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NOTES

Evidence that Rho and NusA Are Involved in Termination in the *rplL-rpoB* Intercistronic Region

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The frequency of transcription of the ribosomal protein and RNA polymerase gene segments of the *rplKAJL-rpoBC* gene cluster was measured for *Escherichia coli* K-12 strains carrying mutations in the genes for transcriptional termination factors. The results of our study suggest that Rho increases and that both NusA and the product of *sfrB* decrease termination frequency in the *rplL-rpoB* intercistronic region.

The concentration of RNA polymerase core enzyme in growing *Escherichia coli* is determined by the rate of synthesis of the β and β′ polypeptides (8, 15). The genes that encode these polymerase subunits (*rpoBC*) are the distal elements in a gene cluster that also contains four ribosomal protein genes (*rplKAJL*) (1, 10, 14, 17, 18, 23, 27, 31, 32). Two major promoters, *rplKp* and *rplJp*, are present in this gene cluster (Fig. 1) (14, 27, 32). Hence, the *rpoBC* genes are cotranscribed with at least the *rplJL* genes. Moreover, it has been reported that many transcripts initiated at *rplKp* do not terminate after *rplA*, but continue through *rplJp*, thus suggesting that all six genes may be cotranscribed (6).

Even though the RNA polymerase subunit genes are cotranscribed with upstream ribosomal protein genes, their frequency of transcription is about 20% of that of the upstream genes (7). This difference is accounted for by a transcriptional attenuator (*atn*) in the *rplL-rpoB* intercistronic region which terminates approximately 80% of the transcripts during steady-state growth (2, 27). DNA sequence analysis has suggested that there is a sequence characteristic of a transcriptional terminator at 43 to 73 base pairs (bp) beyond the end of *rplL* (22, 25). The proposal that this indeed functions as a terminator was further strengthened by S1 nuclease and RNA sequence analyses of an in vivo transcript whose 3′ end mapped 69 bp beyond *rplL* (3). The RNA transcript from this region can form an 11-bp stem followed by five consecutive uridine residues (see Fig. 3). This is the canonical structure of a simple or factor-independent terminator. However, 20% of the transcripts continue through this sequence, and various experiments have suggested that the termination frequency may be altered under certain conditions (5, 19, 20). These results suggest that the function of this sequence may be more complicated than that of a simple constitutive terminator.

A number of factors in addition to the RNA polymerase core enzyme and the RNA sequence have been demonstrated to participate in transcriptional termination. The best studied of these is the Rho protein (28). A general class of terminators, referred to as Rho-dependent terminators, has been recognized (13, 29), but no simple consensus sequence is evident. What appear to be required are both a nucleotide sequence which causes RNA polymerase to pause and an adjacent stretch of RNA devoid of secondary structures (29). Other factors involved in regulating termination are the products of the *nusA*, *nusB*, and *nusE* genes. These were first identified as host genes necessary for N-mediated antitermination of lambda transcription (11). The NusA protein (12) is the best studied of these three, and it has been shown to be involved in termination and antitermination decisions in a number of bacterial operons (9, 13, 16, 30). More recently, the existence of an additional host factor regulating transcriptional termination has been suggested. The *sfrB* gene product appears to be required for antitermination in the *tra* operon of the F plasmid (4). We have performed assays to determine whether any of these termination factors are involved in regulating the frequency of termination at the attenuator between *rplL* and *rpoB*.

To determine the frequency of transcription of the ribosomal protein gene segment versus that of the RNA polymerase gene segment of this composite transcriptional unit, pulse-labeled RNA was hybridized to single-stranded DNA probes complementary to *rplJL* and *rpoB* (Fig. 1). The RNA of mutant strains and their isogenic parents was labeled by the addition of [5,6-^{3}H]uridine to an exponentially growing culture as described previously (26). Hybridizations were done in a volume of 100 μl at 45°C for 18 h. Each reaction contained 2.0 μg of RNA and 0.2 μg of DNA (providing at least a 10-fold excess of DNA, data not shown) in hybridization solution (50% formamide, 0.6 M NaCl, 80 mM Tris hydrochloride [pH 8.4], 4 mM EDTA, 10 μg of yeast RNA per ml). Unhybridized RNA was then degraded by treatment with RNase A and RNase T_1, and the RNA-DNA hybrids were collected and washed (with 0.5 M KCl, 10 mM Tris hydrochloride, pH 7.2) on nitrocellulose filters. By measuring the level of radioactivity hybridized to each probe, correcting for the number of uridine residues, and expressing the result as a ratio, the effect of a number of mutations on the *rplJL/rpoB* transcript ratio, and hence on the termination efficiency at *atn*, was determined (Table 1).

The parental ratios were slightly less than 5, consistent with previous estimates for wild-type *E. coli* strains (7, 26). Two mutant strains, AD1600 and AD1919, both with lesions in *rho*, showed reduced *rplJL/rpoB* transcript ratios, suggesting that termination at *atn* is less frequent. Strains

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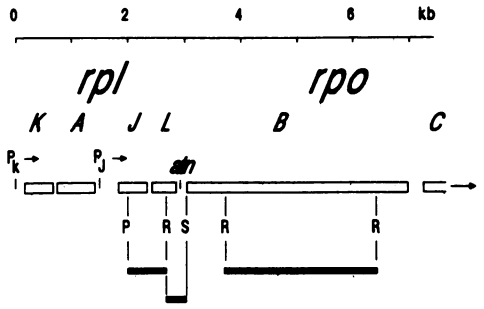

FIG. 1. Hybridization probes. The 658-bp *Pst*I-*Eco*RI fragment (*rplJL*) and the 2,873-bp *Eco*RI fragment (*rpoB*) were ligated into M13mp8 and used as probes for determining the *rplJL/rpoB* transcript ratio. The 503-bp *Eco*RI-*Sal*I fragment was used for S1 nuclease mapping of the 3′ ends of the terminated and processed transcripts. The major promoters, *rplKp* (P_K) and *rplJp* (P_J), and the attenuator (*atn*) are indicated; the structural genes are drawn to scale. Restriction endonuclease cleavage sites for *Eco*RI (R); *Pst*I (P); and *Sal*I (S) are shown.

carrying mutations in *nusA*, *nusB*, and *nusE* were also screened by hybridization. Of these, only the strain carrying the *nusA* lesion showed a ratio significantly different from that of the isogenic parent. The increased ratio for this mutant suggests that termination is more frequent in the absence of normal NusA function. One additional strain, SA2243, carrying a lesion in *sfrB* was examined. This mutant also showed an increased ratio, suggesting that the termination frequency is increased by the alteration.

The location of the attenuator has been previously mapped by Barry et al. (3) by using a combination of S1 mapping and RNA sequencing. The same article was the first to describe an RNase III processing site in the *rplL-rpoB* intercistronic region, but this site was mapped only approximately at 210 bp downstream of the attenuator. Essentially all of the transcripts that read through the attenuator are cleaved at this processing site. To determine both the position of the processing site more accurately and the effect of the above mutations on the relative levels of attenuated versus processed transcripts, a higher-resolution S1 mapping technique was used. We made use of a shorter DNA probe extending 503 bp from the *Eco*RI site in *rplL* to the *Sal*I site preceding

TABLE 1. *rplJL/rpoB* transcript ratios[a]

Strain	Source	Relevant genotype	*rplJL/rpoB* transcript ratio
SA1030	S. Garges	*rho*$^+$	4.8
AD1600	S. Garges	*rho-15*	1.6
AD1919	S. Garges	*rho-112*	2.5
K37	D. Friedman	*nusA*$^+$ *B*$^+$ *C*$^+$	4.9
K95	D. Friedman	*nusA1*	7.8
K450	D. Friedman	*nusB5*	4.5
K556	D. Friedman	*nusE71*	4.9
SA2244	K. Sanderson	*sfrB*$^+$	4.3
SA2243	K. Sanderson	*sfrB*	7.2

[a] Each RNA preparation was hybridized to M13mp8 DNA without a cloned insert to determine the background hybridization. The background level was subtracted from the counts per minute of radioactivity that had hybridized to the *rplL* and *rpoB* probes, and the ratio was calculated as described in the text. The net counts per minute of radioactivity hybridized to the probes ranged from 500 to 1,000 cpm in the individual experiments. Each ratio is the average of three experiments; in all cases, the standard deviation was less than ±0.3.

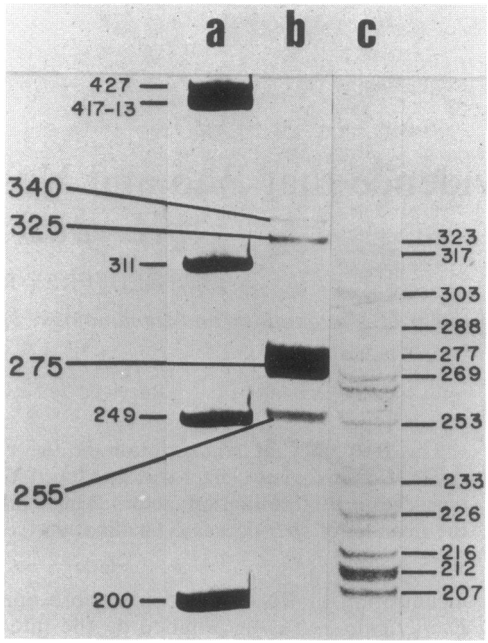

FIG. 2. Sizes of S1 nuclease-resistant *rplL-rpoB* probe-transcript hybrids. After digestion with S1 nuclease, hybrids of the 503-bp probe (3′ end labeled at the *Eco*RI terminus [Fig. 1]) and total RNA were denatured, and the single-stranded DNA probe fragments were resolved by electrophoresis at 55°C on 7 M urea–8% acrylamide gels (lane b). The radioactively labeled bands were visualized by autoradiography. For size standards, φX174 DNA was digested with *Hin*fI, end labeled with [^{35}S]dTTP via the Klenow fragment fill-in reaction, and run alongside (lane a) the S1-resistant fragments. To more accurately size the S1-resistant fragments, the 503-bp probe described above was purified and subjected to limited degradation at guanine residues to produce a nested set of labeled fragments (lane c) with sequences identical to those of the S1-resistant fragments. The larger numbers on the left show the sizes assigned to the S1 nuclease-resistant bands. The estimated error in these sizes is ±3 bp.

rpoB (Fig. 1). Only the *Eco*RI terminus was labeled with [^{35}S]dTTP via the Klenow fragment fill-in reaction. The hybridization protocol was performed as previously described (21), with the following modifications. Each hybridization reaction contained 25 μg of extracted RNA, 10 μg of yeast RNA, and approximately 50 ng of end-labeled DNA. The denaturation step was at 80°C for 10 min, and hybridization was at 53°C for 3 h. The hybrids were then treated with 1,000 U of S1 nuclease for 30 min at 37°C, recovered by ethanol precipitation, and suspended in 90% formamide for denaturing gel electrophoresis.

Transcripts from a wild-type *E. coli* strain were sized by resolving the single-stranded DNA probe fragments surviving S1 nuclease digestion on a denaturing gel in a lane adjacent to a set of single-stranded size markers of the same probe sequence (Fig. 2). The predominant fragment was estimated to be 275 bp long, suggesting that most transcription into the intercistronic space is terminated about 72 bp downstream of *rplL* (Fig. 3) (3). A smaller, much fainter band at 255 bp coincided with a second region of five consecutive uridine residues in the transcript. The larger 325- and 340-bp species corresponded to the readthrough transcripts that had been cleaved by RNase III. However, the size of the fragments seen on these higher resolution gels placed the 3′ ends of the transcripts about 127 and 142 bp

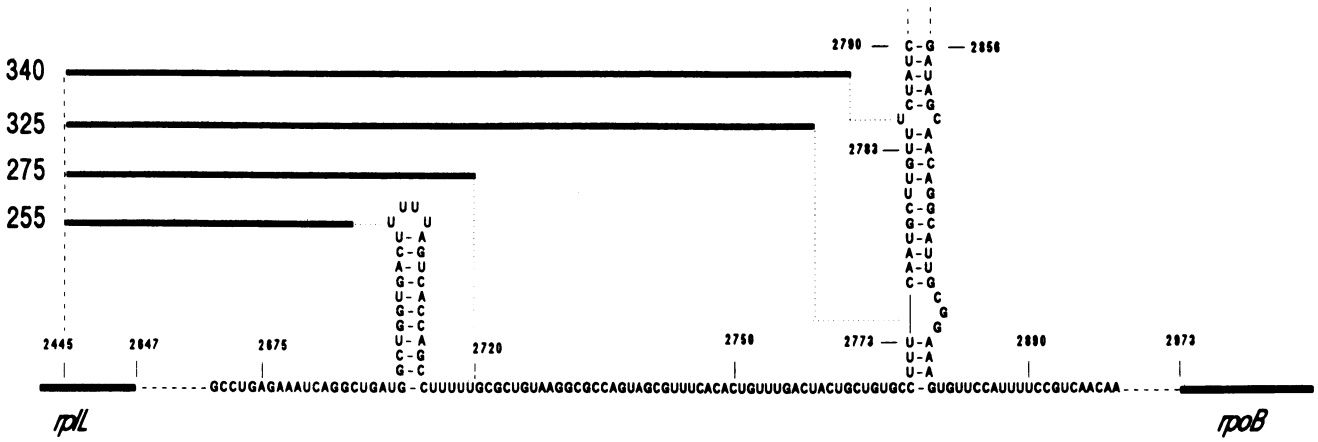

FIG. 3. Alignment of the 3′ ends of the S1 nuclease-protected transcripts with the RNA sequence. From the size of the S1 nuclease-protected probe fragments estimated as described in the legend of Fig. 2 (±3 bp), the most probable ends of the transcripts are indicated on the RNA sequence of the *rplL-rpoB* intercistronic region. The potential secondary structures for the attenuator (positions 2690 to 2715) and the RNase III-processing region (positions 2770 to 2879) are indicated.

downstream of *rplL* (Fig. 3), rather than +210 bp downstream as mapped by Barry et al. (3). When RNA extracted from the *nusA*, *sfrB*, and two *rho* mutants was examined by the same procedure, no dramatic differences were seen between the wild-type and mutant patterns (data not shown). In all cases, the 275-bp fragment was the predominant species, with minor bands at 255, 325, and 340 bp. Moreover, the relative amounts of these species did not appear to vary in any of the strains.

The altered *rplJL/rpoB* transcript ratios we have measured indicate that the products of the *rho* and *nusA* genes affect the frequency of termination in the *rplL-rpoB* intercistronic region. Even though the attenuator has a structure that resembles a simple or factor-independent terminator, these results suggest that Rho normally increases the frequency of termination at this site and that NusA decreases this frequency. These two antagonistic factors could then potentially play a role in modulating the frequency of termination at the attenuator under varying physiological conditions. However, this frequency is not found to vary with changes in the steady-state growth rate (26). The finding that NusA decreases termination in this region is consistent with the earlier observation that L factor (NusA) is required for the efficient synthesis of the β and β′ polypeptides in an in vitro DNA-directed transcription-translation system (33). The transcript ratio is not altered in the *nusB* or *nusE* mutants, suggesting that the products of these genes do not function at the attenuator. However, it cannot be ruled out that these mutant alleles, originally isolated because of their effect on lambda growth, may produce altered proteins which have sufficient activity to function at the attenuator. The increased ratio found in the *sfrB* mutant suggests that the product of this gene can also affect the termination frequency. Unfortunately, relatively little is known about the *sfrB* gene product as compared with Rho and NusA.

Surprisingly, we found no obvious difference in the pattern of S1-resistant hybrids between the *nusA*, *rho*, and *sfrB* mutants and the wild type. In all strains, the 275-bp band was the predominant species (Fig. 2). Moreover, the ratio of the 255- and 275-bp bands to the 325- and 340-bp bands was much greater than the 5:1 ratio expected from the *rplJL/rpoB* transcript ratio. One possible interpretation of this discrepancy is that once a full-length transcript is cleaved at the processing site, the 3′ end is rapidly degraded back to the stable hairpin loop. Thus, the band at 275 bp may represent a combination of both terminated transcripts and transcripts that have been processed and then degraded. A similar situation has been observed in the tryptophan operon in which transcripts terminated at *trpt′* are degraded approximately 250 bp in the 3′-to-5′ direction until an 8-bp hairpin (*trpt*) is encountered (24). Alternatively, there may be a factor-dependent terminator at which Rho and NusA act further downstream of the proposed attenuator and then the true terminated transcript is rapidly degraded back to this stable hairpin. A direct test of these models will require the establishment of an in vitro transcription system for this gene cluster.

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