The NusA and NusG Proteins of *Escherichia coli* Increase the in Vitro Readthrough Frequency of a Transcriptional Attenuator Preceding the Gene for the β Subunit of RNA Polymerase*

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The genes for the β (rpoB) and β′ (rpoC) subunits of *Escherichia coli* RNA polymerase are the distal members of a complex transcriptional unit that contains four upstream ribosomal protein genes. The RNA polymerase subunit genes are transcribed at a lower frequency than the ribosomal protein genes as a result of termination at an attenuator preceding rpoB. A purified in vitro transcription system was developed using linear DNA templates that carry the attenuator. The ability of known termination and antitermination proteins to modulate termination at the attenuator was tested. Both NusA and NusG increase the frequency of transcriptional readthrough at the attenuator whereas NusB, S10, and Rho had no significant effect in this system.

The concentration of RNA polymerase is closely regulated in *Escherichia coli* (reviewed in 1–3). Since the α polypeptide is synthesized in molar excess (4–6) of that required to assemble core RNA polymerase (ββ′αα), it appears that the synthesis of β and β′ controls production of the core enzyme. Therefore we have been examining the expression of the rpoB and rpoC genes, which encode the β and β′ polypeptides, respectively, in an effort to understand how the cell controls the level of RNA polymerase. The genes for the β and β′ subunits are part of a complex gene cluster (rplKAJLrpoBC) that includes four ribosomal protein genes and two promoters of approximately equal strength (reviewed in 3, 7). The rplKp (P1) promoter maps upstream of rplJ and is responsible for the transcription of rplJrpoBC. In addition, a promoter upstream of rplK (rplKp or P2) is responsible not only for the transcription of the rplKA genes but also leads to transcription of rplJrpoBC because transcripts initiated at this promoter are not terminated before rplJ (8–10).

The rpoBC genes, however, are transcribed at about one-fifth the frequency of the upstream ribosomal protein genes (11, 12). This differential expression is the result of transcriptional termination in the 319-base pair (bp) rplL-rpoB intercistronic region (7, 13), presumably at the attenuator site (rpoBa) encompassing the sequence located 43–73 bp downstream of rplL (9, 14, 15). This site generates an RNA sequence capable of forming an 11-bp G-C-rich stem followed by a series of 5 uridine residues. Although this resembles the general structure of a simple or factor-independent terminator (16), the frequency of termination in the intercistronic region is only 70–80% during steady-state growth (7, 11, 13), and it has been reported that this level can be altered under certain conditions (17–19).

A series of ancillary factors has been identified in *E. coli* which are involved in termination and antitermination reactions in the host itself and also during λ infection. Rho was the first to be discovered (20) and is required for termination at a general class of rho-dependent terminators (16). The nusA, nusB, and nusE genes were initially identified as *E. coli* genes necessary for N-mediated antitermination of λ transcription (21), but at least nusA and nusB have subsequently been implicated in also controlling termination in the host (reviewed in 16, 22). The nusE gene has since been shown to be rpsJ, the structural gene for the S10 ribosomal protein (23). NusG was recently isolated as an *E. coli* protein that stabilizes the association of λ N protein with λ transcription complexes (55) and makes antitermination by N protein more processive in vitro. It is encoded by an *E. coli* gene, now called nusG (53), in which a mutation suppresses the effects of the nusA1 and nusE71 mutations on the growth of λ.

We attempted previously to ascertain whether some of these proteins played a role in modulating termination frequency in the rplL-rpoB intercistronic region by hybridizing in vivo labeled RNA from rho, nusA, nusB, and nusE mutants to rplL-rpoB-specific probes. Comparison of the ratio of rplJL to rpoB transcription in these mutants with that found in wild type suggested that normally Rho increases and NusA decreases termination in the intercistronic region (15). The nusB and nusE mutations did not significantly alter the transcript ratio, implying that they do not function in the transcription of this region. From these results we tentatively concluded that either rpoBa itself was responsive to Rho and NusA even though it had the structure of a simple terminator or there was an additional factor-dependent terminator(s) in the intercistronic region downstream of rpoBa.

The present study endeavors to distinguish between these models by using a purified in vitro transcription system to examine transcription of the rplL-rpoB intercistronic region. Using a variety of linear templates it was found that termination within this region occurs exclusively at rpoBa. The addition of purified Rho, NusA, NusB, S10, and the newly

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1. J. Greenblatt, R. Horwitz, S. McCracken, and J. Li, submitted for publication.
identified NusG (24) to this system demonstrated that both NusA and NusG increase readthrough at rpoBa, but the others have no significant effect.

**Experimental Procedures**

In Vitro Transcription—Standard single round transcription reactions (50 µl total volume) were carried out by first incubating 20 mM Tris acetate (pH 7.9), 100 mM KCl, 4 mM magnesium acetate, 0.1 mM EDTA, 0.1 mM dithiothreitol, 500 µM CTP, 2 µM ATP, 2 µM GTP, 0.1 pmol of DNA template, 0.5 pmol of RNA polymerase, and additional purified proteins as indicated at 37 °C for 5 min. This allows initiation and formation of a short oligonucleotide transcript at the rplP promoter. Further elongation was allowed by the simultaneous addition of CTP, GTP, and UTP to 200 µM final concentration; ATP to 30 µM; 5 Ci [α-32P]ATP; and rifampicin to 10 µg/ml. After 15 min at 37 °C the reaction was completed by the addition of 50 µl of stop solution (0.6 M sodium acetate, 20 mM EDTA, and 1 mg/ml tRNA). The samples were immediately ethanol precipitated and the pellets dissolved in loading dye (90% formamide, 50 mM Tris-HCl (pH 8.0), 0.05% bromophenol blue, and 0.05% xylene cyanol). After heating at 90 °C for 2 min and quick chilling, the samples were electrophoresed on polyacrylamide gel (40 cm × 30 cm, 0.5 mm). Dried gels were autoradiographed using Kodak XAR-5 x-ray film, and the transcripts were quantitated by laser scanning densitometry (LKB Ultrascan XL). Several exposures of different lengths were made from each gel, and the resulting autoradiograms were scanned to ensure that there was a linear relationship between radioactivity and transcript length, as estimated by comparison with 5'-end-labeled HinfI-generated fragments of pX74 DNA.

The RNA polymerase used in the experiments described here was from Pharmacia LKB Biotechnology Inc. Comparable reactions have also been done with RNA polymerase from Boehringer Mannheim and RNA polymerase purified from E. coli MRE 600 by the method of Burgess and Jendrisak (25). Rho and NusA were generously provided by T. Platt and S10 by R. Zimmerman. NusB was purified as described previously (26), and the purification of NusG will be described elsewhere.

DNA Templates—The DNA templates were derived from a plasmid (pRJ1) carrying the rplKAlrpoOB DNA extending from an engineered EcoRI site at approximately 680 in pl61 to the EcoRI site at 3528 in rpoB (numbering according to Refs. 27 and 28). The plasmid was linearized at the unique SmaI site in pl61 and then treated with Bal31 to generate a series of deletions. Eight-bp XbaI linkers were added before recircularizing the plasmid. The exact end points were determined by DNA sequencing. A deletion of 1161 bp in plVT1 extends from nucleotide 1399 to 2560. The IVT1(E-S) template was excised from this plasmid with EcoRI, which cleaves approximately 670 bp upstream of P2 and S10, which cleaves 226 bp downstream of the rpoBa hairpin. A deletion of 1273 bp in plVT2 extends from nucleotide 1399 to 2672. The IVT2(E-S) template was excised from this plasmid with the same enzymes used above while the IVT2(T) template was generated with T4K, which cleaves approximately 600 bp upstream of P2 and 76 bp beyond the rpoBa hairpin. The fragments were resolved by agarose gel electrophoresis and extracted from the gel matrix using the GeneClean protocol (Bio 101 Inc., La Jolla, CA).

Primer Extension Analysis—Unlabeled RNA was produced in the absence of [α-32P]ATP using the in vitro transcription system described above. An oligonucleotide (5'-GCCACAACCTGGAAATTCTCCTGATA-3') complementary to nucleotides 1376-1399 of the rplKAl intercistronic region was radioactively labeled at its 5'-end and annealed to the in vitro synthesized RNA by incubation at 25 °C for 12 h. Primer extension was carried out by the standard procedure (29). To identify the 3'-terminus of the extension product a set of dyeoxy sequencing reactions (Sequenase, U. S. Biochemical Corp.) was conducted using the same primer and as M13mp19 recombinant clone containing the rplA region. Products were resolved by electrophoresis and visualized by autoradiography as described above.

RNA Chain Termination Using 3'-dUTP—The sequence of U residues in the nascent RNA was determined essentially by the method of Axelrod et al. (30) with modifications suggested by Reisberg and Hearst (31) and Morgan et al. (52), except that the 3'-O-methyl-UTP was replaced with 3'-dUTP. IVT2(T) was used as the template, and the transcription reactions were as described above with the following modifications. The reaction temperature was 30 °C. Elongation was started by the addition of ATP to 20 µM (15 µCi of [α-32P]ATP) and CTP, GTP, and UTP to 10 µM. After 90 s, CTP and GTP were increased to a final concentration of 100 µM, and 3'-dUTP was added to a concentration of 200 µM. After 15 min the reaction was completed by the addition of 50 µl of stop solution and processed as above. The addition of 3'-dUTP 90 s after the start of elongation was determined from pilot experiments to give optimal termination in the desired region. Dried gels were autoradiographed at −70 °C using Kodak XAR-5 x-ray film and a single Du Pont Cronex Lightning Plus intensifying screen.

RESULTS

In Vitro Transcripts and Location of Initiation and Termination Site—Previously reported RNA polymerase binding studies (33), in vitro transcription experiments (27), and S1 nuclease analysis of in vivo transcripts (9) have all indicated that the major promoter for cotranscription of the rplKAlrpoBC genes (P2) is located approximately 374 bp upstream of rplK. Each individual study suggested slightly different initiation sites varying from nucleotide 1346 to 1348 (numbering used throughout this report is according to Post et al. (27) with corrections by Morgan et al. (28)). This region contains a cluster of 5 adjacent C residues downstream of recognizable −35 and −10 promoter sequences (Fig. 1a). In the studies presented here the transcripts were initiated specifically at P2 by supplying the dinucleotide Cpc with low levels of ATP and CTP. This potentially allows initiation at any of the 4 C residues from 1346 to 1349 with formation of a 7-nucleotide transcript, respectively. A single round of transcription was then continued by the simultaneous addition of all four nucleoside triphosphates and rifampicin. For the purpose of listing the size of the final transcripts, the initiation site was assigned as 1346, which is 5 bp downstream of the most highly conserved T residue of the −10 region. Evidence consistent with that assignment is presented below. To produce transcripts that were small enough to be analyzed on high resolution polyacrylamide-urea gels much of the DNA between P2 and the attenuator (rpoBa) was removed by deletion. Several deletion plasmids were constructed as described under “Experimental Procedures.” The templates produced from two of these plasmids are diagrammed in Fig. 1b. All templates retain 54 bp of the DNA normally downstream of P2 (to nucleotide 1399). In the template derived from plVT1, the triangle in IVT2(E-S) and IVT2(T) represents a 1273 bp deletion from nucleotide 1399 to 2672. The dried gels were autoradiographed using Kodak XAR-5 x-ray film and a single Du Pont Cronex Lightning Plus intensifying screen.

**Fig. 1. Transcripts.** Panel a, promoter (rplpL) and attenuator (rpoBa) sequences. The lower line presents the Nontemplate DNA strand in the vicinity of the initiation site of rplpL; the −10 sequence is underlined. The upper line indicates the sequence of the template DNA strand and a indicate the promoter and attenuator, respectively. The triangle in IVT1(E-S) and IVT2(T) represents a deletion of 1273 bp. Restriction endonuclease cleavage sites used to generate linear templates are: E, EcoRI; S, Sall; T, TaqI.
this is followed by 130 bp of DNA upstream of the start of the rpoBa hairpin whereas the templates produced by pIVT2 carry 18 bp upstream of rpoBa.

Two transcripts were produced in vitro from the IVT1(E-S) template (Fig. 2). The migration of the slower band was consistent with a 448-nucleotide transcript predicted from initiation at P1 and runoff at the SalI terminus which is located at the end of the rplL-rpoR intercistronic region only 25 bp upstream of rpoBa. The migration of the faster band was consistent with transcripts of approximately 219-223 nucleotides predicted from initiation at P2 and termination within the set of 5 U residues following the rpoBa hairpin (Fig. 1). The expected lengths of the terminated transcripts listed in Fig. 1 were calculated by designating the termination site as the central U residue (see below).

To help confirm that these two bands corresponded to the runoff and rpoBa-terminated transcripts initiated at P2, additional reactions were done with two different DNA templates. The IVT2(E-S) template (Fig 1b) has 112 fewer bp between P2 and rpoBa than IVT1(E-S), hence both the terminated and runoff transcripts produced from IVT2(E-S) should be 112 bp shorter (Fig. 2). Finally, the IVT2(T) template carries the same sequences between P2 and rpoBa as IVT2(E-S) but has only 76 bp beyond the hairpin of rpoBa (Fig. 1b). Therefore, IVT2(T) would be expected to produce the same size rpoBa-terminated transcript as IVT2(E-S) but a runoff transcript that is 154 bp shorter, as is seen in Fig. 2. The increased resolution that results from a decrease in transcript size when using the shorter IVT2(E-S) or IVT2(T) templates shows that the rpoBa-terminated transcript band is actually made up of several species (Fig. 2).

The relative sizes of the transcripts produced with the various templates are consistent with initiation occurring in vitro at P1 and a significant fraction of these transcripts terminating at rpoBa. However, the exact size of RNA transcripts cannot be determined unambiguously with DNA standards. Therefore, primer extension analysis was used to map more precisely the initiation site. An oligonucleotide complementary to bases 1376-1399 of the rplKAJL sequence was annealed to the in vitro synthesized RNA and primer extension conducted by standard procedures (29). The main band seen on the gel (Fig. 3) corresponded to initiation at nucleotide 1346, with a weaker band corresponding to 1347. This is the same region used for initiation in vivo (9). The weaker band at 1347 may be a premature termination product of the reverse transcriptase as the gel analysis of short runoff transcripts using other templates truncated before rpoBa showed only a single band, indicative of initiation at a single nucleotide (data not shown). The same start site is seen when transcription is initiated with all four nucleotide triphosphates in place of Cpc, therefore it is not an artifact of Cpc-primed initiation (data not shown).

To map more precisely the 3' end of the in vitro terminated transcripts the RNA produced was partially sequenced using 3'-dUTP and E. coli RNA polymerase essentially by the method of Axelrod et al. (30). Incorporation of 3'-dUTP into the growing RNA chain will stop further extension and provide a marker lane for the U residues in the transcript when the products are resolved by electrophoresis on a sequencing gel. As shown in Fig. 4, electrophoresis of the normally terminated transcripts in a lane adjacent to the 3'-dUTP products indicates that termination is occurring in vitro in the cluster of five U residues following the rpoBa hairpin.

Effect of NusA, NusB, NusG, S10, and Rho on the Termination Frequency at rpoBa—Previous in vivo experiments had suggested that NusA increased the frequency of readthrough into rpoB, but Rho decreased that frequency (15). To test whether not only Rho and NusA but also NusB, NusG, and NusE (S10) had any effect on the in vitro termination fre-

**FIG. 3. Primer extension analysis of in vitro transcription initiation site.** The product of the primer extension analysis described under “Experimental Procedures” was resolved on an 8% polyacrylamide gel and visualized by autoradiography (lane 1). The same primer was used for dyeoxy sequencing of the rplK-rpl intercistronic region (lanes labeled T, G, A, and C indicate the bases complementary to the dyeoxyribonucleotide added to that reaction). The 5 consecutive C residues correspond to the sequence at nucleotides 1346-1350 shown in Fig. 1.

**FIG. 4. In vitro transcription termination sites.** Lane 1, 3'-dUTP-terminated products correspond to the region between nucleotides 2693 and 2720 of the rplL-rpoB intercistronic space. This region is identified by the set of 5 U residues at 2700-2704 followed by the second set of 5 U residues at 2716-2720 as these are the only runs of 5 consecutive U residues in the rplL-rpoB intercistronic sequence. Lane 2, products from the standard in vitro transcription reaction show termination in the cluster of U residues at 2716-2720.
frequency at rpoBa or at any other sites in the rplL-rpoB intercistronic region, these proteins were added separately and in various combinations to the purified system. The template used for these experiments was IVT1(E-S), which includes essentially the complete rplL-rpoB intercistronic region. Transcriptional readthrough frequency was determined by laser densitometry of the autoradiograms with correction for the sizes of the terminated and runoff transcripts. RNA polymerase alone read through rpoBa at a frequency of 38\% (Fig. 5 and Table I). The addition of either NusA (3.7 pmol) or NusG (1.7 pmol) increased the frequency of readthrough to 48 and 46\%, respectively (Table I). Titration studies indicated that the effect of NusA and NusG on readthrough reached saturation at approximately these concentrations (Fig. 6). Neither NusB nor S10 alone had any significant effect on readthrough either at 2.8 and 3.4 pmol, respectively.

![Fig. 5. Effect of termination and antitermination proteins on termination at rpoBa. The indicated proteins were added to in vitro transcription reactions containing the IVT1(E-S) template and RNA polymerase. The rpoBa-terminated transcripts are designated with a, the runoff transcripts with r. Lane 1, no additions; lane 2, NusA, NusG; lane 3, NusA, NusB, NusG, NusG, Rho; lane 4, NusA, NusB, NusG, S10; lane 5, NusA, NusB, NusG, S10, Rho; lane 6, Rho. Amounts added: NusA, 3.7 pmol; NusB, 2.8 pmol; NusG, 2.5 pmol; S10, 3.4 pmol; Rho, 4.3 pmol.](image-url)

**TABLE I**

**Effect of Nus proteins and Rho on transcriptional readthrough at rpoBa**

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<tr>
<th>Line no.</th>
<th>NusA</th>
<th>NusB</th>
<th>S10</th>
<th>NusG</th>
<th>Rho</th>
<th>% Readthrough</th>
</tr>
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<tr>
<td>1.</td>
<td></td>
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<td>38 ± 1.9</td>
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<tr>
<td>2.</td>
<td>+</td>
<td></td>
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<td>58 ± 2.7</td>
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<tr>
<td>3.</td>
<td>+</td>
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<td>48</td>
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<td>4.</td>
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<td>40</td>
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<td>5.</td>
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<td>38</td>
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<td>6.</td>
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<td></td>
<td>+</td>
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<td>46</td>
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<td>7.</td>
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<td>8.</td>
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<td>9.</td>
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<td>10.</td>
<td>+</td>
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<td>13.</td>
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<td>17.</td>
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<td>18.</td>
<td></td>
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<td>19.</td>
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**DISCUSSION**

The transcription experiments presented here demonstrate that the rpoBa sequence functions in *vitro* as a transcriptional terminator. Several previous studies which had examined *in vivo* transcripts by S1 nuclease analysis identified 3' RNA termini in the cluster of U residues following the rpoBa hairpin (9, 14, 15). However, S1 nuclease mapping studies cannot distinguish whether these termini are the original products of *in vivo* termination or if they represent degradation products of transcripts terminated further downstream. An apparent example of this occurs in the trp operon in which the 3' terminus seen by S1 mapping of *in vivo* RNA is located.
just downstream of the trpt hairpin. However, it has been suggested that this terminus is the result of not only a low level of termination directly at this site but also of the predominant termination at trpt' approximately 250 bp downstream followed by a 3' to 5' exonucleolytic degradation to the trpt hairpin which also functions as a barrier to further degradation (34, 35).

Prior studies have shown that transcripts which read through rpoBa are cleaved at an RNase III processing site approximately 55 bp further downstream (14). Yet, the observed ratio of 3' termini at rpoBa to that at the RNase III site was much greater than that expected for the 70-80% in vivo termination frequency (15). This suggests that these RNase III-cleaved transcripts are rapidly degraded back to the rpoBa hairpin. This finding combined with the current in vitro transcription results indicates that rpoBa functions as both a terminator and an exonuclease barrier.

Termination in vitro at rpoBa by RNA polymerase alone is relatively inefficient with approximately 38% readthrough. When NusA alone was added to this system the level of readthrough was increased to 48% whereas the addition of NusG alone led to 46% readthrough. When the combination of both NusA and NusG was present, either alone or together with the other Nus proteins, the level of readthrough ranged from 61 to 56% (Table I, lines 2, 9, 14, and 15). Thus, the increase in readthrough in the presence of both proteins was roughly equivalent or just slightly greater than the sum of the separate effects of NusA and NusG. Sigmund and Morgan (54) demonstrated that although equimolar amounts of NusA can cause maximal increases in pausing of RNA polymerase at a variety of sites, a 10-fold molar excess of NusA is required to affect termination maximally at the rRNA polymerase or the various sites in the λ tr, terminator. On the basis of this observation they raised concerns as to whether the effects of NusA on termination in vitro at high molar excess over RNA polymerase are physiologically meaningful. The effects of NusA and NusG in our system are likely to be biologically relevant because the concentration of NusA and NusG required at rpoBa (4 and 1.2 µg/ml, respectively) are the same as those required for antitermination in vitro by the N protein of λ, i.e. about 3–6 µg/ml for NusA and about 1–2 µg/ml for NusG. NusB and S10 had no significant effect on termination frequency when added separately or together. Moreover, neither protein nor the combination of the two significantly altered the level of readthrough seen in the presence of NusA and/or NusG.

The finding that NusA increases the frequency of readthrough at rpoBa in vitro is consistent with our previous in vivo studies that showed a decreased ratio of rpoB to rplL mRNA in the nusAI mutant (15). Both of these results are qualitatively similar to earlier experiments which demonstrated that L-factor, which was later identified as NusA, stimulated the synthesis of β and β' 2–3-fold in a DNA-dependent protein synthesis system (36). Altogether these observations are consistent with NusA functioning as an antitermination factor at rpoBa.

NusA was originally identified genetically as one of several genes necessary for N-mediated antitermination of λ-delayed early gene transcription (21). This has been confirmed biochemically by the demonstration of antitermination with a complex of RNA polymerase, N, NusA, NusB, NusG, S10, and nut site RNA (55, 56). Also, the Q-mediated antitermination of λ late gene transcription is made much more efficient by NusA (37). However, in most other systems that have been examined in which NusA acts independently of the λ N or Q proteins it appears to function as a termination factor. In vitro experiments have shown that NusA increases transcriptional pausing at the 1:2 stem of the trp attenuator (38), the λ tr1 (39) and tr2 (40) terminators, rrnB3 (41), and the T7 bacteriophage early region (42). Moreover it directly enhances termination in vitro at trpt (43), λ tr3 (44), rrrnBT (44), and tr' (45). In the S10 ribosomal protein operon NusA has been shown to be essential for the in vitro stimulation of premature termination in the leader region caused by excess L4 (52). Also in vivo experiments with the nusAI mutant suggested that NusA normally is involved in the transcriptional termination that occurs as a result of a polar mutation (46).

NusA has been implicated in antitermination in the transcription of ribosomal RNA operons (47) and the operon that encodes the 8 subunit of RNA polymerase (48). But the only site other than rpoBa at which NusA has been shown to reduce directly termination in vitro is site I of the λ tr, terminator (39), where it was found to slow transcript release and as a result reduce termination frequency. These previous experiments and our current results suggest that subtle features of the individual termination site may influence whether NusA increases or decreases the frequency of termination.

NusG also increases readthrough at rpoBa in vitro although at a slightly lower level than NusA. NusG interacts with RNA polymerase and makes N-mediated antitermination in λ more processive. In the absence of N, S10, and NusB, NusG also reverses the suppressive effect of NusA on termination at site I of tr, of λ. Therefore, as is the case with NusA, the effect of NusG may depend on other subtle features of the termination site. The effect of NusG on other termination sites has not yet been tested. Currently we have no evidence as to whether NusG influences the in vivo termination frequency at rpoBa.

The lack of any effect of NusB or S10 on termination in the in vitro transcription system is also consistent with our earlier in vivo experiments in which no significant change in the ratio of rpoB to rplL transcription was observed in either a nusB or nusE mutant (15). NusB did not significantly alter the pattern of termination when added to the in vitro system containing only RNA polymerase. However, when Rho was added to reactions containing at least NusA, a series of faint bands which migrated between rpoBa-terminated and runoff transcripts was more evident. This observation suggests that the combination of Rho with at least NusA causes a low level of termination in vitro at multiple sites downstream of rpoBa. But apparently the addition of Rho does not change the frequency of termination at rpoBa itself because the ratio of the rpoBa-terminated band to the sum of the faint and runoff bands indicates 56–59% readthrough of rpoBa (Table I, lines 17 and 18), which is not significantly different from those reactions in which Rho is absent (Table I, lines 2, 9, 14, and 15).

This result is in contrast with our previous observation of an increased ratio of rpoB to rplL mRNA in rho mutants (15). Although those experiments could not determine if termination was occurring specifically at rpoBa, they did suggest that in vivo Rho normally increases the frequency of termination somewhere in the rplL-rpoB intercistronic region. However, subsequent experiments involving the construction of a series of transcriptional fusions of different segments of the rplL-rpoB intercistronic DNA upstream of lacZ have shown that rpoBa is the only termination site used in vivo in this region (49). Therefore the low level of termination seen

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1. J. Greenblatt and J. Li, unpublished data.
in vitro downstream of rpoBa with the combination of Rho and NusA apparently does not occur to any significant extent in vivo. To try and reconcile the disparate in vivo and in vitro observations we speculate that either Rho can increase termination frequency at rpoBa but requires additional factors that are not present in this purified system, or the effect of the Rho mutations on rplJL and rpoB transcription in vivo is indirect. The pleiotropic nature of rho mutations is consistent with the second explanation (50, 51).

Together these experiments suggest that both NusA and NusG decrease the frequency of termination at rpoBa. However, in all cases, either with or without these proteins, the level of readthrough observed in vitro is greater than the 20-30% that occurs in vivo during steady-state growth. Therefore, the ionic conditions of our in vitro reactions may not be ideal, or there may be additional as yet unidentified factors which also play a role in modulating the termination frequency at this site.

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REFERENCES