ON THE INITIATION OF TRANSCRIPTION OF THE TRYPTOPHAN OPERON IN ESCHERICHIA COLI

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Little information is available at present about how RNA polymerase initiates transcription at a specific chromosomal site. If the initiation site (the promoter) determines the frequency of initiation of transcription, what is the mechanism by which the enzyme is told the proper frequency at which to transcribe the operon? With the intent of eventually solving this problem, experiments were performed to determine the timing of transcription initiations in the *trp* operon. This paper will present evidence demonstrating that the frequency of initiation of transcription of the *trp* operon is determined by a specific chromosomal site located at the operator end of the operon.

Materials and Methods.—Bacterial and phage strains: The following strains of *E. coli* K12 were used as the sources of RNA: W3110 (wild-type strain), A9952 (CRM$^+$ mutant, *A* gene), AE5, AE8, AD28, AD5, AC4, and AB7 (deletion mutants). The *trp* operon segments that remain in the deletion mutants are shown in Figure 1. Dr. C. Yanofsky kindly supplied A9952, AE5, AE8, AD28, AD5, and AB7, and AC4 was kindly donated by Dr. I. P. Crawford. The following phages were used as DNA sources: ϕ80, the non-transducing parental phage, and nondefective transducing phages ϕ80ptA–E, ϕ80ptD–E,

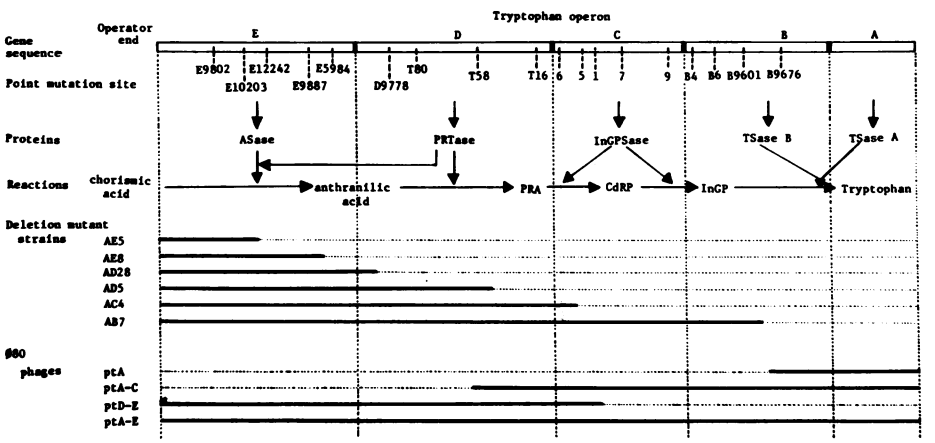

FIG. 1.—*Trp* operon segments in deletion mutants and ϕ80pt's. Each region indicated by a solid line is carried by the deletion mutant or the ϕ80pt. Abbreviations used are: ASase, anthranilate synthetase; PRTase, phosphoribosyl anthranilate transferase; InGPSase, indoleglycerol phosphate synthetase; TSase A and B, subunits of tryptophan synthetase; InGP, indole-3-glycerol phosphate; PRA, N-5′-phosphoribosyl anthranilate; CdRP, 1-(0-carboxyphenylamino)-1-deoxyribulose-5-phosphate. The relative size of the tryptophan genes is estimated from the molecular weights of the corresponding proteins (cf. Fig. 2). The order of the mutationally altered sites indicated on the map is based on previous studies.[2, 11, 12] The distance between the alterations is arbitrary. The relative location of the deletion terminus in phage ptA–C is taken from the data of Deeb *et al.*[13] Locations of the termini in ptA and ptD–E are arbitrary.

ϕ80ptA–C, and ϕ80ptA. The *trp* operon segments carried by the ϕ80pt's are shown in Figure 1. The letter designation of each ϕ80pt indicates the region of the *trp* operon that it contains. The phages were kindly supplied by Drs. A. Matsushiro (ϕ80), S. Deeb and B. Hall (ϕ80ptA–E and ϕ80ptA–C), M. Taylor (ϕ80ptA), and K. Sato (ϕ80ptD–E).

Preparation of pulse-labeled RNA: Conditions were as described elsewhere,[2] except that in derepression studies the cells were grown at 30° in Vogel-Bonner medium[3] supplemented with 19 amino acids (each 0.5 mM), but not tryptophan.

Phage DNA preparation, specific DNA-RNA hybridization technique, and sucrose density-gradient analysis: Conditions were described in detail elsewhere.[1, 2] The background hybridization values obtained with labeled RNA from repressed cultures and with DNA from ϕ80pt's are usually about one-fourth greater than the values obtained with DNA from ϕ80.[2] This is true with labeled RNA prepared from repressed cultures of all strains, including the wild-type, CRM^+ mutants, and deletion mutants.

Reagent: H^3-uridine at 15–20 c/mmole was purchased from Schwarz BioResearch Laboratories, Orangeburg, N.Y., or Daiichi Chemical Co., Osaka, and was used without the addition of carrier. Other materials were the same as described previously.[1, 2]

Results.—(1) *Detection of trp mRNA:* Specific hybrid formation between the DNA of phage ϕ80 carrying the tryptophan region and *trp* mRNA (i.e., mRNA specifically hybridizable with ϕ80 ptDNA) has been demonstrated.[1, 2, 4] Approximately 0.5 per cent of the RNA labeled under derepression conditions is mRNA specified by the whole *trp* operon. The amount of specific mRNA hybrid-

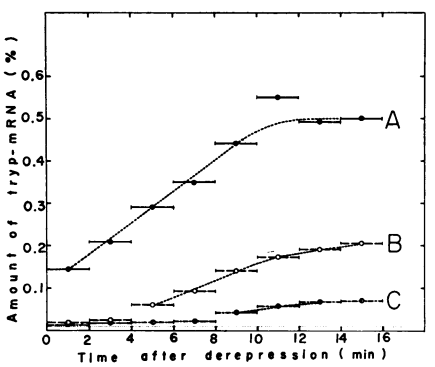

FIG. 2.—Sequential transcription of the *trp* operon. Tritiated RNA was prepared from strain W3110 pulse-labeled for 2-min periods after the commencement of derepression. The ordinate is the percentage of *trp* mRNA relative to the total labeled RNA. Hybridizable RNA was assayed in a 0.25-ml reaction mixture with 5 μg of ptA–E (*A*), ptA–C (*B*), or ptA DNA (*C*). The values are the average of duplicate determinations. The background value with ϕ80 DNA was subtracted from each hybrid value. The horizontal bars represent the pulse periods. The background value (ptDNA value–ϕ80 DNA value) found with H^3-RNA prepared from repressed cultures of W3110 is represented by the horizontal dashed line (- - - - - - -).

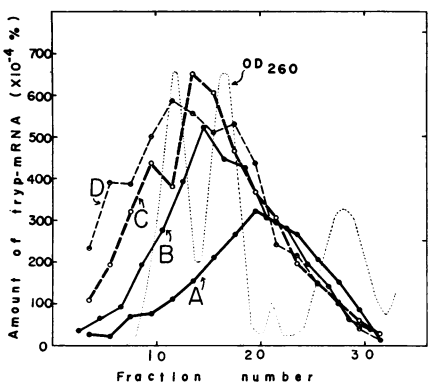

FIG. 3.—Sedimentation profiles of *trp* mRNA from strain W3110 pulse-labeled at various times after derepression. Tritiated RNA was prepared from derepressed cultures pulse-labeled for 1 min, starting at 0.5 (*A*), 2.5 (*B*), 4.5 (*C*), or 14 min (*D*) of incubation. In a linear sucrose gradient, 364 μg (*A*), 272 μg (*B*), 360 μg (*C*), or 301 μg (*D*) of H^3-RNA (spec. act., 4400 cpm/μg, 8000 cpm/μg, 7630 cpm/μg, or 8760 cpm/μg, respectively) were sedimented. Two neighboring RNA fractions were combined, and a portion was hybridized with 5 μg of ptA–E DNA in a 0.25-ml reaction mixture. Values presented were obtained with a three-eighths portion of each fraction, and are expressed as percentage of total labeled RNA in that portion of the RNA sedimented.

ized with the DNA of the different ϕ80pt's agrees well with the size of the segment of the *trp* operon carried by the transducing phage.[2] *Trp* mRNA produced by the deletion mutant AC4 (lacking genes *A*, *B*, and part of *C*) contains only regions corresponding to the remaining segment of the operon.

(2) *Chromosomal location of initiation site for transcription:* Synthesis of the polycistronic tryptophan messenger RNA initiates at the *E*-gene end of the operon and proceeds to the other end.[4-6] Sequential transcription of the *trp* operon is demonstrated in Figure 2. Following a shift from repression conditions to derepression conditions, the rate of *trp* mRNA synthesis measured by hybridization with ptA–E DNA increases until the tenth minute after derepression, at which time it reaches a steady-state level (*A*). The amount of *trp* mRNA hybridizable with ptA–C DNA is extremely low during the initial four minutes, while it increases after the fourth minute (*B*). *Trp* mRNA hybridizable with ptA DNA is barely detectable until the eighth minute after the start of derepression (*C*). These results suggest that transcription initiated at the *E*-gene end of the operon progresses sequentially to the *A*-gene end, taking about ten minutes for completion of the first set of full-length *trp* mRNA molecules.

From the data presented in this paper (see also Fig. 4*B*), and on the basis of more extensive unreported data, it can be concluded that the first five minutes after the initiation of derepression are required for transcription of the *E* and *D* genes, under conditions where it takes about ten minutes to transcribe the entire *trp* operon.

The largest molecules of *trp* mRNA detected to date have a sedimentation constant estimated at 33*S*, which corresponds to a molecular weight of the order of 2×10^6.[5] This value is equivalent to 6,700 nucleotides and 2,200 amino acids and agrees with the estimate of 244,000 for the total molecular weight of the polypeptides specified by the operon. These considerations support the view that there is but one initiation site for transcription of the *trp* operon. If this is true, the *trp* mRNA molecules should grow in size as transcription proceeds from the *E*-gene end to the *A* gene. This expectation has been realized, as shown in Figure 3. The sedimentation profiles are presented for *trp* mRNA from the wild-type strain pulse-labeled for 1 minute at 0.5, 2.5, 4.5, and 14 minutes after derepression. It is evident that the *trp* mRNA exhibits a gradual increase in size to the maximum size exhibited by the 14- to 15-minute *trp* mRNA (pulse-labeling during the steady-state period). These results support the view that transcription is initiated exclusively at a specific chromosomal site located at or near the extremity of the *E* gene of the *trp* operon.

(3) *Frequency of initiation of transcription:* (a) *Wild-type strain:* Changes in the rate of *trp* mRNA synthesis during the initial period after derepression were determined by hybridizing RNA from wild-type W3110 pulse-labeled successively for 30-second periods after the start of derepression, with DNA from ϕ80pt's. The results obtained are presented in Figure 4. The rate of synthesis of *trp* mRNA hybridizable with ptA–E DNA increases until the second minute after derepression, at which time it reaches the first steady-state level (*A* and *B*). Little change in the rate of synthesis can be seen during the subsequent two minutes, i.e., until the fourth minute after derepression. After the fourth minute

the rate again begins to increase, until it reaches the second steady-state level (*B*). The rate of synthesis remains constant when pulse-labeling is performed for different 30-second periods after the second steady-state level has been established (*C*). Sequential transcription is indicated by the fact that *trp* mRNA hybridizable with ptA–C DNA is scarcely detectable during the initial five minutes, while it appears after the fifth minute (*B*).

The simplest and most plausible interpretation of these results is that RNA polymerase starts to transcribe the operon at the initiation site soon after derepression, and that when the enzyme reaches the middle of the operon, another enzyme molecule attaches at the initiation site and starts a second round of transcription. The gradual increase in the rate of synthesis observed during the initial two minutes after derepression is presumably due to nonsynchrony of transcription starts in the bacterial population. Since the rate of synthesis remains constant after it has attained the second steady-state level, one can assume that when the third RNA polymerase is about to commence transcription at the initiation site, the first enzyme molecule has completed transcription of the operon; i.e., at any particular moment, two or three RNA polymerase molecules might be transcribing the *trp* operon.

(b) *Deletion and CRM$^+$ mutants:* Similar experiments were performed with deletion mutants and with a CRM$^+$ mutant to determine whether or not the intervening period for the initiation of transcription was normal. AB7, a deletion mutant lacking gene *A* and part of gene *B*, exhibits essentially the same transcription initiation pattern as that of the wild-type strain. This is shown in Figure 5*A*. The rates of synthesis of *trp* mRNA hybridizable with ptA–E DNA and ptD–E DNA increase until the second minute after derepression, when they attain the first steady-state level. During the subsequent two minutes the rate of synthesis changes very little, but immediately thereafter the rate begins to increase to the second steady-state level. Since the *trp* mRNA produced by this mutant has been shown to lack mRNA corresponding to the *A* and *B* genes[4] (*ca.* 20% of the operon is missing), the frequency of initiation of transcription appears to be unchanged by shortening the operon. When the RNA is hybridized with either ptA–E DNA or ptD–E DNA, two steps in the rate of *trp* mRNA synthesis can be observed. This is consistent with the finding that the distance between two RNA polymerase molecules actively transcribing the operon is within the region of the *E* and *D* genes.

With the CRM$^+$ mutant, A9952, the change observed in the rate of *trp* mRNA synthesis during the initial several minutes after derepression (Fig. 5*B*) was essentially the same as in the wild-type strain.

In order to clarify further the effect of operon size on the frequency of initiation of *trp* mRNA synthesis, several deletion mutants lacking larger regions of the *trp* operon were examined in hybridization studies with ptD–E DNA. As shown in Figure 5*C* (curve *a*), a wavy curve of change in the rate of *trp* mRNA synthesis was observed during the initial eight minutes after derepression with the deletion mutant, AE5 (this strain has only half of the *E* gene (see Fig. 1)). The rate of synthesis attained a maximum level immediately after derepression and then decreased gradually until 3.5 minutes, when it began to increase again

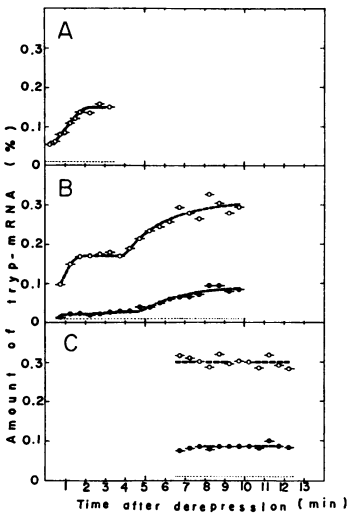

FIG. 4.—Changes in the production of *trp* mRNA during the initial stages of derepression of strain W3110. Tritiated RNA was prepared from cultures pulse-labeled for 30-sec periods during the initial 3.5 min (*A*), between 0.5 and 10 min (*B*), and between 6.5 and 12.5 min (*C*) after derepression. Other conditions are as described in Fig. 2. —○—, *trp* mRNA hybridizable with ptA–E DNA; —⊙—, *trp*-mRA hybridizable with ptA–C DNA.

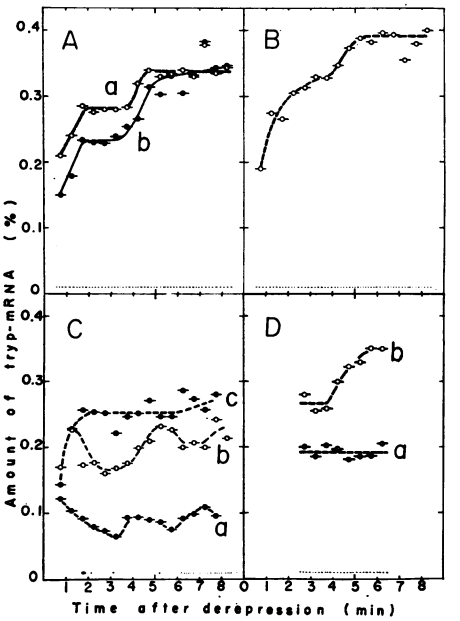

FIG. 5.—Changes in the production of *trp* mRNA by deletion mutants and a CRM$^+$ mutant during the initial stages after derepression. Tritiated RNA was prepared from strains AB7 (*A*), A9952 (*B*), AE5 (*C*, curve *a*), AE8 (*C*, curve *b*), AD28 (*C*, curve *c*), AD5 (*D*, curve *a*), or AC4 (*D*, curve *b*) pulse-labeled for 30-sec periods after derepression. The other conditions are as described in Fig. 2. —●— (*A*, curve *b*), *trp* mRNA hybridizable with ptA–E DNA (a somewhat lower hybridization efficiency was observed in hybridization assays with ptA–E DNA; —○—, —⊙—, —⊕—, *trp* mRNA hybridizable with ptD–E DNA.

To understand this result it is necessary to consider the previous finding that AE5 produces short *trp* mRNA molecules that are only one-eighth the length of the intact *trp* mRNA.[2] These fragments of *trp* mRNA presumably correspond to the first half of the E gene. Under the experimental conditions employed, transcription of this region of the *E* gene should be completed in one minute or so. Thus, the second round of transcription would not be initiated until after a sufficient time elapsed (3.5 min in this experiment), even though this period is longer than that required to transcribe the part of the *E* gene present in this mutant. The somewhat shallow change in the rate of synthesis observed is presumably due to heterogeneity in transcription starts in the bacterial population.

Similar changes in the rate of *trp* mRNA synthesis were observed with deletion mutant, AE8 (Fig. 5*C*, curve *b*), which contains most of the *E* gene but lacks genes *A–D* (see Fig. 1). AE8 also exhibits a wavy change in the rate of synthesis

during the first several minutes after derepression. A point that should be emphasized is that the rate which decreases to a minimum at the third minute begins to increase again after the fourth minute of derepression. The time required to transcribe the entire *E* gene would be about 2.5 minutes under the conditions used. Thus, the second round of transcription is initiated only after a time delay (4 min in this experiment), which is longer than the period required to transcribe the part of the *E* gene retained by this mutant.

An analogous experiment was carried out with AD28, a deletion mutant in which the deletion terminus is located at the beginning of the *D* gene (see Fig. 1). *Trp* mRNA produced by this mutant has an *S*-value estimated to be approximately 16*S*, which would correspond to a molecule of one fourth the length of the intact *trp* mRNA.[2] If the transcription behavior observed with the above strains is also characteristic of AD28, it would be expected that in this mutant an RNA polymerase molecule should start the next round of transcription shortly after the first enzyme molecule reaches the location of the deletion terminus in the operon. This expectation was realized. As shown in Figure 5*C* (curve *c*), steady-state production of *trp* mRNA is observed after 1.5 minutes of derepression, at a level which is nearly equivalent to the first steady-state level observed with mutants such as AB7 and A9952. Thus, generally only one molecule of RNA polymerase is transcribing the *trp* operon segment of AD28.

Of particular interest is the finding that transcription of the *trp* operon is initiated at intervals of 3.5–4.0 minutes under conditions where it takes about ten minutes to complete the first round of transcription, and that these intervals are not changed by shortening the transcribed region of the operon. It would therefore be expected that the amounts of *trp* mRNA produced by deletion mutants such as AE5, AE8, and AD28 for an initial period between 0.5 and 3.5 minutes of derepression (during which the first polymerase molecule has progressed over the initial region of the *trp* operon and the second one has not started yet) would correlate with the length of the *trp* operon segments present in these

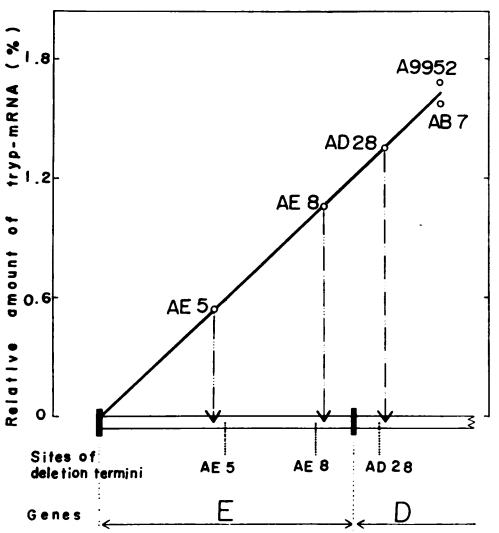

FIG. 6.—Comparison of the amounts of *trp* mRNA produced by deletion mutants during the initial period of derepression and the order of the deletion termini on the genetic map.

The ordinate is the amount of *trp* mRNA obtained by summing the rates detected between 0.5 and 3.5 min of derepression in the experiments of Fig. 5*A*, curve *a* (for AB7 value), *B* (for A9952 value), *C*, curve *a* (for AE5 value), *C*, curve *b* (for AE8 value), or *C*, curve *c* (for AD28 value).

The abscissa presents the relative positions of deletion termini on the genetic map.

The A9952 and B7 values are plotted to indicate the maximum amount of *trp* mRNA produced during this period.

mutants. To examine this possibility, the amounts of *trp* mRNA produced during this period and the order of the deletion termini on the genetic map were compared (Fig. 6). The results clearly indicate that the amounts of *trp* mRNA produced are correlated with the distances from the beginning of the operon to the sites of the deletion termini.

(4) *Determination of chromosomal distance between RNA polymerase molecules acting on the trp operon:* Two deletion mutants, AD5 and AC4, with deletion termini in the vicinity of the end of the *D* gene and the beginning of the *C* gene (see Fig. 1), respectively, were examined for changes in the rate of *trp* mRNA synthesis. The results obtained are presented in Figure 5*D*. AD5 exhibits a constant rate of *trp* mRNA production through the period from 2.5 to 6.5 minutes of derepression (*a*), while AC4 exhibits two step changes; the rate increase leading to the second step starts at the fourth minute of derepression (*b*). As mentioned in the previous section, this behavior is interpreted as indicating that the chromosomal sites of the deletion termini are located before (AD5) or beyond (AC5) the position of the first polymerase molecule, when the second one attaches. Thus, when RNA polymerase comes to the chromosomal region between the mutational sites in the *D* gene in strains T58 and T16 (see Fig. 1), the next RNA polymerase molecule begins transcription at the initiation site of the *trp* operon.

Discussion and Summary.—The results presented in this report have led to the conclusion that an initiation site for transcription of the *trp* operon, located at

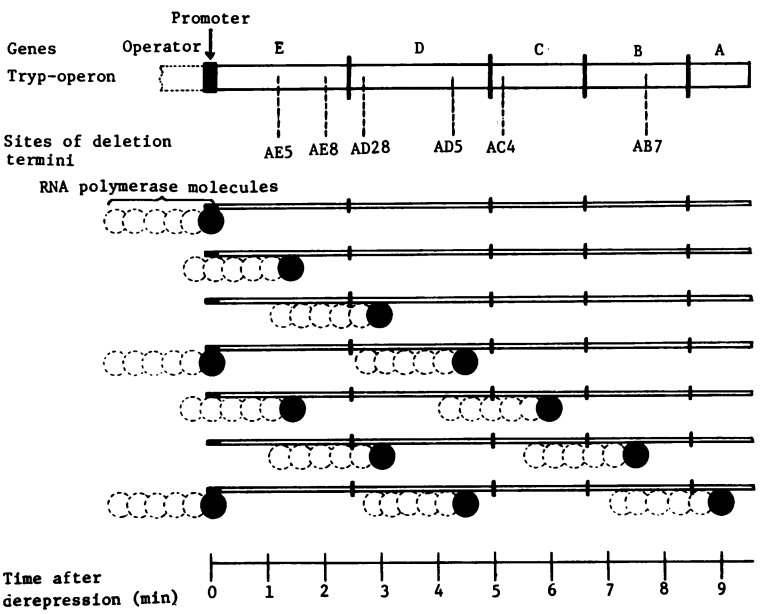

FIG. 7.—Diagrammatic representation of the initiation of transcription of the *trp* operon. Dashed circles do not represent the number of RNA polymerase molecules attached on the single tryptophan operon, but merely indicate heterogeneity in the initiation of transcription of the operon in a given bacterial population. Under the condition employed, the maximal heterogeneity is 2 min in terms of the initiation time, which correspond to the chromosomal distance from the filled circle to the dashed circle at the other end (see time scale).

the operator end of the operon, determines the frequency of initiation of transcription. Rounds of transcription of the *trp* operon are initiated at intervals of 3.5–4.0 minutes under the conditions employed, while it takes about ten minutes to complete the first round of transcription of the entire operon. These transcription events are represented diagrammatically in Figure 7. The diagram assumes the following: RNA polymerase starts transcription at the initiation site soon after derepression but in a somewhat heterogeneous fashion (for 2 min). When the first enzyme molecule reaches the end of the *D* gene, the second one attaches at the initiation site and starts the next round of transcription. When the third enzyme molecule is ready to start at the initiation site, the first enzyme molecule has traveled to the end of the *A* gene. There is a spacing of the order of 3000 nucleotides between each pair of RNA polymerase molecules acting on the *trp* operon, i.e., two or three RNA polymerase molecules might act, at any moment, on the *trp* operon. The present studies establish that the frequency of initiation of transcription is regularly timed with a short time span, whether the operon is intact or most of its terminal portion has been deleted.

The current concept of operon function considers that transcription begins at the promoter site, a site which is closely associated with the operator, with the operator region regulating the rate of transcription of the operon.[7–10] The present findings favor the view that the promoter site determines the rate of transcription of the operon.

Similar observations have been made independently by R. F. Baker and C. Yanofsky and are reported in an accompanying paper.[14]

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