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Evidence for Antitermination in *Escherichia coli* rRNA Transcription

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The stable RNA operons of *Escherichia coli* do not exhibit polarity, even though they make an RNA product that is not translated. By contrast, most *E. coli* operons that specify proteins exhibit polarity if their translation is interrupted. The transcriptional component of this polarity depends on the action of Rho protein on the exposed mRNA, which results in premature transcription termination. Here we examine how a stable RNA operon (*rrnG*) transcript is protected from the Rho protein-mediated polarity response. We compared transcription from the *ara* and the *rrnG* promoters through a 16S DNA segment. In each case, the promoter-16S sequences were joined to a *trp-lac* fusion, and *lacZ* mRNA was examined in *rho*$^+$ and *rho-115* strains. We found significant Rho protein-dependent termination of transcripts from the *ara* promoter but little or no Rho protein effect on transcription from the *rrnG* promoter. We concluded that the transcript of the 16S ribosomal DNA segment does contain Rho protein-dependent transcription terminators, but there is an antitermination system in the *rrnG* control region that allows it to transcribe through those terminators.

In *Escherichia coli*, transcription of stable RNA operons takes place without polarity (27, 28); this is unlike operons that specify proteins, in which polarity often results when translation is interrupted. Polarity is defined as the decreased expression of a distal gene (or sequences) of an operon resulting from specific genetic signals in proximal sequences. There are both translational (2, 31, 32) and transcriptional (1) components of polarity. The transcriptional component requires Rho protein and exposed mRNA. In addition, it is thought that specific Rho protein binding sites and RNA polymerase pause sites on the exposed mRNA segment are required for premature transcription termination (1). The absence of transcriptional polarity in stable RNA genes might be caused either by factors intrinsic to the structure of the stable RNA (e.g., absence of Rho-specific termination sites) or by a system similar to bacteriophage lambda N gene antitermination (14).

There is evidence suggesting that antitermination mechanisms exist in *E. coli*. The transposons Tn*9* and Tn*10* and the insertion element IS*1* cause severe polarity when they are inserted into translated operons (20). Morgan and co-workers (5, 26, 34) have shown that these elements are less polar when inserted into the *rrnC* operon. They suggest that the control region of this rRNA operon causes the decreased polarity. Liebke and Speyer (22) have studied a temperature-sensitive *E. coli* mutant that they suggest affects an antitermination protein that is required for stable RNA synthesis. The mutant blocks synthesis of stable RNAs at nonpermissive temperatures. They say that transcription initiation occurs in the mutant but that mRNA elongation is defective. In recent experiments with the RNA polymerase sigma subunit operon (*rpsU dnaG rpoD*) (7, 23), a sequence similar to the λ *nut* site has been located. No information is available on whether there is an antitermination protein.

We wanted to determine whether transcription termination signals are missing from the *rrn* structural genes, or whether something unique about the *rrn* transcription complexes renders them able to ignore termination signals. Our system compares transcription from the *ara* (arabinose operon) and the *rrnG* promoters through a segment of 16S gene DNA (16S DNA). In each case, the promoter-16S DNA sequences were joined to a *trp-lac* fusion, and *lacZ* mRNA was examined in Rho protein wild-type (*rho*$^+$) and mutant (*rho-115*) backgrounds. We found that there is significant Rho-dependent termination when the 16S *trp-lac* sequences are transcribed from the *ara* promoter. However, Rho protein has little or no effect when the same 16S *trp-lac* sequences are transcribed from the *rrnG* promoter. These results provide evidence that segments of rRNA are not intrinsically resistant to Rho-mediated termination and suggest that an antitermination system is involved in transcription of the *rrnG* operon.

MATERIALS AND METHODS

Bacterial strains, plasmids, and phage. Bacterial strains DW319 (F$^-$ *ilv lacZ*::IS*1*-MS319) and M41 (F$^-$ *ilv lacZ*::IS*1*-MS319 *rho-115*) (11, 15) were provided by D. Calhoun. Plasmid pMC81 (ColE1 *araC*$^+$ *araI*$^+$ *B'*-'Mu'-*trp'CB*$^+$*A'-lac'ZY*$^+$*A*$^+$ *bla*$^+$) and strain MC1009 [Δ(*lacIPOZY*) *galU galK* Δ(*ara-leu*) *rpsL srl*::Tn*10 recA56*] (8) were obtained from M. Casadaban. MC1009 is a derivative of MC1000 (8) that was constructed by P1 transduction (M. Casadaban, personal communication). Plasmid pLC23-30 (9) was obtained from M. Nomura (18). Plasmids pGB8111 (3), pSU8110 (2), and pGB81218 (3) were constructed in this laboratory. Bacteriophage SUM18(+) was made in this laboratory by cloning a 1.8-kilobase-pair (kbp) *lacZ Hin*cII fragment (2) from pMC81 into M13mp7 (24). φ80d3 *ilv*$^+$ *su-7*$^+$ was a gift of L. Soll (30).

Hybrid fusion plasmids. (i) pMC81. Since all of the plasmids used in this study (Fig. 1) were derived from pMC81, a brief description and account of facts not contained in the original report of that plasmid (8) is necessary. pMC81 contains a series of gene fusions that join the *ara* promoter to the Δ(*trp-lac*)*W209* fusion. Casadaban (8) has shown that the Δ*W209* fusion contains all of *trpB* and the intact *trpB-trpA* intercistronic junction. The DNA sequence of the *trp-lac* junction in Δ*W209* (M. Berman, personal communication) shows that amino acid 59 of the *trpA* protein is fused to amino acid 3 of the *lacZ* protein. The resulting hybrid *trpA'-lac'Z* protein has β-galactosidase activity. However, the

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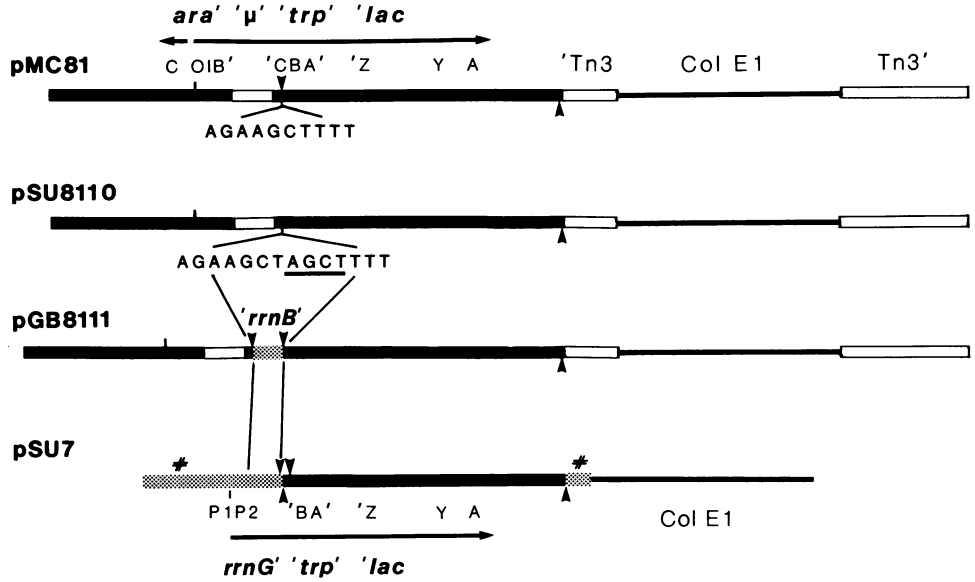

FIG. 1. Hybrid plasmids. The plasmids used in this work were pMC81 (8), pSU8110 (2), pGB8111 (3), and pSU7. *E. coli* chromosomal DNA in plasmid pMC81 and its derivatives is shown as a thick black line. Open boxes denote bacteriophage Mu (μ) or transposon Tn*3* sequences. Vector ColE1 DNA is represented by a thin black line. Thick stippled lines indicate *E. coli* chromosomal sequences from the rRNA operons (*rrnB* and *rrnG*), as well as sequences flanking the *rrnG* operon. The octathorp (#) marks these flanking segments in pSU7. The *Hin*dIII in the *trpB* gene of pMC81 was altered by a 4-bp insertion to give pSU8110 and by insertion of a 567-bp *rrnB* 16S DNA sequence to give pGB8111. Horizontal arrows show the direction of transcription of *ara* and *rrnG* promoters. ↓ marks *Hin*dIII sites, and ↑ marks *Bgl*II sites in the four plasmids. A description of the *rrnG'-trp'B* junction is presented in the text.

hybrid β-galactosidase activity cannot be used to examine transcriptional polarity in our system because the *trpB* and *trpA* genes are translationally coupled (2, 31). The insertion of nonsense codons into *trpB* causes a strong, Rho-independent translational polarity, and the hybrid β-galactosidase activity is not proportional to the amount of *lacZ* mRNA present in cells (2).

(ii) pSU8110. pSU8110 was made by in vitro alteration of the *Hin*dIII site in the *trpB* gene of pMC81 (2). The four-base-pair (bp) insertion at this site causes a shift in the reading frame that generates a UGA nonsense codon 10 codons downstream at nucleotide positions 117 to 119 in the *trpB* sequence (37). The reading frame shift also results in 21 additional nonsense codons in distal *trpB* sequences.

(iii) pGB8111. pGB8111 has a 567-bp 16S DNA *Hin*dIII fragment from *rrnB* in the *trpB Hin*dIII site in pMC81 (3). The insert was derived from the phage φ80d3 (30) and contains nucleotides 80 to 647 of the mature *rrnB* 16S DNA sequence (data not shown) (6). The insert contains 30 nonsense codons that block all three reading frames.

(iv) pSU7. pSU7 was made by fusing the *rrnG* control region from the plasmid pLC23-30 to the *trp-lac* fusion fragment from pMC81 with a 76-bp *Bgl*II-*Hin*dIII fragment from pGB81218 to join the two segments (35). (pGB81218 is a derivative of pMC81 that carries *E. coli rpl'JL-rpoBC* sequences fused to the *trp'BA'-lac'ZY*$^+$*A*$^+$ sequences at the *Hin*dIII site in *trpB* [3].) The fusion in pSU7 has joined the *Bgl*II site of the mature *rrnG* 16S DNA sequence (at position 704 of that sequence) through the 76-bp *Bgl*II-*Hin*dIII sequence to the *Hin*dIII site in *trpB* (at position 89 of that sequence). pSU7, therefore, includes sequences of 16S DNA that are almost identical to the 567-bp segment in pGB8111. (There are only six base differences between *rrnG* and *rrnB* sequences for positions 80 to 647 [33].) In pSU7, the *Hin*dIII site is followed by the natural 57-bp *Hin*dIII-*Bgl*II sequence of the *rrnG* 16S gene, then the 76-bp *Bgl*II-*Hin*dIII sequence from pGB81218 before the *trp'BA'-lac'ZY*$^+$*A*$^+$ *Hin*dIII-*Bgl*II segment which pSU7, pGB8111, and pSU8110 share in common with pMC81. *E. coli* sequences preceding and following the *rrnG* operon are also present on the ends of the *Bgl*II fragment that carries the ColE1 vector and the *rrnG* promoter. These regions are marked with an octathorp (#) in Fig. 1.

Construction of pSU7. Cleavage of pLC23-30 with *Bgl*II gives four fragments (13.5, 5.5, 3.0, and 1.5 kbp). The 13.5-kbp fragment carries the ColE1 vector DNA, the *rrnG* control region, and 16S DNA structural sequences to position 704 of the mature 16S gene, as determined by heteroduplex and restriction mapping, DNA sequencing, and Southern hybridization analyses (10, 33; data not shown). Digestion of plasmid pGB81218 DNA with *Bgl*II gives four fragments (22.8, 8.5, 2.5, and 2.0 kbp) (3). The 8.5-kbp fragment contains the *trp'BA'-lac'ZY*$^+$*A*$^+$, Δ*W209*, fusion from pMC81. This fragment was purified by reverse-phase chromatography (36). DNA from pLC23-30 (1 μg) was cleaved with *Bgl*II and ligated with the purified 8.5-kbp *Bgl*II *trp-lac* target fragment (0.5 μg). Strain MC1009 was transformed with the ligation mixture, and Lac$^+$ transformants were selected on M9 minimal (25) plates containing lactose (0.4%) and leucine (30 μg/ml). Plasmid DNA from the transformants was screened by *Bgl*II digestion. We found several transformants that contained only the 13.5-kbp vector band plus the 8.5-kbp target band. One of these was designated pSU7 (S. Aksoy, Ph.D. dissertation, Columbia University, New York, 1982). pSU7 was subjected to additional restriction mapping and Southern hybridizations to verify sites in the vector and the target and to determine the orientation of the *trp-lac* fragment with respect to the *rrnG* control region (data not shown).

S1 nuclease mapping and filter hybridization of labeled

mRNA. The S1 mapping experiments were carried out as described previously (2), except that the hybrids were separated by electrophoresis on 5% acrylamide gels instead of 1% agarose gels. The filter hybridizations of labeled RNA were carried out as described previously (2).

RESULTS

We used the unique *Hin*dIII restriction site within *trpB* to link different sequences to the *trp-lac* fusion from pMC81 to study polarity in *rrn* sequences. Two of the plasmids were made from pMC81 by introducing nonsense codons between the *ara* promoter and the *lacZ* gene; pSU8110 contains a 4-bp insertion that causes a frame-shift mutation, introducing nonsense codons early in *trpB*. pGB8111 has a 567-bp 16S DNA insertion that contains nonsense codons in all reading frames. A third hybrid plasmid, pSU7, joined the *rrnG* rRNA control region and early 16S DNA sequences to the same *trp-lac* fusion. pSU7 and pGB8111 were used to compare polarity caused by 16S DNA when transcripts originated from either the *rrnG* or the *ara* control region. pSU8110 was used to compare polarity caused by nonsense codons in the *trpB* gene with that found in pGB8111 (see above for a detailed description of these plasmids).

From our previously reported experiments on translational coupling with pMC81 and pSU8110 (2), we knew that disruption of translation within the *trp* segment of pMC81 had a polar effect on *lac* mRNA expression and that this transcriptional polarity was dependent on the presence of Rho protein in the cell. We therefore compared the effect *rho*$^+$ and *rho-115* strains had on *lac* mRNA transcription from the *ara* and *rrn* promoters in our test plasmids.

In these experiments, plasmid copy number was measured for all of the plasmids used. No variation in plasmid copy number was detected between *rho*$^+$ and *rho-115* strains transformed with the test plasmids (Serap Aksoy, Ph.D. dissertation, Columbia University, New York, 1982). Variations in mRNA levels in these strains thus reflect regulatory properties of the sequences carried on the plasmids rather than differences in plasmid copy number.

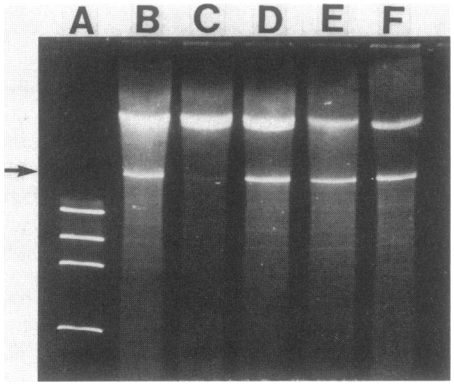

FIG. 2. Nuclease S1 mapping. *lacZ* mRNA was isolated from *rho*$^+$ (DW319) or *rho-115* (M41) strains containing the hybrid plasmids. These mRNAs were probed with single-stranded SUM18(+) DNA, which contains 1,828 bases of *lacZ*. Lanes B through F are RNA-DNA hybrids made with mRNA from the following plasmids and strains: lane B, pGB8111 in M41; lane C, pGB8111 in DW319; lane D, pMC81 in DW319; lane E, pSU8110 in DW319; lane F, pSU7 in DW319. The arrow marks the position of the 1.8-kbp *lacZ* RNA-DNA hybrid. Lane A contains a size standard of φX174 DNA cut with *Hae*III (1,353, 1,073, 872, and 603 bp). The upper band in lanes B through F is plasmid DNA that is present in the mRNA preparations. This double-stranded DNA is not cleaved by S1 nuclease treatment.

TABLE 1. mRNA levels in *rho*$^+$ (DW319) and *rho-115* (M41) strains

Plasmid	Genotype	*lacZ* mRNA (cpm/10 μg of RNA added)[a]
pMC81[b]	*rho*$^+$	9,798 ± 454 (100)
	rho-115	9,047 ± 700 (92)
pGB8111	*rho*$^+$	1,904 ± 218 (19)
	rho-115	10,343 ± 246 (106)
pSU8110[b]	*rho*$^+$	4,695 ± 388 (48)
	rho-115	8,391 ± 66 (86)
pSU7	*rho*$^+$	4,556 ± 268 (100)
	rho-115	5,235 ± 330 (111)

[a] Values are averages ± standard deviation of at least four experiments. Values in parentheses are the percentages of pMC81 *ara* or pSU7 *rrnG* promoter transcription in the *rho*$^+$ strain.
[b] Data are those used in a previous report (2).

Measurement of *lacZ* mRNA. We first examined plasmid-specific *lacZ* mRNA by S1 mapping and then we measured amounts of *lacZ* mRNA by filter hybridization of ^{3}H-labeled mRNA. In both experiments, the *lacZ* probe was single-stranded DNA from SUM18(+), a bacteriophage M13 derivative containing a 1,828-bp *Hin*cII fragment of the *lacZ* gene (nucleotides 1,063 to 2,891 [17]). The S1 experiment is semiquantitative and it shows whether the mRNA is intact for the region being probed. The filter hybridization experiment provides a more accurate measurement of the amount of mRNA present but does not reveal the physical continuity of the mRNA.

The S1 experiment (Fig. 2) was performed on unlabeled mRNA preparations isolated from exponentially growing strains of DW319 (*rho*$^+$) and M41 (*rho-115*) that contained our test plasmids. Except for the mRNA from pGB8111 in the *rho*$^+$ strain, all of the mRNA preparations made high levels of probe-length *lacZ* mRNA. pGB8111 made very little *lacZ* mRNA in the *rho*$^+$ strain; however, in the *rho-115* strain, pGB8111 made high levels, like the other plasmids. These results suggest that the amount of *lacZ* mRNA made from pGB8111 is decreased in the presence of Rho protein. This was not the case for pSU7, which makes high levels of mRNA in the *rho*$^+$ strain. However, previous results have shown that the control strain pSU8110 shows 50% termination of transcription in the *rho*$^+$ strain (2). Since this effect is not obvious in Fig. 2 (lane E), we measured mRNA by filter hybridization to assess the polar effect of 16S DNA in pSU7.

Filter hybridizations were done with [^{3}H]uridine-labeled mRNA prepared from pulse-labeled exponentially growing cells (3-min pulse). The radioactive mRNA was hybridized to a single-stranded SUM18(+) *lacZ* DNA probe immobilized on nitrocellulose filters. The results of this experiment (Table 1) show that less than 20% of the transcripts from pGB8111 originating at the *ara* promoter continue through the 16S DNA and *trp* sequences into *lacZ* in the *rho*$^+$ strain, confirming the result observed in Fig. 2 (lane C). The filter hybridization data also indicate that transcription from the *rrnG* control region through the 16S DNA and *trp* sequences in pSU7 is increased only 11% in the *rho-115* strain. In pSU8110, the 48% read-through found in the *rho*$^+$ strain reflects the amount of transcription termination that occurred in the *trpB* gene. The greater termination (81%) observed for pGB8111 represents the amount of termination that occurred in both 16S DNA and *trpB* sequences.

DISCUSSION

Transcription of stable RNA operons occurs without Rho protein-dependent transcription termination. There are several possible explanations for this lack of transcriptional polarity. The transcripts of these genes may lack Rho binding sites or other features, such as RNA polymerase pause sites (1). Extensive secondary structure of the stable RNAs may prevent Rho from acting. The rapid association of rRNA with ribosomal proteins might block the action of Rho, but this possibility does not explain the lack of polarity in transfer RNA operons. Alternatively, stable RNA operons may have an antitermination system that makes the transcription complex resistant to transcription termination. Such a system has been studied extensively in the bacteriophage lambda, and we have cited work that suggests that there may be also an endogenous antitermination system(s) in *E. coli*.

Transcription from the *rrn* control region of pSU7 showed that the 16S rRNA sequences specified by that plasmid are resistant to Rho-dependent transcription termination. Transcription from the *ara* promoter in pGB8111 showed that the 16S rRNA sequences specified by that plasmid contain Rho-dependent transcription termination signals. Thus, the absence of Rho sites on the 16S rRNA transcript cannot be responsible for the lack of polarity in *rrn* operons. Of the rRNA sequences specified by pGB8111 and pSU7, 561 nucleotides are identical. These sequences from both plasmids should form similar secondary structures. We might also expect them to associate with ribosomal proteins to the same extent (if this portion of the 16S rRNA molecule is capable of doing so). So, it seems unlikely that secondary structure or ribosomal protein association is responsible for the difference in termination of pSU7 and pGB8111. Our results and the data of Morgan and co-workers (5, 26, 34) suggest that signals that function as strong terminators in other operons do not do so in *rrn* operons.

Our results show that sequences 5′ to position 80 of 16S are responsible for making pSU7 resistant to Rho-dependent termination. Siehnel and Morgan have also found that the *rrn* promoter region is necessary for efficient read-through of Tn*9* and IS*2* (34). These combined results suggest that the *rrn* control regions of *E. coli* can promote the formation of transcription complexes, like those made by the lambda p_L and p_R promoters that are resistant to certain kinds of termination signals. In lambda, antitermination requires the N protein and a *nut* site, with associated BoxA and BoxC (12) sequences, as well as factors that are specified by *E. coli*, called Nus proteins (14). NusA protein recognizes the BoxA sequence (13) and is necessary for N protein to function (14).

The analogy between antitermination in lambda and *rrn* or other stable RNA operons allows several predictions to be made (i) *E. coli* has an endogenous N-like antitermination protein that is necessary for the transcription of stable RNA operons. (ii) The control regions of stable RNA operons contain sequences in which the putative antitermination protein(s) act. (iii) Certain classes of terminators are not recognized by the antitermination-elongation complex.

The existence of an antitermination protein in *E. coli* has been suggested by several researchers. There is a terminator within the *rpsU dnaG rpoD* operon (7, 23) that responds to the lambda N protein (29), and it has been proposed that there is an endogenous *E. coli* antiterminator protein normally associated with expression in this operon (23). The *rplJL rpoBC* operon also contains an internal terminator (attenuator) (4), and it has been speculated that this terminator is responsive to an endogenous antitermination protein (16). Finally, Liebke and Speyer (22) have isolated a temperature-sensitive mutant of *E. coli* that does not synthesize stable RNA at nonpermissive temperatures. It maps outside any previously reported mutation known to affect stable RNA transcription. These authors state that the defect is in elongation rather than initiation of transcripts and suggest that the mutation may alter an antitermination protein required for the elongation of rRNA.

The six rRNA promoter regions that have been sequenced contain BoxA and BoxC sequences located between p_2 and the beginning of the 16S gene. It has been shown in vitro, that the NusA protein stimulates *rrn* transcription termination in the region just after BoxC. Kingston and Chamberlin have called this phenomenon "turnstile attenuation" (19). Possibly, NusA protein-dependent pausing and termination occurs because some protein factor (i.e., an endogenous N-like protein) is missing in their in vitro transcription system. It has been shown in bacteriophage lambda that NusA protein can either increase or decrease transcription termination and can also increase pausing at several sites in the lambda t_{R1} region (21). Although NusA protein may be involved in *rrn* antitermination, we now believe that the "turnstile attenuator" site after BoxC is not part of the antitermination system. Recent experiments in our laboratory have shown that the *rrnG* BoxA-BoxC region can confer antitermination properties on transcripts from *lac* as well as *rrnG* promoters. The NusA-interacting region after BoxC is not required for antitermination (S. Li, C. L. Squires, and C. Squires, manuscript in preparation).

Transcription of the 16S *trp-lac* sequences from the *rrn* promoter of pSU7 is not terminated significantly in *rho*$^+$ strains. When initiated from the *ara* promoter in pGB8111, 81% of these same transcripts are terminated in *rho*$^+$ strains. Our results show that there are Rho-dependent transcription terminators within both the *rrn* and *trp* gene sequences. Thus, *rrn* sequences do contain the features necessary for termination. There is little or no polarity in stable RNA transcription, and Rho-dependent transcription terminators that lie within the *rrn* and *trp* gene sequences are not recognized as terminators by the transcription complex initiated from the *rrnG* control region. Therefore, we conclude that there is an antitermination system in the *rrnG* control region that allows it to transcribe through Rho-dependent terminators. This antitermination system may be similar to the one in bacteriophage lambda.

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