Regulation of transcription from tandem and convergent promoters

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ABSTRACT

We have examined transcription on templates containing the trp and lac UV5 promoters arranged in tandem or opposing orientations. These studies have revealed that the strengths of the two promoters are comparable, though the lac UV5 promoter is much more sensitive to the level of initiating purine present. Kinetic experiments have shown that a polymerase molecule poised at the lac promoter, or a lac repressor molecule bound to the lac operator, can temporarily block a polymerase molecule initiated from the trp promoter, though transcription eventually continues through. In the convergent construct, transcription from the lac promoter is hindered only when initiation is suboptimal due to low purine concentrations.

INTRODUCTION

The lactose (lac) and tryptophan (trp) operons of Escherichia coli are well-characterized gene clusters which have been studied extensively as models for the regulation of catabolic and biosynthetic operons, respectively. In both systems, the promoters are strong in vivo, though they are subject to different regulatory control mechanisms. Efficient transcription from the wild-type lac promoter requires the presence of the CAP protein to function, though its derivative, lac UV5 (an "up" promoter mutant) does not. As a consequence of the efficient transcription obtained from both the lac UV5 and trp promoters in the absence of additional regulatory factors, they have been widely used in genetically engineered constructs to drive the expression of cloned genes. However, the strengths and characteristics of the two promoters have never been directly compared. We have constructed two templates containing the lac UV5 and trp promoters on the same segment of DNA, oriented in either tandem or opposing configurations in order to investigate the two under a number of conditions. In addition, we have used these templates to examine a variety of phenomena which may play important roles in the regulation of transcription.

Previous studies have been done in which the process of convergent

transcription was examined in vivo by monitoring the expression of distal genes when two operons of opposing orientation are fused by means of a deletion. In one of the earlier studies, it was found that when the his and roughb operons of Salmonella typhimurium are fused in this way, there is a symmetrical inhibition of expression of each operon (1). Ward & Murray (2) constructed λ trp phages in vitro to examine convergent transcription between λ P_L and the trp promoter of E. coli in vivo. They found that expression from both promoters was impaired; trp expression was blocked completely, while expression from λ P_L was only partially blocked. Unlike either of these findings, Miller et al. (3) found that there was little or no effect on the levels of expression in vivo from the trp and lac promoters when they are fused in opposing orientation. We have reexamined the process of convergent transcription between the lac UV5 and trp promoters by monitoring transcripts produced in vitro from each promoter when present on a template in opposing orientation. Our studies reveal that the strength of each of the opposing promoters may be crucial in determining the transcripts produced from such a region of DNA.

We also wished to ask what effect a repressor molecule bound to DNA downstream from an elongating polymerase molecule would have on the progress of that polymerase molecule. Although it is commonly accepted that the primary mode of repressor action is to preclude the normal binding of the polymerase molecule to the promoter region, several lines of evidence suggest that repressor may also function by blocking the progress of RNA polymerase. Reznikoff et al. (4) examined this problem in deletion strains which fused the trp promoter to the lac operator and distal lacZ, Y, and A genes. They monitored in vivo expression of the distal lacZ gene (due to readthrough transcription from the trp promoter) and found a decrease in the levels of β -galactosidase activity in the presence of lac repressor. In DNase footprinting studies, Schmitz & Galas (5) showed that RNA polymerase can still bind to the lac promoter when repressor is bound to the operator, though it fails to form the same extensive complex as it does in the absence of lac repressor. Since expression of the lac operon in vivo under repressed conditions is minimized, this finding suggests that the lac repressor can prevent the progress of a bound polymerase molecule.

We report here the results of our studies on these regulatory regions in vitro, and discuss the implications that convergent transcription and blockage of transcription by proteins bound to the DNA may have in the regulation of gene expression.

EXPERIMENTAL PROCEDURES

Enzymes and radionucleoside triphosphates. Restriction enzymes were purchased from Biolabs. T4 DNA ligase was purchased from P L Biochemicals.

[α -³²P]GTP (10-50 Ci/mmol) and [α -³²P]UTP (600 Ci/mmol) were obtained from New England Nuclear. RNA polymerase was purified by the method of Burgess & Jendrisak (6). Lac repressor was kindly provided by Tom Steitz and Maureen Leahy.

Construction of plasmids TL12 and TL13. The starting vector, pGC301 is described elsewhere (7). Derived from a deletion mutant which lacks the trp attenuator region, this plasmid contains the trp promoter and 25 bases of the leader region fused to the beginning of the trpC gene. The plasmid contains a single EcoR I restriction site created by the insertion of an EcoR I linker into a Bal I site 75 bases beyond the beginning of the trpC gene.

A 203 base-pair Hae III fragment, with EcoR I linker ends, containing DNA coding for the last 18 amino acids of the lacI gene, the CAP site (with the L8 mutation), the lac UV5 promoter-operator region, and the first 63 bases of the lac message was the gift of M. Fried. This fragment was ligated into the single EcoR I site of the plasmid pGC301 with T4 DNA ligase. The ligation mixture was transformed into the bacterial strain W3110 trp Δ AE1, and transformants were selected for ampicillin resistance. A number of ampicillin-resistant clones were screened on XG plates to identify plasmids carrying the insert: dark blue colonies result when the host lac operon is induced by the molar excess of operator-carrying insert which binds all the intracellular lac repressor. Restriction digests on minilysates derived from sample clones determined the orientation of the insert DNA. Two clones, pTL12 and pTL13 were selected for use (See Figs. 1a and b).

Template purification. Plasmid DNA was isolated from pTL12 and pTL13, digested with the restriction enzymes Sau3A I and Pvu II, respectively, and run on a 5% polyacrylamide gel. The 675 base-pair fragment from pTL12 and the 545 base-pair fragment from pTL13 containing the trp and lac promoters (See Figs. 1a and b) were isolated for use in transcription experiments in vitro.

Standard transcription reaction. Transcription reactions were carried out in a total volume of 10 μ l containing 20 mM Tris acetate pH 7.9, 0.1 mM DTT, 4 mM MgAc₂, 100 mM KCl, 3-5 μ Ci of one [α -³²P]-labeled nucleoside triphosphate, 200 μ M each of the remaining three triphosphates, 0.02-0.06 pmoles of DNA template, and 0.05-0.1 μ g of RNA polymerase. After incubation for 20 minutes at 37°C, the reactions were stopped by the addition of 100 μ l of 0.3M sodium acetate, 1 mM EDTA, and 0.5 mg/ml carrier tRNA. 250 μ l of

ethanol were added, the RNA precipitated, resuspended in formamide plus dyes, and run on 5% acrylamide/7M urea gels. Transcripts were visualized by autoradiography. Experiments done in the presence of lac repressor were performed as above except that 0.5 μ g of lac repressor was incubated with the template at 37°C for ten minutes prior to the addition of RNA polymerase. This quantity was found to be sufficient to completely block transcription from the lac promoter.

Kinetic experiments. The transcription mix was as above except: 1) It was scaled up ten times in volume, and 2) it lacked MgAc₂. RNA polymerase was preincubated with the DNA in the reaction mix at 37°C for ten minutes (in kinetic experiments using lac repressor, a ten minute incubation with the repressor was performed before the ten minute incubation with polymerase). At zero time an aliquot was removed, and transcription initiated by the simultaneous addition of MgAc₂ (to 4 mM) and rifampicin (to 10 μ g/ml). 10 μ l aliquots were removed at the indicated times and placed directly on dry ice to prevent further elongation. 100 μ ls of 0.3 M NaAc, 1 mM EDTA, 0.5 mg/ml tRNA were then added to each sample and the mixture was immediately phenol-extracted, ethanol precipitated, and analyzed as described above.

Fingerprint Analysis. RNA obtained from transcriptions using radioactive GTP was eluted from gels by incubation of gel slices at 37°C in 0.33 M KCl, 10 mM Tris-HCl (pH 7.6), 1 mM EDTA. Eluted RNA samples were subjected to digestion with T1 RNase (10 units) for 30 minutes at 37°C, and then to two-dimensional fingerprint analysis as previously described (8). Individual spots were eluted from the PEI plates, secondary digests were performed with pancreatic RNase, and samples were run on DEAE paper electrophoresis in pyridine-acetate (pH 3.5).

RESULTS

I. Transcription reactions performed in vitro. We isolated a 675 base-pair Sau3A 1 fragment from pTL12 and a 545 base-pair Pvu II fragment from pTL13 for use as templates in transcription experiments (See Fig. 1). The transcription pattern obtained from both these templates is shown in Fig. 2. Lanes 1-3 show the pattern obtained upon transcription of TL12. The three transcripts produced, RNA-1, RNA-2, and RNA-3, were subjected to digestion with T1 RNAase followed by two-dimensional fingerprint analysis (Fig 3). The patterns produced revealed that RNA-1 is the trp-promoted runoff product, RNA-3 is the lac-promoted runoff product, and RNA-2 is a trp-promoted transcript which does not proceed to the end of the template. We have previously reported that

Fig. 1. Templates utilized in transcription experiments. a. TL12 *Sau3A* I template. Top line: *trp* and *lac* promoters, CAP and *lac* repressor binding sites, and the end of the *lacI* gene are indicated. ♦, fusion of the *trp* leader to *trpC* sequence created by the *trp*ΔLD102 deletion. §, fusion points of the *lac* to *trpC* sequence created in the cloning. The source of each RNA species is shown. Bottom: nucleotide sequence of the region. Boxed-in regions indicate dyad symmetry of CAP and *lac* operator sites. DNA protected from DNase digestion by *lac* repressor (5) and RNA polymerase (27) are shown. (The polymerase-protected region shown is the longest contiguous stretch of DNA protected in DNase I experiments. However, footprinting experiments performed by Schmitz & Galas [5] showed that protection extends back as far as the right hand edge of the CAP site. Thus, the RNA-2 endpoint actually extends beyond the most proximal point of polymerase protection [see text]). Numbered brackets above sequence indicate the source of the corresponding T1 oligonucleotide spots in fingerprints shown in Fig. 3. b. TL13 *Pvu* II template. Symbols as in *a*.

Fig. 2. Transcription experiments performed *in vitro*. Lanes 1-5, TL12 template. Lanes 6-10, TL13 template. Lanes 4, 5, 9, and 10 were performed after pre-binding lac repressor (see Methods). All reactions contain 10 μ M [α - 32 P]UTP, 200 μ M CTP and the following purine triphosphate concentrations: Lanes 1, 5, 6, and 10, 200 μ M ATP, 20 μ M GTP; lanes 2 and 7, 20 μ M ATP, 200 μ M GTP; lanes 3, 4, 8, and 9, 200 μ M ATP, 200 μ M GTP. Identities of RNA transcripts are indicated.

RNA-2 may be the product of a termination event beyond the lacI gene or may be due to a collision between the trp-promoted polymerase molecule and a polymerase molecule bound at the lac promoter that has not initiated transcription (9). The 3' end of RNA-2 as determined from fingerprint analysis lies within the T1 oligonucleotide CUUUACACUUUAUG (see Fig. 1a). S1-mapping studies indicated two 3' ends which are indicated in Fig. 1a by arrows (9). Lanes 6-8 show the results of transcription using the TL13 template. RNA-6 and RNA-7 were fingerprinted (Fig. 3) and shown to be lac- and trp-promoted runoff transcripts, respectively.

II. Repressor Effects. Transcription reactions were performed on the TL12 and TL13 templates after preincubating with a saturating amount of lac repressor (see Methods). Lanes 4 and 5 of Fig. 2 shows that in the presence of repressor, transcription off the TL12 template no longer produces the lac transcript, RNA-3. However, a new transcript, RNA-4 (which is slightly longer than RNA-2), is produced. Similarly, transcription off the TL13 template in the presence of lac repressor no longer produces the lac runoff product, RNA-6, but produces a new transcript, RNA-8 (Fig. 2, lanes 9 and 10). RNA-4

Fig. 3. T1 fingerprint analysis of RNA transcripts. Transcripts were isolated from transcriptions utilizing radioactive GTP. a. RNA-1, b. RNA-3, c. RNA-2, d. RNA-4, e. RNA-6, f. RNA-7, g. RNA-8. Spot numbers correspond to T1 oligonucleotides shown in Fig. 1. T1 oligonucleotides absent from fingerprints are indicated by dotted arrows, with the number of the oligonucleotide in brackets.

and RNA-8 were subjected to T1 fingerprint analysis which showed that both transcripts are trp-promoted (Fig 3). The 3' end of each transcript was determined as follows: RNA-4 contains the T1 oligonucleotide, CUCG (spot 32 in Fig 3d), but lacks the adjacent oligonucleotide UAUA AUG (spot 41 in the fingerprint of RNA-1, Fig. 3a). Thus the 3' end of RNA-4 must reside within the oligo UAUA AUG (see Fig 1a).

A T1 fingerprint of RNA-8 does not contain the oligonucleotide UUUCUG, the position of which is indicated as spot 29 in the fingerprint of the run-off transcript, RNA-7 (Fig. 3f). However, RNA-8 does contain the oligonucleotides, AAUCCG (spot 23) and UAAUCAUG (spot 33). The fingerprint of RNA-8 also contains spot 26. Spot 26 in the T1 fingerprint of RNA-7 contains two oligonucleotides: UUCACG (derived from the trp leader region) and UCAUAG (located within the lac insert, adjacent to the T1 oligonucleotide, UAAUCAUG [spot 33]). To determine if spot 26 in the fingerprint of RNA-8 contains one or both of these oligonucleotides, we eluted this spot from both fingerprints and subjected them to secondary analysis by digestion with pancreatic ribonuclease. The spot UUCACG will yield ApCp* while the spot UCAUAG will yield Ap*Gp (asterisks indicate the radioactive phosphate). The secondary digests were electrophoresed on DEAE paper at pH 3.5 and then visualized by autoradiography. RNA-8 spot 26 yielded both ApCp* and Ap*Gp when digested with pancreatic RNase. Thus, it contains both the oligonucleotide UUCACG and UCAUAG. Therefore, the 3' end of RNA-8 must be distal to the oligo UCAUAG, but no further than the G of the oligo UUUCUG (See Fig. 1b).

The extent of repressor blockage of elongation was determined by calculating the per cent of trp-promoted transcripts that are found as RNA-4 or RNA-8 in a transcription assay performed on the the TL12 or TL13 templates, respectively. It was found that under conditions in which the GTP concentration is 200 μ M, repressor blockage was about 25% for both templates. However, under conditions where the GTP concentration is decreased to 15 μ M repressor blockage is increased to about 50% for each template.

III. Kinetics of Lac Repressor and RNA Polymerase Blockage. We wished to examine the process of repressor blockage in kinetic experiments in which only one round of transcription occurs. After lac repressor is pre-bound to the DNA template, RNA polymerase is introduced in the absence of magnesium so that binding occurs but initiation is blocked. Transcription is initiated by the addition of magnesium immediately followed by the addition of the drug rifampicin (which prevents subsequent initiation). Time points are taken, and the time course of the reaction is monitored by gel electrophoresis followed

by autoradiography. Figure 4a shows the results of a kinetic experiment performed on the TL12 template in the presence of lac repressor. The repressor-induced transcript, RNA-4, first appears at about thirty seconds after the onset of transcription, peaks in intensity at around 1.5 minutes, and then decreases again, commensurate with the appearance of the fully elongated trp transcript, RNA-1. (It can be seen that RNA-2 is present as a low-level and long-lived pause band [discussed below]).

Figure 4c depicts a kinetic experiment performed on the TL13 template in the presence of lac repressor. The RNA-8 transcript appears at around thirty seconds after the onset of transcription, peaks around 1.5 minutes, and then decreases again, as the full length trp transcript, RNA-7 is produced.

We also performed kinetic experiments on templates in the absence of lac

Fig. 4. Kinetic experiments (see Methods). a. and b. TL12 template; c. and d. TL13 template. a. and c. were performed after preincubating with lac repressor. Time points: Lane 1. zero time, 2. 15 sec, 3. 30 sec, 4. 45 sec, 5. 1 min, 6. 1.5 min, 7. 2 min, 8. 3 min, 9. 5 min, 10. 10 min. The identity of each species is indicated.

repressor. Fig. 4b shows the appearance of RNA-2 peaking at about 1.5 minutes. Again, the production of this species may be due to a block in elongation of the trp-promoted polymerase molecule by a polymerase molecule bound at the lac promoter, although there is also evidence in support of a DNA-mediated pause (9).

The kinetic experiment performed on the TL13 template is shown in Fig. 4d. It is apparent that there is a prominent pause band, RNA-8, that is barely detectable in the standard twenty minute reaction (Fig. 2, lanes 6-8). The species first appears at approximately thirty seconds after the onset of transcription, peaks at about 45 seconds, and has disappeared by the 5 minute time point. The fingerprint of this species is indistinguishable from that of the repressor-induced transcript, RNA-8 (data not shown), and is caused by blockage of a trp-promoted polymerase molecule by a polymerase molecule bound at the lac promoter. As is shown in Fig. 1b, the edge of lac repressor- and polymerase-protected DNA observed in DNase protection studies is the same. Our results indicate that the elongating polymerase molecule is blocked at the same position on the DNA (in the TL13 template) by bound lac repressor or RNA polymerase. However, because the pause induced by lac repressor is longer-lived than that caused by polymerase blockage, RNA-8 is more prominent in the standard transcription reaction when lac repressor is present.

IV. Relative Strengths of Trp and Lac UV5 Promoters : Effects of Purine Concentration and Convergent Transcription. We utilized the advantage of having both the lac and trp promoters on the same template in order to test the relative strengths of each under a variety of conditions. We performed the standard transcription reaction on the TL12 and TL13 templates, excised the trp- and lac-promoted bands from a gel, and determined the Cerenkov counts per minute per radioactively labeled nucleoside in each transcript. The ratio of trp- to lac-promoted transcripts was calculated as the ratio of the sum of the cpm/labeled residue for each trp transcript to that of the lac transcript. It was found that in the standard reaction (using radioactive UTP, the UTP concentration is about 10 μ M, that of the remaining triphosphates is 200 μ M), the trp: lac ratio for both templates is about one to one (Fig. 2, lanes 3 and 8). However, we found that when we decrease the concentration of GTP or ATP in the transcription reactions on both templates, the intensity of the lac transcript is greatly decreased (Fig. 2, lanes 1, 2, 6, and 7). Figures 5a and b show the effect of increasing the GTP or ATP concentrations (while maintaining the concentration of the other purine triphosphate at 200 μ M) in transcriptions done on the TL13 template. It is clear that production of the

Fig. 5. Effect of purine nucleoside triphosphate concentrations on transcription of TL13. All reactions contain 10 μ M [γ - 32 P]UTP and 200 μ M CTP. a. 200 μ M ATP, varying GTP concentration. b. 200 μ M GTP, varying ATP concentration. Concentration of varying triphosphate in a. and b.: Lane 1, 20 μ M, 2. 40 μ M, 3. 60 μ M, 4. 80 μ M, 5. 100 μ M, 6. 200 μ M (not shown in b.).

lac-promoted transcript is much more sensitive to the concentration of purine triphosphate than is that of the trp-promoted transcript. In transcriptions performed with low GTP concentrations (10-15 μ M), the trp to lac ratio is about four to one for the TL12 template and six to one for the TL13 template. Thus, under these conditions, transcription from the lac promoter is slightly more impaired in the convergent than the tandem promoter template.

DISCUSSION

We have performed a series of experiments to examine the process of transcription as it occurs in complex regulatory regions of the DNA. These studies have provided insight into the subtle control mechanisms engineered into the *E. coli* chromosome which allow for a spectrum of responses to be made under different conditions. Specifically, we have found that several aspects of promoter function are quite different in the trp and UV5 promoters. Both the slow half-time for initiation and the strong dependence on initiating triphosphate concentration of the UV5 promoter-RNA polymerase complex result in a long-lived complex which can block the progress of another polymerase molecule approaching from either direction. We have also seen that a bound repressor molecule can have a similar effect. Our studies show that the apparent strength of a promoter may have important implications in the outcome of transcription from convergent promoters.

I. Promoter Function

Our studies with the TL12 and TL13 templates have enabled us to compare the strengths of the trp and lac promoters under conditions where we know that they are absolutely equimolar. One of the ways in which the two promoters differ is with regard to the lag time between polymerase-promoter open complex formation and the productive initiation of transcription. This difference is most clearly demonstrated in the kinetic experiment shown in Fig. 4d. Open complexes are formed at the two convergent promoters on the TL13 template and transcription is initiated by the addition of magnesium. If the productive initiation rate (rate of formation of full-length transcript) were the same for the two promoters, we would expect to find a pause transcript resulting from collision of the lac-promoted polymerase with a polymerase molecule bound at the trp promoter as well as a pause transcript arising from the reverse collision. However, the only pause band observed is the RNA-8 species, which fingerprint analysis reveals to be a trp-promoted transcript. The absence of a lac-promoted pause transcript indicates that the majority of polymerase molecules initially bound at the trp promoter had proceeded down the template before a significant number of lac-promoted molecules could initiate and traverse this same distance. By the time most of the lac-promoted polymerase molecules reached the trp promoter, there was no longer a polymerase molecule bound there. Since it is improbable that a difference in the elongation rate is responsible for this disparity, the most likely explanation is that the rate of productive initiation from the trp promoter is greater than that from lac UV5.

The slow start-time of the lac UV5 promoter has been observed before in a different system. Stefano & Gralla (10) found that although the formation of the stable RNA polymerase-UV5 complex is very rapid and occurs with a half-time of 0.2 minutes, the complex forms RNA slowly. Their measurements of the initiation rate (determined as the rate of incorporation of radionucleoside triphosphates into acid-precipitable material when added to preformed complexes) indicated that initiation from the complex occurs with a half-time of about one minute. In contrast, the half-time for binding RNA polymerase by the trp promoter is about ten times greater than that observed for lac UV5 (about 2 minutes [11]), and our experiments suggest that the rate of productive initiation occurs more rapidly from open complexes at the trp promoter than from those at lac UV5.

We have also observed a striking difference in the sensitivity of the trp and lac UV5 promoters to the concentration of the initiating nucleoside

triphosphate. The trp promoter initiates transcription with the sequence AAGTT (8), while the lac UV5 promoter has been reported to initiate at two positions in transcriptions performed in vitro at 25°C. 15% of the time the startsite occurs at the sequence GAAUUG, while 85% of the time, it occurs at the adjacent position, AAUUG (12). Whereas a decrease in the concentration of GTP or ATP from 200 to 20 μ M drastically decreases the amount of transcription from the lac UV5 promoter in both the TL12 and TL13 templates, transcription from the trp promoter is barely affected. Gralla et al. performed experiments measuring the rate of abortive and productive initiation from the UV5 promoter and their results also showed a strong dependence on higher ATP concentrations for efficient initiation (13). In addition, the general finding that much higher concentrations of the initiating nucleotide are required for initiation (14, 15) suggests that the sensitivity of the UV5 promoter to purine nucleotide concentration may be a reflection of a particularly high requirement for the initiating triphosphate.

Gralla et al. (13) have suggested that the slow rate of dinucleotide formation observed in abortive initiation experiments performed on the UV5 promoter could arise from a disfavored binding (relatively high K_m) of the penultimate purine nucleotide. These authors predict that if this is true, then promoters possessing penultimate purine residues would be expected to be slow initiators. However, we find that the trp promoter (which contains the penultimate purine, adenosine) does not appear to be as slow in initiating transcription as is lac UV5. In addition, it is not severely inhibited by a decrease in the ATP concentration. We find that lowering the GTP concentration inhibits production of the lac transcript to the same extent as lowering the ATP concentration, even though neither UV5 start contains a G residue in the penultimate position. Thus, although our experiments cannot be interpreted as a direct measurement of the rate of initiation from the two promoters, it is most likely that the inhibition of productive transcription from the lac UV5 promoter at low purine triphosphate concentrations is due mainly to a requirement for a higher concentration of the initiating nucleoside triphosphate.

A high K_m for the first and/or second nucleoside triphosphate could limit the rate of formation of the first phosphodiester bond. Indeed, it has been suggested that the slower rate of formation of the first bond may be one of the main reasons why the lac UV5 promoter exhibits a slow rate of productive initiation (13). Thus, the sensitivity of the UV5 promoter to purine triphosphate concentration may be related to the long lag time that is

observed between the formation of the open complex and the production of RNA.

Many aspects of promoter function ultimately determine the number of transcripts derived from a region of DNA. The rate at which the promoter region binds RNA polymerase and the rate of isomerization from the closed to open complex form will affect the rate of promoter utilization. In addition, the rate of formation of the first phosphodiester bond could be rate-limiting. However, the production of a full-length transcript is also highly dependent on the characteristic number of abortive initiations (premature terminations) which occur at a particular promoter prior to the escape of the RNA polymerase to produce the full-length transcript. This last feature has been suggested to be another important factor in the slow productive initiation rate of the UV5 promoter; a higher number of abortive initiations will result in a longer lag-time between open complex formation and the production of RNA.

Our experiments do not examine the features of initiation separately, but rather they examine the sum of their effects on the production of transcripts from each promoter under specific sets of conditions. In this way, we were able to obtain a rough estimate of the relative strengths of the lac UV5 and trp promoters as measured by the number of transcripts produced by each. We have found that at 200 μ M GTP and ATP, the ratio of trp to lac transcripts is about 1:1. Although it is possible that these ratios may differ at even higher concentrations of purine nucleoside triphosphate, the change would probably be minimal since we observe that the rate of change in the ratio of trp to lac message with increasing GTP concentration levels off by 200 μ M GTP (data not shown).

Clearly, the rate of production of full-length transcripts for each promoter is dependent on a number of potentially rate-limiting steps. Just as the rate-limiting step can be decided by using certain conditions in vitro (13), it is probable that varying conditions (for example, a change in the nucleotide pools) can have a similar effect in vivo. Thus, the "strengths" of the promoters are likely to be different depending on physiological conditions. Our experiments, however, show that under conditions we have found to be optimal for transcription from both promoters in vitro, the lac UV5 and trp promoters are of comparable strengths.

It should be noted, however, that a collision between a trp-promoted polymerase molecule and a polymerase molecule bound at the lac promoter may inactivate the molecule bound at the lac promoter (possibly by causing its release from the template by unwinding the DNA adjacent to the promoter). In this case, we would be underestimating the strength of the lac UV5 promoter.

Such a situation would have significant physiological implications since there are several examples of genes transcribed from tandem promoters. The ribosomal RNA operons that have been studied contain two promoters separated by about 100 base pairs (16, 17, 18). Although it is not known what role is played by each promoter in the regulation of the operon, there is evidence that the second promoter (P2) is characterized by a slow initiation rate and that it is sensitive to the concentration of CTP, the initiating nucleotide (18, 19). Thus, it is conceivable that a polymerase molecule bound at P2 under conditions unfavorable for initiation from that promoter (for example, low CTP levels) might be inactivated when the polymerase molecule initiated from the upstream promoter collides with it. The resulting change in the ratio of transcripts produced may be important in regulation of the operon's expression.

II. DNA-Bound Protein Effects

RNA Polymerase. Studies on the in vitro transcription of ϕ X174 DNA (20) and T7 DNA (21) indicated that in transcription reactions performed with excess polymerase and rifampicin, termination events occur at promoter sites, and are attributed to blockage of elongation by downstream RNA polymerase-rifampicin complexes incapable of initiating transcription. Our experiments show that the slow start-time for initiation from the lac UV5-polymerase complex may also result in the blockage of elongation of another polymerase molecule.

We have previously reported that the production of RNA-2 from the TL12 template may be due to either a true termination event or to polymerase blockage of elongation (9). We identified a region of DNA homologous to two atypical terminators, rrnBt_L and trp t', and found that when the TL12 sequence proximal to the Hpa 2 site (see Fig. 1a) is cloned and a new template isolated which lacks the Pribnow Box, RNA-2 is still produced (9). It seems unlikely that the sequence could bind a polymerase molecule, but this possibility has not been rigorously ruled out. In addition, the observation that the 3' end of RNA-2 is located near the edge of RNA polymerase-protected DNA (as found in DNase I protection studies, see Fig. 1a) is suggestive of a collision event. Whether the mechanism resulting in RNA-2 production is due to polymerase blockage or termination, it may have an important role in preventing readthrough transcription from lacI into the lac operon.

We have also found that a polymerase molecule bound at the lac promoter can block transcription from the other direction (as seen by the production of RNA-8 from the TL13 template in rifampicin pause experiments). Although the nontemplate strand of the lacZ gene would not normally be transcribed (as it

is in the TL13 template), it is conceivable that protein molecules bound at the regulatory regions dispersed throughout the *E. coli* chromosome may function to block readthrough transcription from one operon to another and may decrease undesirable transcription produced by random, spurious initiations.

Additional regulatory functions of polymerase blockage have been suggested in other systems. Kassavetis *et al.* (22) noted that since bacteriophage T7 induces inhibitors that bind to and inactivate the bacterial RNA polymerase, it is possible that binding of the inactive polymerase at T7 minor promoters may play a regulatory role in turning off class I and II T7 RNA synthesis *in vivo*. Another example of polymerase blockage is found in the *rrnB* operon of *E. coli* which contains two promoters separated by about 120 base pairs. Kingston & Chamberlin (23) found that a very strong pause site was present in the leader region of this operon and was due to the blockage of transcription from the upstream promoter by a polymerase molecule bound at the downstream promoter. This second promoter, like the UV5 promoter, is characterized by a slow start-time for initiation (18, 19). In addition, the *rrnB* pause site is enhanced by ppGpp. These authors suggest that the pause may function as a potential regulatory site that would be highly responsive to both RNA polymerase concentration and the rate of RNA chain initiation.

Lac Repressor Effects. By comparing the position of the 3' ends of RNA-4 and RNA-8, we can determine the region of DNA that is "protected" by a bound *lac* repressor molecule. As is illustrated in Figure 1, the positions at which polymerase elongation is blocked are very similar to the edges of protection from DNase cleavage that have been observed (5). Repressor blockage in transcription reactions containing 200 μ M ATP and GTP is about 25% for both the TL12 and TL13 templates. Under conditions of low GTP concentration, transcription is somewhat impaired, and repressor blockage is increased to around 50%. Thus, it is conceivable that fluctuations in the cellular levels of nucleosides and/or other nutrients may result in a modulation of blockage, and, consequently transcription of certain regions of the DNA. Clearly, repressor blockage could have similar functions to those described above for polymerase blockage: preventing readthrough transcription from one operon to the next, and reducing random transcription. In the particular example of the TL12 template, repressor blockage may serve to curtail readthrough transcription from the *lacI* gene into the *lac* operon. Those transcripts that extend beyond the terminator or polymerase blockage site that produces RNA-2 may be terminated approximately 20 bases downstream, as RNA-4.

Reznikoff *et al.* (4) detected a repressor blockage of distal gene

expression in vivo in deletion mutants that fuse the lac operator and lacZ, Y, and A genes to the trp operon. These authors found that transcription of lac (which was presumed to have initiated at the trp promoter upstream) could be partially blocked in the presence of lac repressor. They suggested that one possible explanation for incomplete blockage (which we also find in vitro) is that the separating of the two DNA strands that occurs during transcription might destroy repressor binding to the operator. In addition, they suggested that low-level readthrough from the lacI gene into the lac operon during repressed conditions might account for the basal level of expression of the lac operon observed under these conditions.

Another example of repressor blockage is observed in the regulatory region of the uvrB gene (24). The region preceding the gene contains three promoters arranged in tandem, but only the last two promoters direct transcription into the uvrB gene. Transcription from the first promoter is terminated within a region close to the middle promoter. The lexA repressor binds to the middle promoter, preventing transcription from it and obscuring the normal termination site for transcription from the first promoter. However, transcription from the first promoter still terminates (although at a slightly different position), presumably due to blockage by the bound repressor molecule. This particular example suggests that protein blockage of transcription may play a significant role in regulation at complex sites in the DNA.

III. Convergent Transcription

There are few regions of DNA in which it is known that both strands of the DNA are transcribed. The b2 region and the cro gene of λ are two sites, however, where convergent transcription is possible since they contain promoters in opposing orientations which direct transcription of both strands of a defined stretch of DNA (25, 26). However, it is not known if transcription from the opposing promoters occurs simultaneously on one piece of DNA. The process of convergent transcription has been examined indirectly in studies which monitor the expression of genes in opposing operons which are joined by deletion. As noted in the Introduction, the general finding is impairment of expression from each promoter though some promoters seem to fare better than others. One case, however, that of opposing trp and lac promoters, failed to show inhibition of expression from either promoter (3).

We compared the ratio of trp to lac transcripts produced from the TL13 template with that from TL12 in order to detect any effects attributable to convergent transcription between the two promoters. Our studies indicate that

at high purine triphosphate concentrations, the trp to lac ratio is about one to one for both the tandem and convergent promoter templates. Although equal impairment of transcription from both promoters would result in a one to one ratio, it is unlikely that there is a significant decrease in transcription in the convergent template since: 1) incorporation of radioactive triphosphates per μg of template added is comparable to that seen in the TL12 template, and 2) addition of lac repressor (which prevents opposing transcription from the lac promoter) does not result in increased incorporation in the trp transcripts.

However, we do find that when initiation from the lac promoter is hindered by a decrease in the GTP or ATP concentrations, the trp to lac ratio is greater for the TL13 than the TL12 template. This suggests that under conditions where one promoter is "weaker" than the other, it may suffer greater inhibition in the convergent transcription situation. Our findings are similar to those of Ward & Murray (2) who found that when the very strong λ P_L promoter opposes the trp promoter, transcription from trp p is totally blocked, while expression from λP_L is only partially inhibited. Such a mechanism could provide an important means of switching on and off the transcription of certain regions of DNA and may have important implications in developmental control. Indeed, it has been suggested (2) that the delicate balance of transcription from the opposing P_{RE} and P_R promoters may determine whether λ enters the lysogenic or lytic pathway.

We have shown that selective inhibition of transcription can be achieved by the processes of repressor blockage, polymerase blockage and convergent transcription. Moreover, the efficiency with which transcription inhibition occurs can be modulated by small variations in reaction conditions so that a gradient of responses may be achieved. Our studies indicate that many factors are influential in determining the amount of transcription derived from a region of DNA. Clearly, the situation in vivo is infinitely more complex, being affected by many more regulatory proteins and factors as well as varying triphosphate and salt conditions, to name but a few. Undoubtedly, as we learn more about the process of transcription we will find that it is the complex nature of the interactions involved that dictates the ultimate response elicited, and therefore allows for a precise control of gene expression in the prokaryotic cell.

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