Proc. Natl. Acad. Sci. USA
Vol. 90, pp. 9842–9846, November 1993
Genetics

Use of a gene encoding a suppressor tRNA as a reporter of transcription: Analyzing the action of the Nun protein of bacteriophage HK022

(transcription termination/RNA processing/reporter genes)

SIEGHILD BÖHMER SLOAN AND ROBERT A. WEISBERG

Laboratory of Molecular Genetics, National Institute of Child Health and Human Development, National Institutes of Health, Building 6B, Room 448, Bethesda, MD 20892

Communicated by Howard A. Nash, July 19, 1993 (received for review April 26, 1993)

ABSTRACT **The Nun protein of phage HK022 blocks the expression of genes that lie downstream of the *nut* sites of phage λ. Nun is believed to act by promoting premature termination of transcription at or near these sites. To test this hypothesis and to facilitate mapping the sites of termination, we inserted a gene encoding a suppressor tRNA immediately downstream of the λ *nut*L site and determined the effect of Nun on tRNA level. We found that Nun severely reduced the accumulation of mature, biologically active tRNA and promoted the accumulation of short, promoter-proximal transcripts whose 3′ ends were dispersed over a 100-nucleotide region downstream of *nut*L. These results are consistent with the hypothesis that Nun terminates transcription within the region immediately downstream of *nut*L and are inconsistent with the hypothesis that the only action of Nun is to prevent translation of genes located downstream of the *nut* site. The stability, small size, and easily assayable biological function of suppressor tRNA recommend it as a reporter of transcription in other systems.**

The Nun protein of bacteriophage HK022 prevents growth of the related phage λ by blocking the expression of essential genes that lie downstream of the λ *nut* sites (1). Several lines of evidence suggest that Nun acts by promoting premature termination of early λ transcription at or near the *nut* sites (1). First, Nun blocks expression of *lac* or *gal* reporter genes located downstream of the λ *nut*L or *nut*R sites. Nun has no effect in the absence of an upstream *nut* site. Second, Nun reduces the level of mRNA downstream but not upstream of the λ *nut*R site. Finally, the many similarities between the action of Nun and that of N, the λ antitermination factor, suggest that Nun, like N, acts at a transcriptional level. These similarities include common requirements for the phage λ *nut* sites and the host Nus factors (1–4).

The arguments that Nun directly promotes transcription termination, while strong, do not exclude alternative models. One model assumes that the primary action of Nun is to block translation of genes downstream of *nut* and that the absence of *nut*-distal transcripts is an indirect consequence of the absence of translating ribosomes. It is well established that untranslated mRNA is especially prone to transcription termination (5–7). A second model assumes that Nun recruits an endo- or exonuclease that begins to degrade the message at *nut* and acts processively with a net 5′ to 3′ polarity. Such models are tenable because our previous assays of Nun action relied on reporter genes whose transcripts are translated or on measurements of steady-state RNA level, which do not distinguish between synthesis and degradation. Therefore, to assay Nun action in a more direct way, we have used a gene encoding a small, stable, and biologically active RNA as a reporter of Nun activity. This method obviates some of the problems associated with the usual reporter genes.

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MATERIALS AND METHODS

Strains and Plasmids. A gene encoding suppressor $tRNA^{Gly}$ (*Escherichia coli* isoacceptor GLY1 with a triple mutation in the anticodon loop) inserted via *Eco*RI and *Pst* I linkers into plasmid pGF1B was kindly provided by W. McClain and K. Foss (Fig. 1) (8). The $tRNA^{Gly}$ region was amplified by the polymerase chain reaction using primers carrying *Hin*dIII restriction sites, and the resulting fragment was cut with *Hin*dIII and cloned into the *Hin*dIII-cut λ P_L-*lacZ* fusion plasmids pHA22 and pHA25 (1) to form pSB513 and pSB514, respectively. The insertions were verified by sequencing. The *bla*-P_L-$tRNA^{Gly}$-*lacZ* segments of the two plasmids were transferred by homologous recombination to phage B305 to form phages B387 (from pSB513) and B389 (from pSB514). B305 is a derivative of λ *imm21* that contains segments of the *bla* and *lacZ* genes inserted within the nonessential *b2* region, and the recombinant phages were selected by screening for blue plaques on plates containing the indicator 5-bromo-4-chloro-3-indolyl-β-D-galactoside as described (1). Lysogens of these phages in strain N5947 were constructed by selection for ampicillin resistance after infection. N5947 is deleted for *lacZ* and carries a defective λ prophage (λ *int2 xis1* Δ[*Sal-Xho*] *Nam7 Nam53 cIts857* ΔH1[*cro-uvrB*]) (1). These lysogens were then superinfected with HK022 *nun*::Tn*10*-A or HK022::Tn*10*-I *nun*$^+$ (1), and lysogens carrying these phages were isolated by selection for tetracycline resistance. Derivatives of these strains carrying plasmids pLUX1 (*luxAB*$^+$) or pLUX2 (*luxA*$^+$ *luxBam*) (9) were constructed by selection for chloramphenicol resistance after transformation. We also constructed derivatives of these strains in which the *N* amber mutations carried by the defective prophage were replaced with an *N*::kan disruption mutation through transduction with phage P1. The donor of the *N* disruption mutation was strain TAP106 obtained from T. Patterson (23).

Luciferase Measurements. Bacterial strains carrying active *lux* genes could be identified by their luminescence on closed agar plates under air saturated with *N*-decyl aldehyde (Sigma). For quantitative measurement of luciferase, cells growing exponentially at 32°C in L broth supplemented with chloramphenicol were assayed by the method of Schultz and Yarus (9), except that an LKB model 1251 luminometer was used to measure light emission. Peak values were recorded without integration. When appropriate, the cells were shifted to 42°C for 15 min before assay to allow derepression of the λ P_L operon.

RNA Preparation and Northern Analysis. Cells growing exponentially in L broth at 32°C were harvested with or without a 13-min induction period at 42°C. RNA was extracted by the hot SDS/phenol method of Sarmientos *et al.*

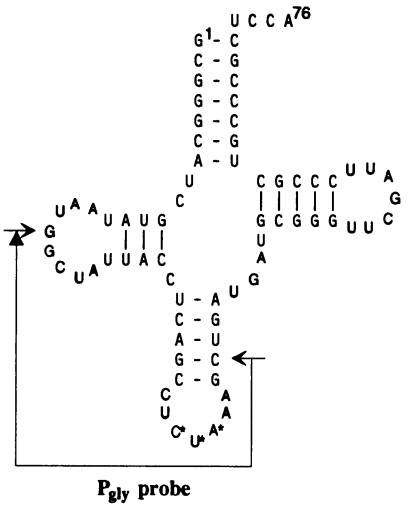

FIG. 1. Cloverleaf arrangement of the nucleotide sequence of the mature suppressor $tRNA^{Gly}$ used in this work. This sequence differs from the wild-type *E. coli* $tRNA^{Gly}$ (isoacceptor GLY1) by the substitutions U34C, C35U, and C36A in the anticodon loop (these positions are starred). The P_{gly} oligonucleotide probe is complementary to positions 40–18, as indicated by the arrows. The corresponding $tRNA^{Gly}$ DNA that was inserted into the P_L-*lacZ* fusions differed from the RNA sequence shown here by the addition of short oligonucleotides containing *Hin*dIII restriction sites at each end.

(11) and resuspended in water to a final concentration of 20–40 $\mu g/\mu l$. Molecular size standards were prepared by transcribing a partial *Hin*fI digest of pGEM-4 plasmid DNA (Promega) with SP6 RNA polymerase in the presence of [α-^{32}P]GTP and by treating DNA molecular weight markers (Boehringer Mannheim) with kinase in the presence of [γ-^{32}P]ATP. The RNA was fractionated by electrophoresis on 6% polyacrylamide/urea sequencing gels, electroblotted onto nylon membranes, and fixed by ultraviolet irradiation. Radioactive probes were prepared by treating 24-base synthetic oligonucleotides with polynucleotide kinase in the presence of [γ-^{32}P]ATP to a specific activity of 10^8–10^9 cpm/μg. Probe P_{pL} is complementary to bases 3–26 of the λ P_L transcript, probe P_{gly} is complementary to bases 17–40 of $tRNA^{Gly}$ (Fig. 1), and probe $P_{4.5S}$ is complementary to bases 40–63 in the conserved loop of *E. coli* 4.5S RNA. Hybridizations were carried out overnight at 37°C in 50% (vol/vol) formamide, and the membranes were processed essentially as described (12). Quantitative estimates of radioactivity were made with an AMBIS Systems radioanalytic imager.

RESULTS

Construction of Fusions. We have previously described a set of plasmids containing fusions of a promoterless *lacZ* gene to various sites in the phage λ P_L operon (1). We modified two of these plasmids by inserting a 93-bp fragment containing a promoterless suppressor $tRNA^{Gly}$ gene into a unique restriction site located at the fusion joint between λ and *lacZ* sequences (see *Materials and Methods*). The insertion point is within the *boxB* sequence of *nut*L in the Δ*nut* fusion carried by pSB514 (immediately after position 53 of the P_L transcript) and is 11 bp downstream of the end of *boxB* in the *nut*$^+$ fusion carried by pSB513 (immediately after position 75 of the P_L transcript; Fig. 2*A*). The $tRNA^{Gly}$ gene carries a 3-base substitution mutation in the anticodon that causes insertion of glycine or glutamine in response to the amber codon UAG, thereby providing a simple biological assay of tRNA abundance by amber suppression (8). In addition, the mutation allowed synthesis of an oligonucleotide probe that

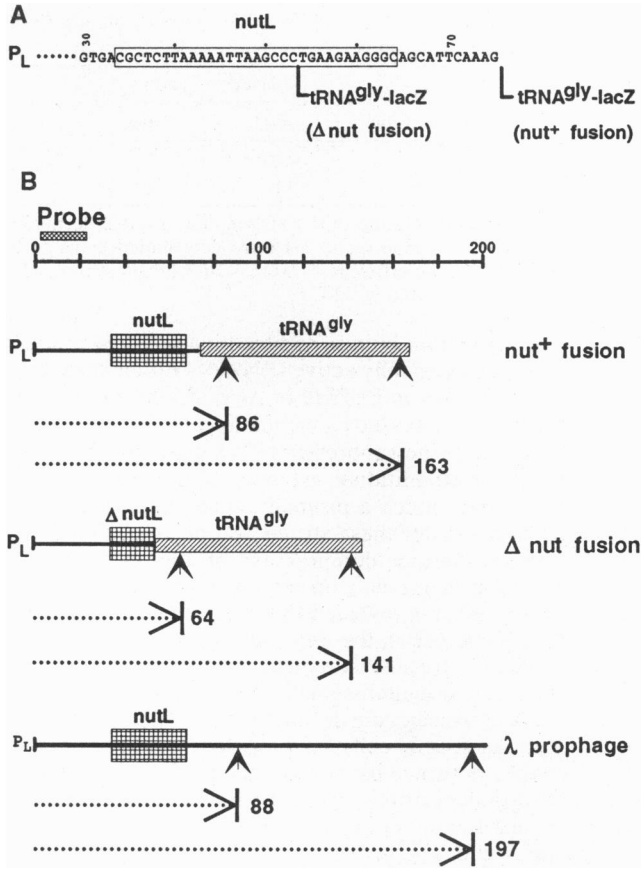

FIG. 2. (*A*) Structure of P_L-$tRNA^{Gly}$ fusions. The sequence of the λ P_L transcript from position 30 to position 75 is shown. The λ *nut*L site is boxed. The *boxB* segment of *nut*L includes positions 50–64. The Δ*nut* and *nut*$^+$ fusions substitute $tRNA^{Gly}$-*lacZ* sequences for λ sequences downstream of positions 53 and 75, respectively (see text). (*B*) Possible promoter-proximal P_L transcripts. The upper scale is the distance in nucleotides from the start of the P_L transcript. The promoter-proximal probe is indicated by the cross-hatched rectangle, the *nut*L site is indicated by the checkered rectangle, and the $tRNA^{Gly}$ gene in the *nut*$^+$ and Δ*nut* fusions is indicated by the hatched rectangles. The vertical arrowheads indicate the locations of known processing sites in the pre-$tRNA^{Gly}$ and λ P_L transcripts (see text). The dotted horizontal arrows indicate possible transcripts that extend from the P_L start site to the processing sites, and the numbers following the arrows give their predicted sizes in nucleotides.

hybridized specifically to the suppressor $tRNA^{Gly}$ (Fig. 1; see below).

To verify that insertion of the tRNA gene did not alter the effect of Nun on expression of *lacZ*, we integrated the fusions into the chromosomes of cells carrying a defective prophage that provides thermosensitive λ repressor. We also lysogenized these cells with HK022, to provide Nun, or HK022 *nun*::Tn*10*, a *nun* disruption mutant. We found that Nun reduced expression of *lac* in the *nut*L$^+$ fusion but had no perceptible effect in the Δ*nut*L fusion: colonies of the *nun*$^+$ *nut*L$^+$ strain remained white on MacConkey-lactose agar following thermal derepression, whereas colonies of the *nun*$^-$ *nut*L$^+$ fusion and both Δ*nut* fusions turned red. These results are identical to those obtained with P_L-*lacZ* fusions that lack the $tRNA^{Gly}$ gene (1), and we conclude that insertion of the $tRNA^{Gly}$ gene upstream of *lacZ* does not appreciably alter the Nun sensitivity of *lacZ* expression.

Assay of $tRNA^{Gly}$ Level by Amber Suppression. We first determined the efficiency of plating of T4 phage carrying amber mutations in various vital genes. We found that Nun inhibited suppression of the amber mutations when the suppressor tRNA gene was carried by the *nut*$^+$ fusion (Table

Table 1. Efficiency of plating of T4 amber mutants on strains carrying P_L-tRNA^{Gly} fusions

	tRNA^{Gly} fusion	
nun genotype	*nut*$^+$	Δ*nut*
nun$^+$	$<10^{-4}$	1
nun::Tn*10*	1	1

The efficiencies of plating of T4 amber mutants B25 (gene *34*), NO22 (gene *48*), and H36 (gene *23*) were determined using plates incubated at 42°C. The efficiencies were $<10^{-4}$ for all strains when the plates were incubated at 32°C.

1). This suggests that Nun in combination with *nut* prevents production of biologically active tRNA^{Gly}. Efficient suppression occurred in the *nun*::Tn*10* or Δ*nut* strains when the P_L promoter was derepressed by incubation at 42°C, as expected if transcription of the suppressor tRNA gene initiates at P_L.

To obtain a quantitative estimate of suppressor tRNA activity, we introduced a plasmid containing a *luxB* amber mutation into each of these strains and measured luciferase activity after thermal derepression of the P_L promoter. Inspection of cells growing on solid medium and exposed to a luciferase cofactor revealed that Nun depressed the production of luciferase in the *nut*$^+$ but not in the Δ*nut* fusion (Fig. 3). Quantitative measurement of light emission showed that Nun reduced luciferase activity to about 5–10% of the *nun*$^-$ level in cells carrying the *nut*$^+$ fusion (Table 2). No such reduction was seen in cells carrying the Δ*nut* fusion. These results were confirmed by using a second strain in which the defective prophage providing the thermosensitive λ repressor carried a disruption mutation instead of amber mutations in the phage *N* gene (Table 2). Therefore suppression of the *N* amber mutations, which could occur in the *nun*$^-$ hosts because they synthesize the suppressor tRNA, does not measurably change tRNA^{Gly} transcription and hence suppressor activity as measured by the luciferase level. We note that Nun inhibition of suppressor tRNA activity could be greater than the 90–95% estimated from the data of Table 2 since light emission by the *nun*$^+$ *nut*$^+$ strain was indistinguishable from that by a strain lacking the suppressor tRNA gene. We conclude that little or no biologically active suppressor tRNA^{Gly} accumulates in the *nun*$^+$ *nut*$^+$ strain.

Assay of tRNA^{Gly} Level by Northern Hybridization. RNA was extracted from uninduced and heat-induced cultures of the fusion strains, fractionated by gel electrophoresis, and hybridized to a mixture of two labeled oligonucleotide probes, one complementary to a segment of tRNA^{Gly} (probe P_{gly}; Fig. 1) and a second complementary to a segment of 4.5S RNA, a stable *E. coli* RNA (probe $P_{4.5S}$) (13, 14). We assume that the level of 4.5S RNA is invariant in these conditions. We found that transfer of *nun*$^-$ cells to 42°C for 13 min induced

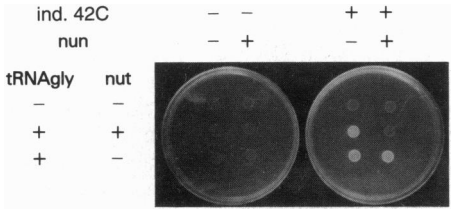

FIG. 3. Detection of suppression by light emission. Cultures were spotted on broth plates and grown 4 hr at 32°C. The plate at the right was then incubated at 42°C for 1 hr to derepress the P_L promoter. Both plates were exposed to *N*-decyl aldehyde vapor and photographed in a darkroom. The strains were N5947/pLUX2 lysogenic for two prophages: (*i*) HK022 *nun*$^+$ or HK022 *nun*::Tn*10* and (*ii*) B387 (Δ*nut* tRNA^{Gly} fusion), B389 (*nut*$^+$ tRNA^{Gly} fusion), or a prophage isogenic to B387 but lacking the tRNA^{Gly} gene. The *nun*, *nut*, and tRNA^{Gly} genotypes of the prophages are indicated in the column and row headings.

Table 2. Quantitative assay of inhibition of suppression by Nun

	tRNA^{Gly} fusion	
nun genotype	*nut*$^+$	Δ*nut*
nun$^+$	3 (7)	60
nun$^-$	23 (64)	45
nun$^+$/*nun*$^-$	0.13	1.3

The first and second rows report light emitted (in arbitrary units) by exponentially growing N5947/pLUX2 lysogens after induction for 15 min at 42°C. The *nun* genotype of the HK022 prophage and the *nut* genotype of the fusion prophage are indicated. The numbers in parentheses are the results of assays of strains that carry an *N*::kan disruption mutation instead of *N* amber mutations (see text). The third row reports the quotient of the first two rows. These strains emitted from 3 to 5 units if they were not thermally induced, from 3 to 6 units if they lacked the tRNA^{Gly} fusion, and from 115 to 376 units if they carried a plasmid with the wild-type *lux* genes.

the accumulation of RNA molecules with the size and hybridization properties expected for tRNA^{Gly} (Fig. 4). We assume that the ends of these molecules are generated by the same nucleases used in normal tRNA processing (15, 16). Accumulation was inhibited by Nun in the *nut*$^+$ but not in the Δ*nut* fusion. Quantitative scanning of the gels and normalization of the observed intensity of hybridization to that found for 4.5S RNA in the same extract revealed that Nun, in the presence of a functional *nut*L site, reduced accumulation of mature tRNA^{Gly} about 10-fold relative to 4.5S RNA (Table 3). No unprocessed tRNA^{Gly} precursor is visible in the autoradiogram of Fig. 4, but bands corresponding to unprocessed transcripts can be seen in longer exposures of membranes probed with a promoter-proximal probe (below). The results in Table 3 show that Nun strongly inhibits production of mature tRNA^{Gly} and are quantitatively consistent with our measurements of tRNA^{Gly} activity.

Transcripts Produced in the Presence of Nun. To determine the effect of Nun on the size of the P_L transcript, we used a probe complementary to a short, promoter-proximal segment of the message (probe P_{pL}). Lanes 1–4 of Fig. 5 display hybridizable RNA extracted from cells containing a P_L-*nut*L segment from a defective λ *cIts857* prophage (λ *int2 xis1* Δ[*Sal-Xho*] *Nam7 Nam53 cIts857* ΔH1[*cro-uvrB*]) but no P_L-tRNA^{Gly} fusion. We found that heat-induced cells that lacked active Nun protein contained a major P_L transcript about 200 nt long (Fig. 5, lane 2). Nun strongly decreased the level of this transcript and promoted formation of a large

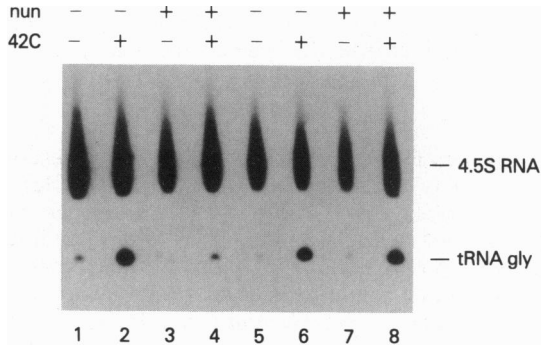

FIG. 4. Northern blot of RNA hybridized to a mixture of two labeled oligonucleotides, one complementary to suppressor tRNA^{Gly} (probe P_{gly}) and the other to *E. coli* 4.5S RNA (probe $P_{4.5S}$). RNA was extracted from N5947 lysogens containing a *nut*$^+$ (lanes 1–4) or Δ*nut* (lanes 5–8) P_L-tRNA^{Gly} fusion prophage and a *nun*$^+$ or *nun*$^-$ HK022 prophage, as indicated, and fractionated on a denaturing polyacrylamide gel. The cells were grown at 32°C or shifted to 42°C for 13 min, as indicated. Cells lacking the suppressor tRNA^{Gly} gene contained no hybridizable RNA with the mobility of the lower band (data not shown).

Table 3. Inhibition by Nun of the accumulation of mature $tRNA^{Gly}$

	$tRNA^{Gly}$ fusion	
nun genotype	*nut*$^+$	Δ*nut*
nun$^+$	46	363
nun$^-$	371	299
nut$^+$/*nun*$^-$	0.12	1.2

Transcription from the P_L promoter was derepressed by transfer of the cultures to 42°C for 13 min, and the level of $tRNA^{Gly}$ and 4.5S RNA was determined by Northern blotting and scanning as described in *Materials and Methods*. The first two rows report the level of $tRNA^{Gly}$ normalized to the amount of 4.5S RNA in the same gel lane in arbitrary units. The numbers are the average of three experiments. An autoradiogram of one of the gels used to collect these data is shown in Fig. 3. The third row reports the quotient of the first two. The relative level of $tRNA^{Gly}$ in repressed cells was 32 to 47.

number of new RNA species (at least 14), each present in low amounts, with lengths between 60 and 200 nt (Fig. 5, lane 4). We will argue that the Nun-dependent transcripts probably result from transcription termination (see *Discussion*). None of these transcripts was found in uninduced cells (lanes 1 and 3), as expected if they originate at the λ P_L promoter.

The 3′ end of the Nun-sensitive transcript is probably produced by RNase III, since this endonuclease is known to cleave the λ P_L message at position 197 (Fig. 2*B*) (17, 18), and we did not see a transcript of this size in a mutant that lacks active enzyme (data not shown). This RNA should be relatively resistant to degradation by 3′ to 5′ exonucleases because nucleotides at the 3′ end can pair with nucleotides further upstream (18). RNase III also cleaves the λ P_L transcript at position 88 (Fig. 2*B*) (10, 17, 18). The promoter-proximal product of this cleavage is known to be extremely

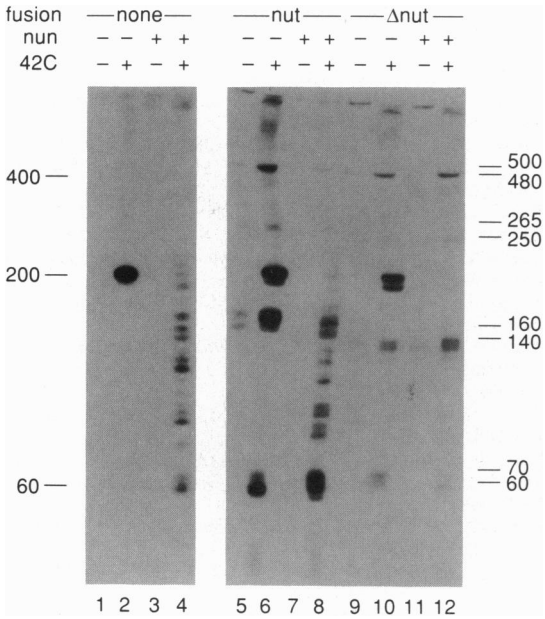

FIG. 5. Northern blot of RNA hybridized to a labeled oligonucleotide complementary to the 5′ end of the λ P_L transcript (probe P_{pL}). RNA was extracted from N5947 lysogens that had been grown at 32°C or shifted to 42°C for 13 min, as indicated, and fractionated on a denaturing polyacrylamide gel. The cells contained *nun*$^+$ or *nun*::Tn*10* HK022 prophage, as indicated. The cells used in lanes 1–4 contained a defective λ *cIts857* prophage (see text), and those used in lanes 5–12 contained in addition a *nut*$^+$ or Δ*nut* P_L-$tRNA^{Gly}$ fusion prophage, as indicated. Equal amounts of RNA, as judged by OD_{260}, were applied to each lane except for lanes 2 and 3, which received one-third the amount. Different preparations of labeled probe and different exposure times were used for the two gels shown.

unstable (19), which can account for our failure to detect it, and the more stable promoter-distal product does not hybridize to our probe.

Lanes 5–12 of Fig. 5 display hybridizable RNA extracted from cells containing a P_L-$tRNA^{Gly}$ fusion in addition to the P_L-*nut*L segment of the defective prophage. We found that the *nut*L$^+$ fusion generated a major new transcript of about 160 nt and a less abundant transcript of 60–65 nt (Fig. 5, lane 6). [The shorter transcript can also be seen in cells lacking the fusion, but in lower levels (data not shown).] Nun reduced the level of the major transcript and promoted formation of a heterogeneous collection of transcripts with sizes in the range of 65–200 bases (Fig. 5, lane 8). Some of these Nun-dependent fragments differed in size from those seen in the *nun*$^+$ strain lacking the P_L-$tRNA^{Gly}$ fusion (Fig. 5, lane 4). The major new RNA species in cells containing the Δ*nut* fusion was about 140 bases long and was insensitive to Nun (Fig. 5, lanes 10 and 12). The 200-nt transcript that is transcribed from the P_L-*nut*L segment of the defective prophage can be seen in cells lacking Nun (lane 10) but not in cells that contain it (lane 12), which provides additional evidence that Nun is still active in these cells. It is likely that the 160- and 140-base transcripts seen in the *nut*$^+$ and Δ*nut* fusions, respectively, are intermediates or by-products of tRNA maturation because their sizes are close to those expected from cleavage at the 3′ pre-tRNA processing sites of the two P_L transcripts (Fig. 2*B*). Interestingly, the comparable fragments produced by cleavage at the 5′ tRNA processing sites, which would be 64 bases long for the Δ*nut* fusion and 86 bases long for the *nut*$^+$ fusion (Fig. 2*B*), are less abundant or absent, and we suggest that these fragments are rapidly degraded by 3′ to 5′ exonuclease action. The 60- to 65-nt transcript noted above may be an intermediate in such degradation since its 3′ end is located at or near the base of a stem–loop formed by the promoter-distal end of the *nut*L transcript (18). The longer fragments produced by cleavage at the 3′ processing site probably resist such exonucleolytic degradation because of the protective effect of base pairing of the tRNA sequence. There were several minor large transcripts in the cells containing a $tRNA^{Gly}$ fusion that were absent in strains without one. We are unable to identify these transcripts with certainty, but some are clearly encoded by the fusion, as revealed by changes in their sizes between the *nut*$^+$ and Δ*nut* fusions.

DISCUSSION

We found that phage HK022 Nun protein prevented the accumulation of mature, biologically active $tRNA^{Gly}$ in cells containing a $tRNA^{Gly}$ reporter gene fused immediately downstream of the λ *nut*L site. This shows that Nun can act without blocking translation and therefore that translation inhibition cannot be the sole mechanism of Nun action. Nun promoted the accumulation of short, promoter-proximal transcripts whose 3′ ends were distributed over a 100-bp region downstream of *nut*L. This result is consistent with two models of Nun action: (*i*) transcription termination that begins shortly downstream of *nut* and is largely complete within 100 bp or (*ii*) transcript degradation that begins shortly downstream of *nut*. Several arguments favor model (*i*). First, to explain Nun blockage of $tRNA^{Gly}$ accumulation by degradation, we would have to assume that degradation is so rapid as to preclude tRNA maturation. Second, the requirements for Nun action largely overlap with those for λ N, a well-characterized transcription antitermination protein (1, 3, 4). Third, Nun action is directional and distance independent (1). To explain this, the degradation model requires an additional assumption—for example, that the nucleolytic activity becomes a stable part of the transcription complex and continues to cleave the RNA as it travels along the

template. We note that E. Hung and M. Gottesman (personal communication) have recently obtained evidence that Nun prevents transcript elongation in a multiprotein *in vitro* transcription system.

It is often difficult to decide if a change in the level of a gene product *in vivo* is the direct result of a change in transcription initiation, transcription termination, mRNA stability, or translation. This difficulty arises because of our incomplete understanding of the factors and sites governing message stability (20–25) and because of the coupling between transcription termination and translation in prokaryotes (5, 6, 26, 27). Conclusions based on *in vitro* studies may be misleading if appropriate mutants are unavailable or if some of the relevant factors are unknown. A reporter gene that encodes a suppressor tRNA has some advantages over other reporter genes in deciding among these alternatives. First and foremost, the gene product is stable once it has been cleaved from the primary transcript. Such stability is a consequence of the highly folded secondary structure of mature tRNA, and it is likely that partial folding of immature or partially synthesized tRNA also impedes degradation. We anticipate that a tRNA reporter will be less responsive than standard reporters to sites that alter transcript stability (28, 29), although this prediction has not yet been tested. If so, tRNA reporters will complement standard reporters and direct measurements of RNA abundance as a means of identifying and mapping transcription termination and transcript processing sites in poorly characterized transcripts. Second, the final gene product can be detected without translation through the use of an appropriate oligonucleotide probe or with translation through nonsense suppression. In the latter case, the gene containing the nonsense mutation can be placed on a separate replicon from the transcription unit whose expression is under investigation. The data reported in this article confirm that the two kinds of measurement give concordant reports of transcription. Third, the small size of the tRNA reporter facilitates the construction of fusions to different points within a gene of interest and therefore the identification of both 5′ and 3′ control sites.

If in fact Nun promotes transcription termination rather than transcript degradation, then the multiplicity of Nun-dependent transcripts we observe could be a consequence either of multiple sites of termination or of partial 3′ to 5′ exonucleolytic degradation of a single terminated transcript. Our ability to detect significant steady-state levels of short, Nun-dependent transcripts in wild-type cells already suggests that these transcripts are unexpectedly stable. If they were as unstable as the promoter-proximal fragments produced by RNase III cleavage at position 88 of the wild-type P_L message or by RNase P cleavage at the 5′-end of $tRNA^{Gly}$ in the P_L-*nut*L-$tRNA^{Gly}$ fusion message, it is unlikely that they would have been detected by our techniques. It is tempting to speculate that Nun binding to the terminated transcripts partially protects them from degradation.

We found that a promoter-proximal probe detects roughly similar numbers of transcripts in the presence and absence of Nun (Fig. 5). This observation suggests that Nun does not act by arresting the transcription complex downstream of *nut*, since such arrested complexes will retain the association between template, polymerase, and nascent mRNA and should therefore sterically block subsequent rounds of transcription. This conclusion is supported by studies of transcription in the λ right operon, which showed that Nun did not prevent synthesis of either the P_R-*nut*R transcript or Cro, the protein encoded by this transcript (1). However, the conclusion rests on the assumption that transcripts made in the presence of Nun are not much more stable than those produced in its absence, since such a difference will lead to an overestimate of transcription in the presence of Nun.

If the multiplicity of the Nun-dependent 3′ ends we have detected is not the result of partial degradation, there must be multiple sites of termination. We have previously suggested that Nun, like λ N protein, binds to nascent *nut* mRNA and forms a multiprotein complex that includes Nus factors and RNA polymerase (1–4). The N antitermination complex changes polymerase to a terminator-resistant form [reviewed by Das (30)]. We argue that substitution of Nun for N in the complex sensitizes polymerase to nucleotide sequences that would not normally cause termination. Perhaps Nun actively promotes disassociation of the nascent RNA chain from the template strand in a manner similar to that proposed for the rho termination protein (31), and termination occurs at sequences that slow elongation and thereby facilitate complete disassociation (see ref. 32). Alternatively, Nun might change polymerase to a form that is more responsive to weak rho-independent terminators (see ref. 33).

We are grateful to Asis Das for suggesting the use of a gene encoding a stable RNA, to Donald Court and Max Gottesman for their comments on the manuscript, and to William McClain and K. Foss for their advice.

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