we may calculate approximate nucleotide lengths along the messenger. With this information, and the data indicating the position of the first ribosome, we may calculate the rate of ribosome movement. These rates are roughly the same for any region of the messenger, as can be seen in Table 2. Messenger-hybridization studies[1] indicate that transcription of the E-D length is completed at 3.5 minutes after derepression, and the entire E-A length is completed by 6.5 minutes. The first ribosome, then, must be located immediately behind the polymerase on the nascent messenger chain; it travels in this position just behind the site of RNA polymerization over

TABLE 2. *Rate of ribosome travel.*

Ribosome	mRNA region translated	Nucleotides/minute
First	E, D	1310 ± 310
First	E, D, C, B, A	1200 ± 190
First	C, B, A	1120 ± 220
Hundredth	C, B, A	1350 ± 320
Average		1245 ± 260

Rates are calculated from the lengths of the mRNA segments determined from the molecular weights of the gene products[20] and from the translation times estimated in Fig. 4. The indicated uncertainties reflect only the experimental errors in the determinations of these two parameters. The lengths of the E-D, C-A, and E-A segments were taken as 3280 (± 330), 3370 (± 340), and 6650 (± 670) nucleotides, respectively.

the entire operon length. The somewhat shorter times estimated from the translational data compared to those obtained in transcription studies may reflect a small systematic effect of RNA degradation upon the recovery of mRNA used for hybridization.

The estimated time of completion of the first round of translation (i.e., completion of translation by the last ribosome to attach to that message) is independent of the accuracy with which the absolute number of ribosomes/mRNA has been determined; it rests upon the precision with which the enzyme activity increment per message can be measured, and upon the accuracy in the determination of the time at which this increment has appeared. The last ribosome attaching to the first copy of mRNA has finished translation of the *D* region about 3.5 minutes after the start of derepression. Thus we can be confident that we are measuring only the contribution from translation of the first messenger produced, since synthesis of the second round of mRNA will not be initiated until 2.5 minutes after the start of derepression (ref. 1 and Figs. 2 and 3), and its first contribution of anthranilate synthetase could not be expected until 2.5 minutes after that.[21] Calculations based on the kinetics of movement of the last ribosome indicate that it travels the distance between the 3′-ends of the D and A regions of the messenger at a rate of about 1350 (± 320) nucleotides/minute (Table 2). Therefore, the first and last ribosomes are translating the bulk of the messenger at approximately the same rate, and this rate is in good agreement with the estimate of 1000 nucleotides/minute for the rate of travel of RNA polymerase in transcribing the tryptophan operon under these conditions.[1]

Studies of the degradation of *trp*-mRNA indicate that degradation commences from the 3′-end about seven minutes after the start of derepression,[17] while the present study demonstrates that translation of the message is completed by about six minutes after the start of derepression. It can be concluded that degradation of the messenger does not begin until after the RNA chain has been released from the complex with RNA polymerase and DNA, and has been completely translated by the entire complement of ribosomes with which it had been loaded. The sequential translation of the polycistronic *trp*-mRNA by a limited and constant number of ribosomes indicates that there is no appreciable further attachment of new (intact) ribosomes to the mRNA at either terminal or internal positions after about one or two minutes following the start of transcription. Sequential translation commencing from the operator-proximal gene has pre-

viously been observed for the lactose,[22-25] histidine,[26-28] and galactose[29] operons under conditions similar to those employed here.

Since the last (100th) ribosome has passed the end of the D region (at 3.5 min) before the first ribosome has reached the end of the A region (at 5.5 min), it is apparent that all the ribosomes travel as a tightly clustered group along the mRNA immediately behind the RNA polymerase molecule. The length of RNA occupied by this cluster is estimated roughly from the foregoing data to correspond to about 20–40 per cent of the length of the extended polycistronic messenger.[30] Assuming an internucleotide spacing of 6.8 Å for an extended RNA chain,[31] this estimate would suggest that each ribosome occupies an average RNA length on the order of 100–200 Å, comparable to the dimensions of the ribosomal subunits.[32] This is in agreement with the recent observation[33] that ribosomes are very closely packed in bacterial polysomes.

It should be noted that interpretation of all the results obtained in such kinetic experiments is limited by the asynchrony of derepression and subsequent events. Thus, the degree to which asynchrony contributes to the observed time intervals will introduce a systematic error in the estimation of the rate of ribosome travel and, correspondingly, of the length of mRNA occupied by the ribosomes. Furthermore, if protein synthesis was not arrested immediately upon exposure to chloramphenicol and NaN_3, the rapidity of ribosome movement and the closeness of ribosome packing would be systematically overestimated; however, the control experiment measuring the arrest of C^{14}-leucine incorporation under these conditions suggests that this effect is not large.

The rapidity of ribosome movement in a cluster immediately behind the RNA polymerase accounts for the suggestion of discontinuous steps which may be discerned in the kinetics of enzyme appearance following derepression (Fig. 3). These may reflect the successive waves of translation of each new mRNA formed with a regular periodicity. Similar waves may be detected in the data reporting synchronous derepression of the lactose,[22-24] histidine,[26, 27] and galactose[29] operons.

Although the ribosomes travel along the nascent mRNA chain immediately behind the polymerase, it is not yet clear if the polymerase merely limits ribosome travel or if there is a more complicated relationship between the two. The uncertainties of the measurements do not permit us to decide from our data whether or not ribosome travel is significantly increased after the polymerase has completed transcription of the operon. However, it is possible that completion of the first round of synthesis of tryptophan synthetase α is somewhat faster than that of anthranilate synthetase (Fig. 4), which might indicate that translation of the terminal region of the messenger is only partially limited by transcription.

In a recent study, Ito and Imamoto[34] have found that all of the gene products are synthesized in a temporal sequence corresponding to the gene order in the tryptophan operon. Their data on the kinetics of translation are consistent with much of that reported here.

* This investigation was supported by grants from the National Science Foundation and the U.S. Public Health Service.

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[12] That derepression for 30 min results in little more synthesis than that obtained after derepression for only 9 min apparently reflects the result of partial repression by newly synthesized tryptophan. It seems likely that the observed level of tryptophan synthetase rapidly produces sufficient intracellular tryptophan to partially overcome the effect of the added IP.

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