

## Gene Q antiterminator proteins of *Escherichia coli* phages 82 and $\lambda$ suppress pausing by RNA polymerase at a $\rho$ -dependent terminator and at other sites

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Communicated by Michael J. Chamberlin, April 20, 1989 (received for review January 31, 1989)

**ABSTRACT** The Q genes of phages  $\lambda$  and 82 encode transcription antiterminators that are active *in vitro* in a purified transcription system. Transcription termination is thought to involve two distinct steps: pausing of the transcription complex at the terminator and release of enzyme and RNA; either or both steps might be inhibited by Q protein. We show that Q-modified RNA polymerase pauses much less efficiently than does unmodified enzyme at the natural pause sites of a  $\rho$ -dependent terminator as well as at other pause sites. This changed behavior can account for the termination properties of Q-modified RNA polymerase and reflects a fundamental alteration of the elongation properties of the enzyme.

Transcription antiterminators are regulatory proteins that allow RNA polymerase to transcribe genes otherwise blocked by termination sequences. Only two antiterminators, the products of *Escherichia coli* bacteriophage  $\lambda$  genes Q and N, have been purified and studied, but it is likely that antitermination or related mechanisms exist in various organisms (for review, see refs. 1-3). The  $\lambda$  Q and N proteins regulate phage late and early gene expression, respectively. Each protein modifies RNA polymerase during transcription at a site that is specific to the antiterminator; the modified enzyme then can elongate RNA chains through the downstream terminators that are natural barriers to gene expression or through different and unrelated terminators that are put downstream artificially. N protein is thought to enter a complex with RNA polymerase at the N protein engagement site (4, 5), and it is likely that Q protein does the same, although this has not been shown. Purified  $\lambda$  Q protein efficiently modifies RNA polymerase into an antiterminating form *in vitro*, with the required addition of only one other protein, the transcription factor NusA (6). Q protein of the related lambdoid phage 82 does not even require NusA for activity *in vitro*, although NusA is stimulatory (ref. 7; J. Goliger, X. Y. and J.W.R., unpublished data). Thus, Q function can be analyzed readily *in vitro*.

What property of the Q-modified RNA polymerase lets it go through termination sites? It is believed that termination by *E. coli* RNA polymerase occurs in two distinguishable steps: pausing of the elongation complex and then release of enzyme and RNA from the template at the pause site (reviewed in refs. 3 and 8). Pausing often is induced by hairpins that form in the transcript, centered  $\approx 20$  bases before the pause site. Release at the pause site then occurs in two ways. First, if there exists a run of uridines or a similar sequence at the end of the transcribed RNA, RNA polymerase and RNA dissociate spontaneously from the template. One theory suggests that weak base-pairing between the uridines and the template deoxyadenosine stretch allows the transcript to dissociate (9), although this simple view is challenged by

experiments that imply a more direct effect of such sequences on the enzyme rather than the transcript (10). Second, if the emergent RNA contains appropriate sequences, perhaps consisting of some number of cytidines in an available stretch of unstructured RNA (11, 12), the termination factor  $\rho$  can interact with both enzyme and RNA to cause their release. Considering how terminators work, one can imagine two different, although not necessarily exclusive, ways that Q might act: it might inhibit pausing at terminators, or it might prevent release of the paused enzyme.

An immediate effect of Q on RNA polymerase at the Q engagement site (named *qut*) suggests that it does affect pausing. The *qut* sites of both phage  $\lambda$  and the related lambdoid phage 82 overlap the late gene promoters and include  $\approx 20$  nucleotides of the transcribed regions (see Fig. 1). Point mutations in the nontranscribed region of *qut* $\lambda$ , at -13 and -15, prevent Q function, suggesting recognition of DNA by Q in this segment (unpublished data). Sequences in the transcribed region are required for an early pause during which Q modifies RNA polymerase (X.Y., J. Goliger, and J.W.R., unpublished data). For  $\lambda$ , Q acts while RNA polymerase pauses at nucleotide 16 of the  $\lambda$  late gene transcript (13); in a similar way, Q of phage 82 acts during pauses at both nucleotide 15 and nucleotide 25 (unpublished data). Although the primary effect of Q is to allow RNA polymerase to read through downstream transcription terminators, it also drives the enzyme out of the early pauses as modification occurs; thus, Q changes the elongation behavior of RNA polymerase even at the *qut* site.

Here we ask if Q affects pausing at a terminator well downstream from the *qut* site. Since termination factor  $\rho$  is required for transcript release but not for pausing at  $\rho$ -dependent terminators (14), it is possible to isolate and observe the pausing step of termination at  $\rho$ -dependent terminators by omitting  $\rho$ . Thus, by using appropriate template constructions containing the Q site of action, we can ask if Q interferes with the ability of RNA polymerase to pause at a terminator. We first show that Q does act against a  $\rho$ -dependent terminator *in vitro*, in addition to the  $\rho$ -independent terminators we have used previously (6, 7, 13, 15); this result is expected from the fact that Q suppresses polarity (16), an affect of  $\rho$  function *in vivo* (17). We then show that Q protein strongly reduces pausing at  $\rho$ -dependent terminators in the absence of  $\rho$ , and, in fact, reduces pausing at many, if not all, sites it encounters during transcription. Thus, the antiterminator fundamentally alters the elongation behavior of RNA polymerase throughout a long transcription unit.

### MATERIALS AND METHODS

**Proteins and DNA.** RNA polymerase was purified by the method of Burgess and Jendrisak (18) as modified by Lowe

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*et al.* (19). NusA protein was purified by the method of Schmidt and Chamberlin (20).  $\lambda$  Q protein was purified by a modification of the published procedure (ref. 21; J. Goliger and X.Y., unpublished data). 82 Q protein was purified as described (7).  $\rho$  protein was purified in this laboratory by L. F. Lau, as described by Finger and Richardson (22).

Plasmids used to make DNA fragments illustrated in Fig. 1 were derivatives of pXY303, pXY306, and pXY312 (15); pXY311 is identical to pXY312 except that the DNA segment encoding nucleotides +6 to +51 of the 82 late mRNA was removed by exonuclease III digestion (J. Goliger and J.W.R., unpublished data). Plasmids pXY303-2, pXY306-2, pXY311-2, and pXY312-2 were constructed from pXY303, pXY306, pXY311, and pXY312 by replacing their *EcoRI/Sma I* fragments, which contain the  $\lambda$   $t_0$  terminator, with the 950-base-pair *EcoRI/Sma I* fragment of pLS1 (23), which contains  $\lambda$   $t_{RI}$ .

DNA fragments were isolated by precipitation of larger fragments with polyethylene glycol (24).

**In Vitro Transcription.** Purified DNA fragment (0.5–1.5 nM) and proteins (20 nM RNA polymerase, 150 nM NusA, 200 nM Q $\lambda$  or Q82 where present, and 54 nM  $\rho$  protein hexamer where present) were preincubated 10 min at 37°C in 20 mM Tris-HCl, pH 7.9/120 mM KCl/0.1 mM EDTA/1.0 mM dithiothreitol/10% (vol/vol) glycerol/200  $\mu$ M ATP, CTP, and GTP/25 or 50  $\mu$ M UTP containing 2.5–5  $\mu$ Ci of [ $\alpha$ -<sup>32</sup>P]UTP (1 Ci = 37 GBq) in a vol of 25  $\mu$ l. RNA synthesis was begun by simultaneous addition of 5 mM MgCl<sub>2</sub> and rifampicin at 10  $\mu$ g/ml. RNA was processed and analyzed by electrophoresis and autoradiography as described (13). RNAs were measured by scanning films with a Bio-Rad model 620 video densitometer or by cutting out RNA bands and counting in a scintillation counter.

## RESULTS

**Q Antagonizes  $\rho$ -Dependent Termination.** To analyze the effect of Q on elongation of RNA chains, we transcribed the DNAs drawn in Fig. 1. Each contains the  $\rho$ -dependent terminator  $t_{RI}$  taken from the  $\lambda$  early rightward operon, connected to a DNA segment containing the  $\lambda$  or 82 late gene promoter ( $p_{R'}$  or  $p_{82}$ ) with or without an active *qut* site. The natural terminator against which the antitermination activity of each Q protein acts ( $t_{R'}$  or  $t_{82}$ ) has been removed. The  $\lambda$  *qut* site includes DNA on either side of the RNA initiation site,

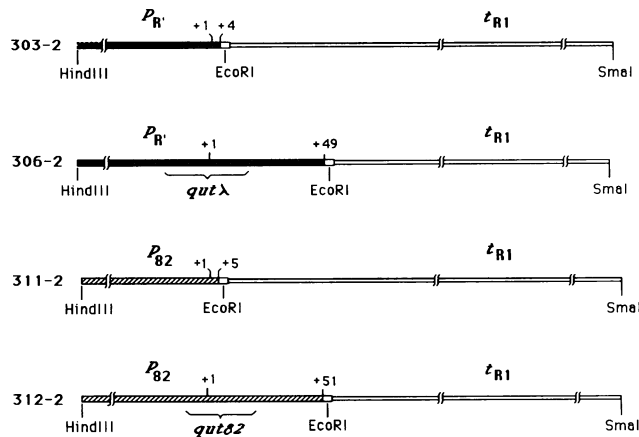


FIG. 1. Maps of DNA templates containing the  $\rho$ -dependent terminator  $t_{RI}$ . DNA fragments are named after plasmids from which they were derived. The *EcoRI/Sma I* segment is the same in all DNAs. The *HindIII/EcoRI* segments of pXY306-2 and pXY312-2 contain *qut $\lambda$*  and *qut $_{82}$* , respectively, along with the corresponding phage late gene promoters. The DNA fragments 303-2 and 311-2, which contain inactive or only slightly active *qut* sites, are described in the text.

and, in particular,  $\approx 20$  nucleotides of the transcribed segment where the pause at +16 occurs (13, 15, 25); the 82 *qut* site similarly extends into the transcribed region (J. Goliger and J.W.R., unpublished data). The shorter promoter segments in pXY303-2 and pXY311-2 are active promoters but are defective in supporting Q function, whereas the longer segments in pXY306-2 and pXY312-2 have intact *qut* sites.

We consider first the effect of Q on  $\rho$ -dependent termination at  $t_{RI}$ . In all experiments, RNA synthesis was synchronized by preincubating proteins with the template in the absence of magnesium and was restricted to one round by adding rifampicin with the magnesium. For the experiment of Fig. 2, we used Q82 and template pXY312-2, which contains an intact *qut $_{82}$*  site; the activity of Q $\lambda$  on its proper template is described below. NusA protein was added, because it increases antitermination  $\approx 50\%$  at  $t_{82}$ , the natural barrier to late gene expression in phage 82 (ref. 8; X.Y., J. Goliger, and J.W.R., unpublished data).  $\rho$  induces termination at the subsites I, II, III, and IV of  $t_{RI}$  (26, 27): compare the 1st and 10th lanes, showing RNA made in 10 min with and without  $\rho$ . Site I is barely visible, whereas site II is prominent, as expected for synthesis in the presence of NusA protein (26). The second lane shows that Q inhibits termination at all subsites of  $t_{RI}$  except possibly site IV. However, only a

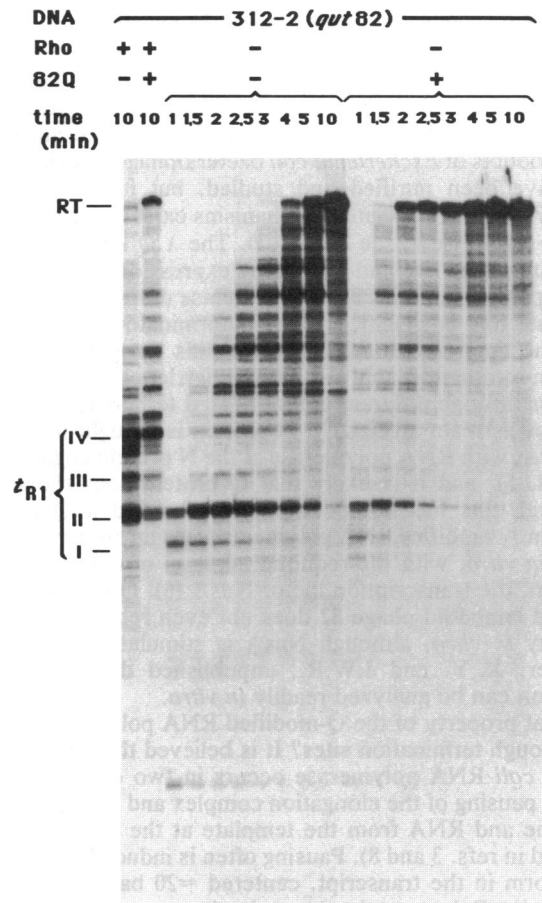


FIG. 2. Effects of Q82 on termination, pausing, and elongation by purified RNA polymerase. The 312-2 *HindIII/Sma I* template containing *qut $_{82}$*  was transcribed in the presence of 150 nM NusA protein, 54 nM  $\rho$  (where present), and 200 nM Q82 (where present). An autoradiogram of a gel analysis of transcription products is shown. Positions of read-through RNA (RT) and RNA terminated at the four subsites of  $\rho$ -dependent terminator  $t_{RI}$  are shown. Total RNA synthesis, measured as the final yield of RT in the absence of  $\rho$ , was 30% less in the presence than in the absence of Q82. The lengths of RNAs, in nucleotides, are as follows: RT, 1000; sites I–IV of  $t_{RI}$ , 272, 292, 325, and  $\approx 360$ .

fraction ( $\approx 10\%$ ) of the transcripts reach the end of the fragment, because many stop at other sites between site IV and the end. Most of these sites are  $\rho$ -dependent terminators (27) that appear only faintly in the absence of Q because most RNA stops at the  $t_{RI}$  cluster. Thus, Q antagonizes not only  $\rho$ -independent terminators, as shown previously (13, 15), but also  $\rho$ -dependent terminators. However, the antiterminating effect of Q is not uniform across this field of  $\rho$ -dependent terminators.

**Q Inhibits Pausing and Speeds Elongation.** To determine how Q affects pausing in the absence of termination, we left out  $\rho$  and followed the progression of RNA polymerase through pXY312-2 DNA during a single synchronized round of RNA synthesis. Lanes 3–10 and 11–18 of Fig. 2 show RNAs present at intervals during 10 min. In the absence of Q (lanes 3–8), RNA polymerase pauses at many sites including the subsites of  $t_{RI}$ , but particularly at subsite II. The  $\rho$ -dependent termination sites beyond  $t_{RI}$  that are more prominent in the presence of Q also appear as pause sites. Lanes 11–18 show the progression of RNA polymerase through this DNA in the presence of Q but still in the absence of  $\rho$ . Two effects of Q protein are clear: the occupancy of all pause sites is less, and RNA polymerase reaches the end of the DNA fragment more quickly.

We showed previously that Q discharges RNA polymerase from pause sites very close to the RNA start: at +16 of  $\lambda$  DNA (13) and at +15 and +25 of 82 DNA (not shown in the gel of Fig. 2; unpublished data). RNA polymerase thereby

escapes the promoter region faster in the presence of Q, accounting in part for its faster transit of the DNA fragment. However, if Q affected *only* these promoter-adjacent sites and not downstream sites, then its effect should be to synchronize the wave of RNA synthesis from the promoter region, allowing more RNA polymerase to accumulate somewhere downstream in the presence than in the absence of Q; but, in fact, the opposite is observed, the occupancy of all downstream sites being less with Q than without it. Thus, Q must shorten pausing at downstream sites as well as at the early pause sites near the promoter, consistent with the fact that antitermination occurs at far downstream terminators.

**The Effect of  $\lambda$  and 82 Q Proteins on Elongation Requires an Active *qut* site.** To show that Q affects elongation *in vitro* through its authentic engagement of Q at the *qut* site, rather than through some nonspecific effect on elongating RNA polymerase, we used deletion derivatives of each *qut* site that are inactive or much reduced in Q response but unaltered as promoters. Thus, the *qut* $\lambda^-$  plasmid pXY303-2 and the *qut*82 $^+$  plasmid pXY311-2 (Fig. 1) have lost DNA in the transcribed regions between +4 and +49 or +5 and +51 where the early pauses occur but retain all of the natural promoter sequences. We examined pausing from all four templates with or without the proper Q protein, and we show a densitometer trace of an autoradiogram of one time point in the elongation series (Fig. 3). It is clear that Q $\lambda$  speeds elongation on the *qut* $\lambda^+$  template (pXY306-2) and not on the *qut* $\lambda^-$  template (pXY303-2), and that Q82 speeds elongation

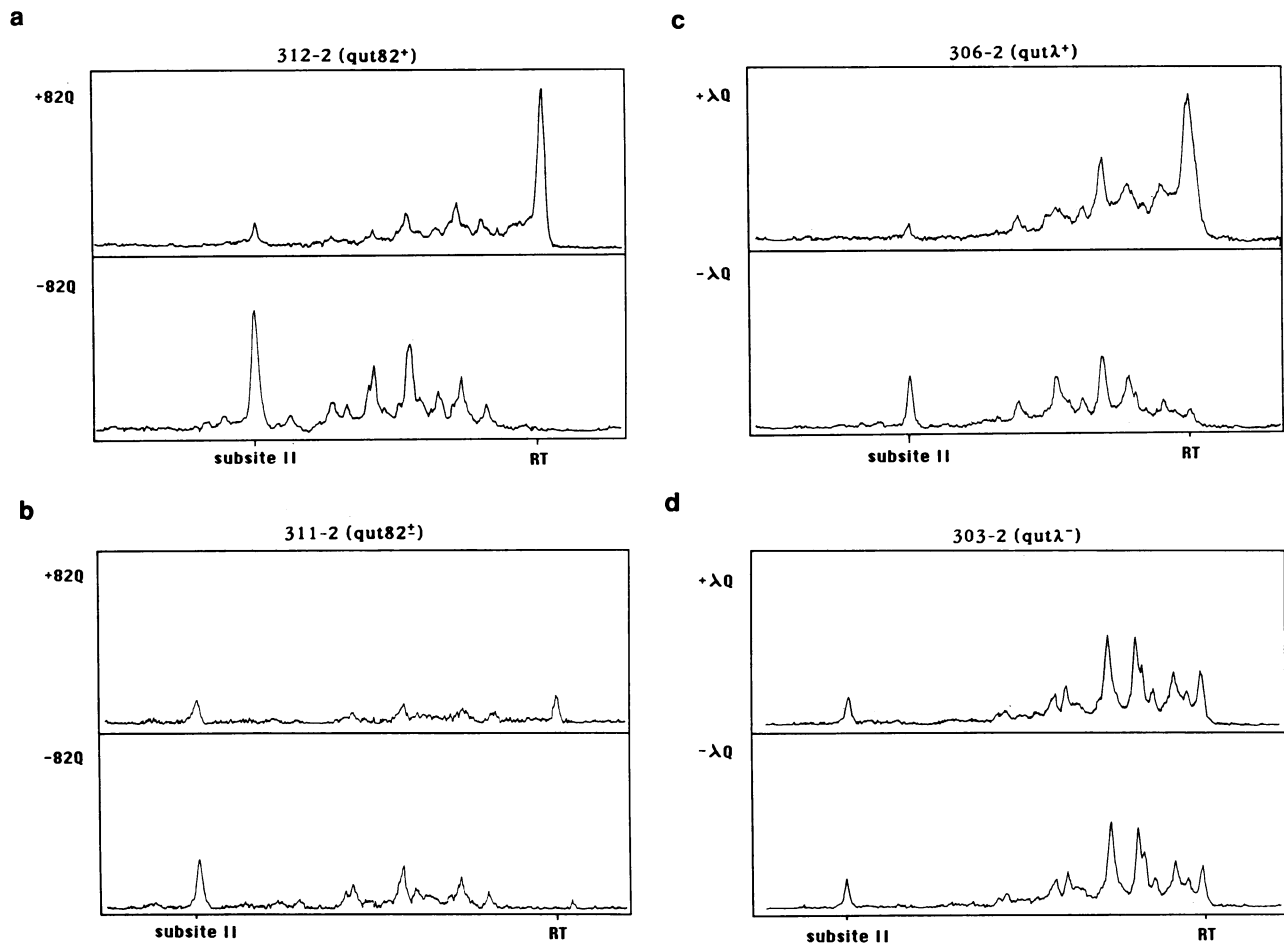


FIG. 3. A functional *qut* site is required for modification of RNA polymerase by Q protein. Densitometric traces of transcripts resolved by gel electrophoresis are shown. Transcripts of 312-2 (a) and 311-2 (b) are from a 2.5-min reaction; Q82 was present where indicated. Transcripts of 306-2 (c) and 303-2 (d) are from 4-min reactions; Q $\lambda$  was present where indicated. The positions of pause subsite II within  $t_{RI}$  and of read-through RNA (RT) are indicated. NusA protein was present in all reactions. These traces have not been normalized to total RNA synthesis, so that only relative peak heights within a trace are comparable.

much more on the *qut82*<sup>+</sup> (pXY312-2) than the *qut82*<sup>-</sup> (pXY311-2) template. The slight effect of Q82 on the latter DNA is consistent with the fact that pXY311-2 also supports ≈20% as much antitermination as the wild type (J. Goliger and J.W.R., unpublished data), whereas the *qutλ*<sup>-</sup> template pXY303-2 is completely inactive in antitermination (15). In similar experiments, we have shown that each Q affects elongation of RNA synthesis only from a template containing its own *qut* site (unpublished data).

**The Efficiency of RNA Polymerase Modification by Q.** To describe more quantitatively the effect of Q82 on pausing, we measured RNA at the *t*<sub>RI</sub> terminator subsite II pause at each time for the experiment of Fig. 2. Fig. 4 shows RNA at subsite II in arbitrary units, after normalization to total RNA synthesis at 10 min, when most RNA polymerase has reached the end of the DNA fragment. First, note that the shape of the curve, and in particular the time of maximum occupancy of the pause, are changed by Q82, implying that RNA polymerase molecules that still pause are not simply an unmodified fraction of the enzyme. Of those enzyme molecules in the pause between 3 and 5 min in the absence of Q82, 85–90% are missing if Q82 is present during transcription and therefore must have been modified. If we assume that all RNA polymerase molecules are equivalent, this result means that most molecules are modified by Q82; but there is still a substantial pause at subsite II, suggesting that modified enzyme pauses. A second possibility is that there is a subset of molecules that both is refractory to modification by Q82 and pauses differently from the rest, whereas Q-modified molecules do not pause detectably at all.

We offer three arguments that most RNA polymerase molecules are modified by Q82, yet do still pause, although for a shorter time than does unmodified enzyme. (i) Fig. 2 shows that Q82 allows considerable synthesis beyond site II at 1 min that is not present without Q82; enzyme transcribing this segment at 1 min must therefore be modified, yet it still exhibits the characteristic pattern of pausing. (ii) In Fig. 5 we plot the (relative) number of molecules that reach the end of the DNA fragment with or without Q82. The data for reactions with NusA protein are the same as shown in Fig. 2. The 4-min data show that >90% of RNA polymerase molecules have reached the end of the DNA in the presence of Q82 at a time when only 10% reach the end without Q82. Thus, at least 80% of all RNA polymerase must be modified, although this is an underestimate because any modified molecules that are still in transit are not counted. Yet, Fig. 4 shows that a large fraction of the enzyme is found at the subsite II pause at early times in the presence of Q82. (iii) The asynchrony of progression of modified enzyme molecules—the fact that

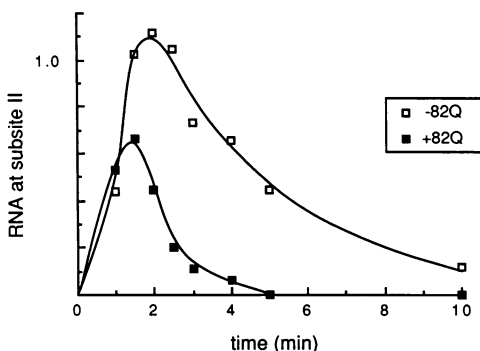


FIG. 4. Effect of Q82 on RNA polymerase pausing at subsite II of *t*<sub>RI</sub>. RNA present at subsite II of *t*<sub>RI</sub> during transcription of 312-2 (*qut82*) DNA is shown, in arbitrary molar units; the data are from the experiment of Fig. 2. Values were normalized to total RNA synthesis, measured as the amount of read-through RNA at 10 min. RNAs were measured by densitometry of the autoradiogram. All reaction mixtures contained NusA protein.

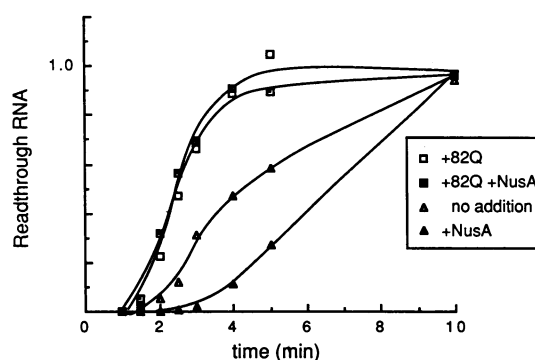


FIG. 5. Effect of Q82 on elongation of RNA synthesis. Accumulated read-through RNA during transcription of 312-2 (*qut82*) DNA is shown in arbitrary molar units. All values are normalized to total RNA synthesis, as described in the legend to Fig. 4. The times required for the accumulation of one-half the maximum amount of read-through RNA are as follows: 4.3 min for RNA polymerase alone; 6.8 min with NusA added; and 2.4 min with Q82 added, both in the presence and in the absence of NusA.

they arrive at the end over a period of 2–3 min, even though initiation should be very fast—implies that they still pause at many sites during synthesis.

We observe these same effects, but less clearly, for Q<sub>λ</sub>, which acts less efficiently *in vitro*; it is very likely that both regulators work the same way.

**Q Suppresses the Effect of NusA Protein on Pausing.** We have included the transcription protein NusA in the reactions described above. NusA is essential for substantial antitermination by Q<sub>λ</sub> but only modestly stimulates antitermination by Q82; this difference is detected at the engagement site, where NusA is required for Q<sub>λ</sub> to chase RNA polymerase from the early +16 pause of *qutλ* (13), but not for Q82 to chase RNA polymerase from the +25 pause of *qut82* (unpublished data). Besides its role at antiterminator engagement sites, however, NusA has distinct effects on termination and elongation of transcription in the absence of antiterminators. In particular, NusA increases pausing at certain pause sites on natural templates that have been tested, thus slowing the progress of RNA polymerase (20, 28–31). Fig. 5 shows this effect on DNA template pXY312-2, where, in the absence of Q82, NusA delays the first appearance of read-through RNA by ≈2 min. This delay is associated with an obvious enhancement of pausing at major pause sites in experiments like that of Fig. 2 (data not shown). Fig. 5 shows two further aspects of the relation of NusA, Q, and pausing. First, it is clear that Q82 speeds elongation even in the absence of NusA; thus, Q82 does not simply counteract the effect of NusA. This is consistent with the fact that Q82 is active in antitermination in the absence of NusA and with evidence that NusA acts in Q<sub>λ</sub>-dependent antitermination primarily at the engagement site. Second, and most important, NusA does not significantly slow RNA polymerase that is modified by Q82: there is no detectable delay in the appearance of read-through RNA caused by addition of NusA to reaction mixtures containing Q82. Thus, Q appears to neutralize the delaying effect of NusA on transcription elongation.

## DISCUSSION

Although termination is not well understood, it is clear that sequences that make RNA polymerase pause during transcription are essential components of natural terminators. A mutation that eliminates termination at site II of  $\rho$ -dependent terminator *t*<sub>RI</sub> also reduces pausing at site II (14) and completely eliminates the ability of NusA protein to enhance this pausing (30). At  $\rho$ -independent terminators, sequences encoding a hairpin that forms in the transcript just before the

release site are both necessary for termination and capable of inducing pausing by themselves (reviewed in ref. 8). For a pause site in the *E. coli trp* operon, it is particularly clear that the hairpin actually forms in the paused transcription complex, that the length of pausing is directly related to the stability of the hairpin (31), and that the pause exists both *in vitro* and *in vivo* (32). Finally, mutations in the RNA polymerase  $\beta$  gene that reduce pausing also reduce termination (33), suggesting strongly that the processes are related. It is not necessarily true that the kinetic aspect of pausing is important for termination: the structural change that induces pausing might also induce a termination-competent state whose effect is not strongly related to its duration. Furthermore, the duration of a pause—i.e., how much longer RNA polymerase actually stays at the pause than at the average nucleotide—may be considerably less *in vivo* than *in vitro* (28, 32). Whatever the kinetics may be *in vivo*, it seems clear that the signal that induces pausing *in vitro* is an essential part of termination and that its suppression by Q underlies anti-termination.

It appears that the different  $\rho$ -dependent termination sites within the DNA segment we used do not respond equally to Q; thus, site IV of  $t_{RI}$  remains prominent in the presence of Q, whereas termination at both sites II and III is efficiently suppressed. However, it is complicated to measure antitermination accurately at farther downstream sites, because Q activity at early sites allows more RNA polymerase to reach them. We see no obvious direct relation between the occupancy of a pause and either the efficiency of  $\rho$ -dependent termination at the site or the effect of Q on  $\rho$ -dependent termination at the site. This result suggests, as noted before, that termination efficiency is not related in a simple way to the duration of a pause.

Although at least 80% of the transcribing RNA polymerase molecules are modified by Q82 in these experiments, only  $\approx 10\%$  reach the end of the  $\rho$ -dependent termination segment. Thus, antitermination at the  $\rho$ -dependent terminators is not absolutely efficient *in vitro*. In apparent contrast, experiments in which two tandem  $\rho$ -independent terminators fused to *qut* $\lambda$  or *qut*82 were used and transcribed in the presence of the proper Q showed that most enzyme that passed the first terminator also passed the second, suggesting high efficiency of antitermination by modified enzyme (ref. 15; J. Goliger and J.W.R., unpublished data). It will require further work to determine whether Q acts differently at  $\rho$ -dependent and  $\rho$ -independent terminators, or if, instead, the difference reflects properties of the particular terminators tested and the sequence contexts in which they were placed.

It seems likely that the efficiency of Q-mediated antitermination at  $\rho$ -dependent terminators is higher in the cell than we detect *in vitro*. Our minimal purified system might lack some essential components. Several proteins besides NusA, such as NusB and NusE, are required for activity of the N protein antiterminator in most conditions (2), and the genetic analysis that originally identified these factors has not been applied to Q function. Possibly similar, or even the same, proteins are required for full natural activity of the Q proteins. Nonetheless, it is clear that Q alone displays the essential antitermination activity. It is noteworthy that the  $\lambda$  N protein is active at certain terminators *in vitro* with the required addition only of NusA protein (34).

Finally, we note that Q acts not only against pauses induced by RNA hairpin formation but also against those induced by DNA sequence in an unknown way, like the early pauses at the Q engagement site. This generality of the activity of Q is also implied by its ability to neutralize the effect of NusA. Schmidt and Chamberlin (20) showed that NusA acts not only to accentuate particular strong pauses, but also as a competitive inhibitor of the nucleoside triphos-

phate substrates in the concentration range we have used here (25–200  $\mu$ M). Q must counteract both these effects of NusA, since it appears to reverse completely the effect of NusA on the elongation rate; thus, it seems likely that Q changes the elongation proficiency of RNA polymerase in a fundamental way unrelated to how elongation is constrained. It may be possible to find some simple kinetic manifestation of the effect of Q, such as a more favorable  $K_s$  for interaction of nucleotide triphosphates with the elongating enzyme.

We thank Jeff Goliger for gifts of materials and for many discussions; John Lis, Jeff Goliger, Bill Yarnell, Craig Hart, and Hwai-Chen Guo for criticizing the manuscript; and Mike Chamberlin for helpful suggestions. We also thank Elizabeth Grayhack and Lester Lau, who started these experiments several years ago. This work was supported by Grant GM 21941 from the National Institutes of Health.

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