JOURNAL OF BACTERIOLOGY, Dec. 2003, p. 7085–7091 Vol. 185, No. 24
0021-9193/03/$08.00+0 DOI: 10.1128/JB.185.24.7085–7091.2003

# Suppression of Factor-Dependent Transcription Termination by Antiterminator RNA

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Received 13 June 2003/Accepted 22 September 2003

**Nascent transcripts of the phage HK022 *put* sites modify the transcription elongation complex so that it terminates less efficiently at intrinsic transcription terminators and accelerates through pause sites. We show here that the modification also suppresses termination in vivo at two factor-dependent terminators, one that depends on the bacterial Rho protein and a second that depends on the HK022-encoded Nun protein. Suppression was efficient when the termination factors were present at physiological levels, but an increase in the intracellular concentration of Nun increased termination both in the presence and absence of *put*. *put*-mediated antitermination thus shows no apparent terminator specificity, suggesting that *put* inhibits a step that is common to termination at the different types of terminator.**

After initiating RNA synthesis, RNA polymerase (RNAP) continues to elongate the transcript until it reaches a termination site. At such sites, the enzyme has a high probability of dissociating from the transcript and the template (reviewed in references 26 and 32). Bacteria have two basic types of transcription termination signals, which differ in their requirements for halting elongation. Intrinsic terminators can stop transcription through the action of the nascent transcript. Formation of an RNA stem-loop immediately upstream of a U-rich stretch in nascent RNA disrupts RNA-DNA base pairs within the transcription elongation complex, and this destabilizes the complex (15, 20, 47). By contrast, factor-dependent terminators recruit a termination factor to the nascent transcript. Two termination factors have been well characterized: the bacterial Rho protein and the bacteriophage-encoded Nun protein. After binding to nascent transcripts, they both act on the nearby elongation complex. Rho has an ATP-driven RNA-DNA helicase activity, which is thought to destabilize the elongation complex (7, 30). Nun is transferred from its RNA binding site to the elongation complex, where it is thought to anchor RNAP to the DNA template within a few hundred nucleotides downstream of the binding site (16, 39, 43). Dissociation of Nun-arrested polymerase from the template and the transcript has not been observed in vitro and appears to require an additional factor or factors. Recent evidence suggests that the *Escherichia coli* Mfd protein can stimulate the dissociation of Nun-arrested complexes (42).

*E. coli* and its bacteriophages alter the efficiency of transcription termination in order to control the expression of genes located downstream of terminators (reviewed in reference 44). For example, the phage λ antitermination proteins N and Q modify RNAP so that it reads through intrinsic and rho-dependent terminators. Both N and Q recognize specific phage sequences (*nut* and *qut*, respectively) before they modify polymerase, and this limits antitermination to RNAP molecules that are transcribing phage DNA. Elongating RNAP that has been modified by interaction with either protein retains the modification as it translocates, as shown by its ability to read through multiple, sequential terminators. The action of both proteins is enhanced by host-encoded factors. Transcription of the rRNA operons in *E. coli* is also subject to antitermination control and, as in the case of λ N and Q, *cis*-acting sequences (*boxA* sites) located near rRNA promoters limit antitermination to polymerase molecules that are transcribing rRNA operons (6, 11). Ribosomal antitermination also requires *trans*-acting factors (41, 48).

Bacteriophage HK022 is a relative of λ that also antiterminates transcription in order to increase the expression of genes located downstream of termination sites (reviewed in reference 45). However, in contrast to the factor-dependent antitermination mechanisms outlined above, transcription of *cis*-acting, promoter-proximal phage sequences (*put* sites) is sufficient to convert RNAP into a termination-resistant form; no dedicated factors are absolutely required. We refer to this as intrinsic antitermination. *put* differs in sequence from the *nut*, *qut*, and ribosomal operon *boxA* sites. Computer modeling and enzymatic probing of RNAs synthesized in vitro suggest that the *put* transcripts fold into two stem-loops separated by an unpaired base (4). The stems are required for *put* function, since mutations that prevent base pairing reduce antitermination, and additional mutations that reestablish base pairing but not the original sequence restore antitermination (18). Nascent *put* RNA binds to the transcription elongation complex and remains associated with it through subsequent translocation. Stable binding is required for antitermination (35).

The distinction between intrinsic and factor-dependent antitermination is highlighted by the following observations. First, *E. coli* mutants that are defective in *put*-mediated antitermination supported N-mediated, Q-mediated, and ribosomal operon antitermination (10). Conversely, host mutants that are defective in λ N-mediated antitermination supported

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TABLE 1. Bacteria, plasmids, and phage strains[a]

| Strain | Relevant genotype and/or reference |
|---|---|
| **Bacteria** | |
| TAP114 | W3110 ΔM15[*lacZ*] *lacI*$^q$; T. Patterson and D. Court, unpublished |
| RW3926 | TAP114 *rpoC-Y75N btuB*::*Tn10*(*kan*) |
| MC1000 | 37 |
| MOC23 | MC1000 *rpoC-Y75*N; reference 10 |
| **Plasmids** | |
| pJT6 | $P_{TAC}$-*putL*-λ$T_{R1}$-*lacZ* fusion in pRS415 (Amp$^r$); contains HK022 sequence from +2 to +174 relative to start of $P_L$ transcription |
| pML042 | pGB2ts (Cm$^r$); M. Lobocka |
| pMOC170 | Expresses HK022 cI repressor (Spc$^r$); reference 9 |
| pNUN | $P_{Lac}$-*nun* (Cm$^r$); reference 5 |
| pNUNΔ | $P_{Lac}$ (Cm$^r$); reference 5 |
| pNL150 | $P_{TAC}$ *psu*$^+$, Cm$^r$, in pGZ119EH; reference 23 |
| pNL151 | $P_{TAC}$ Δ*psu*, Cm$^r$, in pGZ119EH; reference 23 |
| pRAK31 | A pRS415 derivative that contains HK022 sequences from −144 to +150 relative to the start of $P_L$ transcription |
| pRAK122 | $P_{TAC}$-*putL-lacZ* fusion in pRS415; contains HK022 sequence from +2 to +174 relative to start of $P_L$ transcription |
| pRAK161 | $P_{TAC}$-Δ*putL-lacZ* fusion in pRS415; contains HK022 sequence from +2 to +21 relative to the start of $P_L$ transcription |
| pRAK166 | $P_{TAC}$-Δ*putL*-λ$T_{R1}$-*lacZ* fusion in pRS415; pRAK161 with a phage λ DNA segment from bp 38042 to 38360 inserted between the HK022 sequences and *lacZ* |
| pRAK262 | HK022 cI inserted into pML042 |
| pRAK292 | $P_{TAC}$-*putL*$^{LS}$-λ$T_{R1}$-*lacZ* fusion in pRS415; contains HK022 sequence from +2 to +174 relative to start of $P_L$ transcription; the sequence contains linker scanning mutation G in *putL* (18) |
| pRAK296 | $P_L$(HK022)-*putL*-λ *nutL-lacZ* fusion in pRS415; the λ *nutL* site (bp 35506 to 35558) was inserted into pRAK31 between *putL* and *lacZ* |
| pRAK381 | $P_L$(HK022)-*putL*-λ *nutR*-$T_{R1}$-*lacZ* fusion in pRS415; the λ *nutR* site (bp 38241 to 38292) was inserted into pRAK31 between *putL* and *lacZ* |
| pRS415 | Reference 38; *lacZ* transcriptional fusion vector; confers ampicillin resistance |
| pSB513 | λ *nutL* clone; reference 39 |
| **Phage** | |
| λ RS88 | 38 |

[a] Many derivatives of TAP114 and RW3926 are not listed.

the growth of HK022 (3). The two types of mutations changed different host proteins: those defective in HK022 antitermination altered the β′-subunit of RNAP, and those defective in λ antitermination altered the *E. coli* Nus proteins or the β-subunit of RNAP (13, 24). Finally, purified wild-type polymerase efficiently read through multiple sequential intrinsic terminators that were fused to a wild-type *put* site. Efficient readthrough did not require additional protein factors but was prevented by a β′ mutation that is defective for antitermination in vivo (10, 18).

The different factor and site requirements of the antitermination systems cited above could influence the spectrum of terminators that each is capable of suppressing. The N/*nut* and Q/*qut* pathways prevent termination at both intrinsic and Rho-dependent terminators, suggesting that they interfere with a step that is common to both types. The ribosomal *boxA* pathway promotes efficient readthrough of Rho-dependent terminators but is ineffective or less effective against intrinsic terminators (1). The *put* pathway is known to suppress several intrinsic terminators. Here we show that *put* also promotes readthrough of three factor-dependent terminators, one that requires Rho (λ $T_{R1}$) and two that require Nun (λ *nutL* and λ *nutR*).

## MATERIALS AND METHODS

**Bacteria, phages, and plasmids.** The strains, phages, and plasmids used in this study are listed in Table 1.

**Bacterial growth, media, biochemicals, and antibiotics.** Cell cultures were grown in Luria-Bertani (LB) or tryptone broth (TB) (25). Antibiotics were added (when required) at the following concentrations: ampicillin, 100 μg/ml; kanamycin, 50 μg/ml; chloramphenicol, 30 μg/ml; spectinomycin, 25 μg/ml. Fusions that contained the $P_{TAC}$ or $P_{Lac}$ promoters were induced with isopropyl-β-D-thiogalactopyranoside (IPTG; 1 mM final concentration; purchased from Gold Biotechnologies). *o*-Nitrophenyl-β-D-galactopyranoside was purchased from Sigma. Restriction enzymes, Klenow, and ligase were purchased from New England Biolabs. Bacteriophage were grown and assayed as described previously (2). Oligonucleotides were purchased from BioServe (Laurel, Md.).

**Cloning of the λ$T_{R1}$ terminator.** A fragment containing the λ$T_{R1}$ terminator was amplified by PCR from λ DNA using oligos RK76 (5′-CATCGGATCCTG GAACAACGCATAACCC-3′) and RK78 (5′-TGCAGGATCCCTATGTAAG TATTTCC-3′). RK78 primes downstream of the λ$T_{R1}$ near the cII translation start site. RK76 primes at the beginning of the cro coding sequence and changes the initiating codon to prevent cro translation. The incorporated *Bam*HI restriction sites used for cloning are underlined. The amplified fragment was digested with *Bam*HI and cloned into the reporter constructs shown in Table 1. All fusions made in this study were sequenced at the University of Maryland Biopolymer Laboratory. All fusions that contain the cro-TR1 region of lambda are signified by a $T_{R1}$ notation.

**Cloning the λ *nutL* and *nutR* sites.** The λ *nutL* site was amplified from pSB513 with primers RK88 (5′-CAGCGAATTCTGAAGGTGACGCTCTTAAAAAT T-3′) and SBS59 (5′-CGCCGGAGATCTCTGCAGTGGAGCGGGCAGCGG G-3′). The incorporated *Eco*RI site in RK88 is underlined. The purified PCR

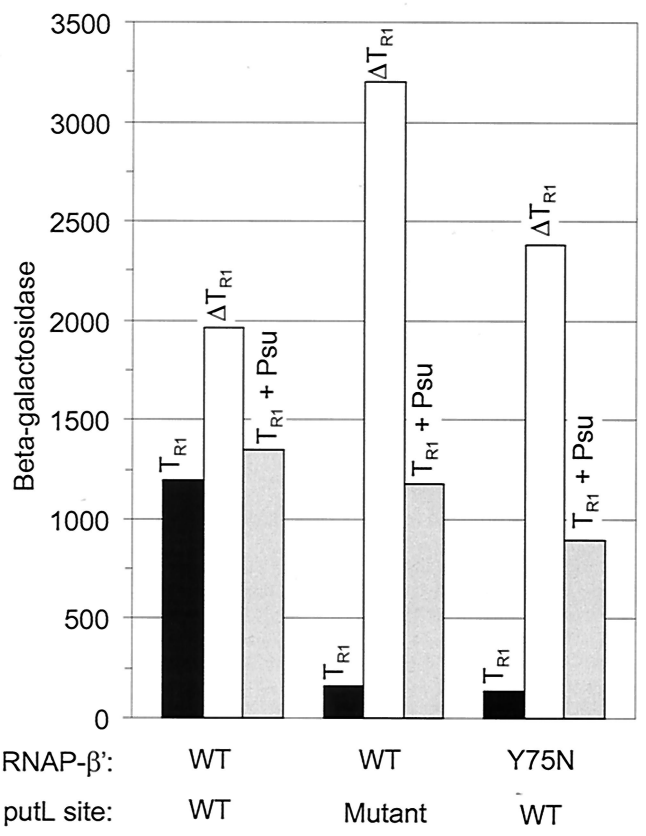


FIG. 1. *put*-mediated antitermination of the Rho-dependent terminator, $\lambda T_{R1}$. The strains were *rpoC*$^+$ or *rpoC-Y75N* derivatives of TAP114 and contained single copies of a $P_{TAC}$-*putL*-$T_{R1}$-*lacZ* or a $P_{TAC}$-*putL*-*lacZ* fusion, as indicated. The mutant *putL* site has a multiple base substitution that prevents antitermination of Rho-independent terminators (linker scanning mutation G [18]). β-Galactosidase activities (in arbitrary units) were measured 1 h after addition of IPTG to growing cultures and are the means of at least four independent assays. The open bars ($\Delta T_{R1}$) report the activities from fusions lacking $T_{R1}$, the shaded bars ($T_{R1}$ plus Psu) report the activities from those carrying $T_{R1}$ in cells that contained a plasmid with a $P_{TAC}$-*psu* fusion (pNL150), and the filled bars ($T_{R1}$) report the activities from fusions containing $T_{R1}$ in cells that contained a $P_{TAC}$-Δ*psu* fusion (pNL151). The standard error of the mean was less than 20%.

products were digested with *Eco*RI, and the ends of the resulting 64-bp *nutL*-containing fragment were filled with Klenow and cloned into the *Sma*I site of pRAK31 (18).

The λ *nutR* sequence was amplified from plasmid pJT6 with RK95 (5-TACG GATATCAATAACCCCGCTCTTAC-3′) and RK96 (5′-GCTGGATCCGTTT AATTTGATGCCCTTTTTC-3′). The *Eco*RV and *Bam*HI sites used for the cloning are underlined. The purified products were digested with *Eco*RV and *Bam*HI and cloned into *Sma*I-*Bam*HI-digested pRAK31. All fusions that contain only the nutR-TR1 region are signified by a *nutR*-$T_{R1}$ notation.

**Crossing *lacZ* fusions onto λ.** The *lacZ* fusions in pRS415 were crossed from the plasmids in which they were constructed onto λ RS88 as described previously (18, 38). The copy number of λ prophage was determined as described elsewhere (31).

**β-Galactosidase assays.** β-Galactosidase activities of cells carrying $P_{TAC}$-*lacZ* fusions were assayed in microtiter plates as described previously (18). Overnight TB broth cultures were diluted into fresh TB containing antibiotics and incubated for approximately 2 h. The exponentially growing cultures were then diluted into TB alone or TB supplemented with 1 mM IPTG. The cultures were incubated for 1 h more and then assayed for β-galactosidase activity. β-Galactosidase activities of cells carrying $P_L$(HK022)-*lacZ* fusions were determined as described elsewhere (25). Overnight cultures were diluted into LB broth with or without 1 mM IPTG and grown for 3 to 4 h to cell densities of $2 \times 10^8$ to $5 \times 10^8$/ml before assay.

TABLE 2. Rho-mediated termination and *put*-mediated antitermination at $\lambda T_{R1}$[a]

| putL | RNAP-β′ | Termination (%) |
|---|---|---|
| WT[b] | WT | 39 |
| Mutant | WT | 96 |
| WT | Y75N | 95 |
| Δ | WT | 96 |
| Δ | Y75N | 95 |

[a] The *putL* site, when present, was located between the promoter and *lacZ* and $T_{R1}$, when present, was located between *putL* and *lacZ*. Termination = [1 − (β-galactosidase activity in a fusion containing $T_{R1}$)/(β-galactosidase activity in a comparable fusion lacking $T_{R1}$)] × 100. β-Galactosidase activities are from Fig. 1 except for those of the Δ*putL* fusions with and without $T_{R1}$, which were 268 and 6,688 U, respectively, in the *rpoC*$^+$ strain and 296 and 6,252 U, respectively, in the *rpoC-Y75N* strain (see Fig. 1 legend for more details).

[b] WT, wild type.

**Plasmid curing.** To measure steady-state levels of β-galactosidase in strains that carried single-copy HK$P_L$-*putL*-*nutL*-*lacZ* (or *nutR*-*lacZ*) fusions, the plasmid that expresses HK022 repressor (pRAK262) was removed by growing cultures at 42°C in LB for several generations. The loss of pRAK262 was confirmed by screening for chloramphenicol sensitivity and for immunity to HK022.

## RESULTS

***put*-mediated suppression of a Rho-dependent transcription terminator.** Lambda $T_{R1}$ is a well-characterized Rho-dependent terminator (14, 21, 34). We measured the efficiency of termination at $T_{R1}$ by comparing the activity of β-galactosidase produced after induction by cells containing a single copy of a $P_{TAC}$-$T_{R1}$-*lacZ* transcription fusion to that produced by cells containing a $P_{TAC}$-*lacZ* transcription fusion. We measured *put*-mediated antitermination of $T_{R1}$ in two ways. First, we compared β-galactosidase activity produced by a $P_{TAC}$-*putL*-$T_{R1}$-*lacZ* fusion to that produced by comparable fusions that lacked a functional *putL* site. Second, we compared β-galactosidase activity produced by a $P_{TAC}$-*putL*-$T_{R1}$-*lacZ* fusion in *rpoC*$^+$ cells to that produced by the same fusion in *rpoC-Y75N* cells. This mutation, which alters the β′-subunit of RNAP, prevents *put*-mediated antitermination at intrinsic terminators (10, 18). The two methods gave similar results.

$T_{R1}$ terminated transcription with an efficiency of 94 to 95% in the absence of *put* or when the cells contained the *rpoC-Y75N* mutation (Fig. 1 and Table 2). The presence of a functional *putL* site between the promoter and $T_{R1}$ in *rpoC*$^+$ cells reduced termination to 39%. We conclude that *put* partially suppresses termination at $T_{R1}$ and that suppression is prevented by *rpoC-Y75N*. These conclusions are supported by measurements made with another fusion (see below).

The apparent termination efficiency of $T_{R1}$ was severalfold higher in our fusions than in phage λ (12). It has previously been observed that the activity of $T_{R1}$ depends on its context (14, 29; D. Court, personal communication), and this might explain the difference. Nevertheless, to confirm that our fusions indicate Rho-dependent termination rather than termination at an uncharacterized intrinsic terminator, we measured β-galactosidase activity in the presence of phage P4 Psu, a protein that antagonizes Rho activity (22, 23). We found that Psu significantly increased β-galactosidase activity if the re-

porter fusion carried an inactive *put* site or if the strain carried the *rpoC-Y75N* mutation (Fig. 1). Therefore, the $T_{R1}$-containing reporter fusions do, indeed, report Rho-dependent termination. Psu had no such effect on a *put*$^+$ fusion in *rpoC*$^+$ cells, as expected if *put* suppresses Rho-dependent termination (Fig. 1). Psu did not increase *lacZ* expression as much as did deletion of $T_{R1}$, perhaps because Psu did not completely prevent Rho termination, or not enough time was allowed to reach the steady-state level of β-galactosidase after Rho action was blocked. The *psu* gene was derepressed for only 1 h before β-galactosidase was measured, because continuous high-level expression is lethal (23). Another possibility, discussed more fully below, is that deletion of $T_{R1}$ increases the stability or translation efficiency of the *lacZ* message.

We note that a deletion and a base substitution mutation of *put* increased the accumulation of β-galactosidase 1.5- to 3.3-fold in fusions that lack $T_{R1}$ (Fig. 1). (We consider possible explanations below.) The increased activity did not significantly change our estimate of $T_{R1}$ termination, which was 95 to 96% regardless of whether antitermination was prevented by a *put* mutation or by *rpoC-Y75N* (Table 2).

***put* suppresses Nun-dependent termination.** The phage HK022-encoded Nun protein terminates transcription after binding to a nascent transcript of the λ *nutL* or *nutR* sites (see introduction). To measure Nun-dependent termination, we compared the steady-state levels of β-galactosidase produced by cells containing a single copy of a $P_L$(HK022)-*putL-nutL-lacZ* transcription fusion in the presence and absence of Nun. In this fusion, the *putL* site is in its natural location, immediately downstream of the HK022 $P_L$ promoter. To see if *put* suppresses Nun-dependent termination, we inactivated *putL* by mutation or prevented *put* action with the *rpoC-Y75N* mutation. A plasmid that contains a $P_{Lac}$-*nun* fusion (pNUN) provided Nun at either low or high concentration according to whether the culture was grown in the absence or presence, respectively, of the *lac* operon inducer IPTG. The low concentration was similar to that found in a single-copy HK022 lysogen, and the high concentration was about 100 times greater (reference 19 and other data not shown).

The efficiency of termination at a low Nun concentration was 33%, and mutation of *rpoC* or *putL* increased this efficiency to 94 or 83%, respectively (Fig. 2 and Table 3). The efficiency of termination at a high Nun concentration was 83%, and mutation of *rpoC* or *putL* increased this efficiency to 99.7 or 99.5%, respectively. We conclude that *put* antiterminates Nun-dependent termination at *nutL*, and that increasing the Nun concentration increases termination in both the presence and absence of *put*-mediated antitermination.

The effect of Nun on *lacZ* expression was, as expected, site specific: Nun, even when present at a high concentration, reduced the activity of a reporter fusion that lacked a *nut* site by only a small amount, and the *rpoC-Y75N* mutation did not further reduce β-galactosidase production (Fig. 2 and Table 3). Unexpectedly, β-galactosidase activity in the fusion lacking *nutL* was 0.3 to 0.4 that of the *nutL*$^+$ fusion in the absence of Nun (Fig. 2). Therefore, we did not attempt to estimate the efficiency of Nun-dependent termination by comparing the activities of these two fusions in the presence of Nun (see below).

We used a $P_L$(HK022)-*putL-nutR-T*$_{R1}$-*lacZ* fusion to measure *put* suppression of Nun-dependent termination at the λ

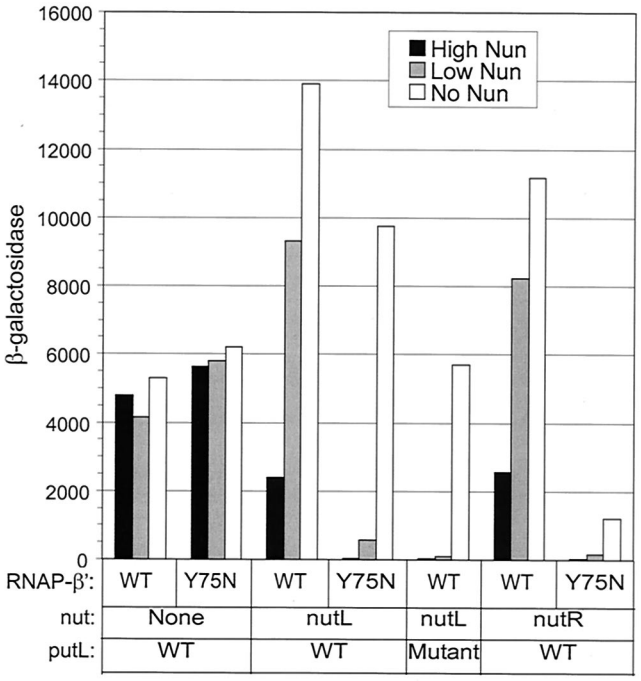


FIG. 2. Antitermination of Nun-dependent terminators. The strains were derivatives of TAP114 carrying a single copy of a $P_L$(HK022)-*putL-lacZ* fusion. Different fusions contained *nutL*, *nutR*, or neither *nut* site between *putL* and *lacZ*, and Nun was provided by a plasmid containing a $P_{Lac}$-*nun* fusion (pNUN). The shaded and filled bars show enzyme activity from mid-log cultures carrying pNUN grown in the absence and presence, respectively, of 1 mM IPTG. The open bars show enzyme activity from mid-log cultures carrying the vector plasmid (pNUNΔ) grown for 3 to 4 h in the presence of 1 mM IPTG. The mutant *putL* site (linker scanning mutation G [18]) has a multiple base substitution that prevents antitermination of Rho-independent terminators. Activities are averages of assays of at least two independent cultures at two different times during exponential growth. The standard errors of the mean ranged from 3 to 33%.

*nutR* site. The *nutR* region also contains the Rho-dependent λ$T_{R1}$ terminator; the *rutA* and *rutB* sequences, which are required for Rho action, lie immediately upstream and downstream, respectively, of the *boxB* element of *nutR* (14). *nutL* lacks *rut* sequences but is otherwise very similar to *nutR*. We hoped that comparison of the *nutL*- and *nutR*-containing fusions would tell us if Rho affects termination or antitermina-

TABLE 3. Nun-mediated termination and *put*-mediated antitermination at the λ *nutL* site[a]

| nut | putL | RNAP-β′ | Nun termination (%) | |
|---|---|---|---|---|
| | | | High Nun | Low Nun |
| *nutL* | WT | WT | 83 ± 3 | 33 ± 4 |
| *nutL* | WT | Y75N | 99.7 ± 0.1 | 93.9 ± 0.2 |
| *nutL* | Mutant | WT | 99.5 ± 0.1 | 98.3 ± 0.1 |
| None | WT | WT | 9 ± 9 | 22 ± 3 |
| None | WT | Y75N | 9 ± 20 | 6 ± 10 |

[a] The *putL* site was adjacent to the promoter and the *nutL* site, when present, was between *putL* and the *lacZ* coding sequence. Nun termination = [1 − (β-galactosidase activity with Nun)/(β-galactosidase activity without Nun)] × 100, and the errors are standard errors of the mean. β-Galactosidase activities are from Fig. 2 (see Fig. 2 legend for more details). WT, wild type.

TABLE 4. *put*-mediated antitermination of interdigitated Nun- and Rho-dependent terminators[a]

| RNAP-β′ | Nun termination (%) | | Rho termination (%) |
|---|---|---|---|
| | High Nun | Low Nun | |
| WT | 77 ± 3 | 26 ± 4 | <1[b] |
| Y75N | 98 ± 0.5 | 86 ± 2 | 80[c] |

[a] All of the fusions contained *putL* immediately downstream of the promoter and either contained or lacked the *nutR*-$T_{R1}$ region upstream of *lacZ*. The efficiency of Nun-dependent termination = [1 − (β-galactosidase activity with Nun)/(β-galactosidase activity without Nun)] × 100, and the errors are standard errors of the means. The efficiency of Rho-dependent termination, measured in cells lacking Nun, = [1 − (β-galactosidase activity in a fusion containing $T_{R1}$)/(β-galactosidase activity in a comparable fusion lacking $T_{R1}$)] × 100. β-Galactosidase activities are from Fig. 2. WT, wild type.

[b] The level of β-galactosidase was 2.1-fold greater in the fusion containing *nutR* than in the fusion lacking *nutR* (Fig. 2).

[c] This could be an underestimate of the termination efficiency (see text).

tion at the *nutR* site. We note that the fusions used for these experiments have a different promoter, a different transcription start, and less transcribed DNA between *putL* and *nutR* than the $P_{TAC}$-*putL*-$T_{R1}$-*lacZ* fusion used to measure Rho-dependent termination in the experiments of Fig. 1 (see Materials and Methods). These differences could, in principle, alter termination and antitermination efficiencies.

We estimated Nun-dependent termination and *put*-mediated antitermination by measuring β-galactosidase activities in the presence or absence of Nun in *rpoC*$^+$ or *rpoC-Y75N* cells, as described earlier for *nutL*. The efficiencies of Nun-dependent termination at *nutR* at low and high Nun concentrations were similar to those observed at *nutL*, and *put* suppressed Nun action at both sites to approximately the same extent (Fig. 2 and Table 4). Therefore, the presence of *rut* sites and their interaction with Rho do not appreciably alter Nun termination or *put* antitermination at *nut* (see also reference 33).

We estimated the efficiency of Rho-dependent termination and *put*-mediated antitermination at $T_{R1}$ by measuring β-galactosidase produced by fusions with or without the *nutR*-$T_{R1}$ region in *rpoC*$^+$ or *rpoC-Y75N* cells. Since these cells did not contain Nun, there was no Nun-dependent termination at *nutR*. The estimated efficiency of termination in the *rpoC* mutant host was 80%, and termination was completely suppressed in the wild-type host (Table 4). These estimates are somewhat lower than those presented in Table 2, in which different fusions were used. The differences might be the result of differences in the fusions, but there are also uncertainties in our estimates of termination efficiency, as discussed below. Nun caused an additional reduction in β-galactosidase activity produced by fusions containing the *nutR*-$T_{R1}$ region (Fig. 2), but the experimental uncertainties preclude quantitative estimates of any effect of Nun on Rho-dependent termination.

We wish to call attention to several unexpected observations. Mutation or deletion of *putL* increased the specific activity of β-galactosidase 1.5- to 3-fold in certain fusions (Fig. 1 and Table 2 footnote [$\Delta T_{R1}$ fusions]). In other fusions, deletion of *nutL* or *nutR*-$T_{R1}$ decreased the specific activity of β-galactosidase to 0.3 to 0.4 (Fig. 2, No Nun). We speculate that these changes increased the stability and/or the translation efficiency of the *lacZ* message, but we have no independent evidence for this hypothesis. Because of this, estimates of termination and antitermination that are based on comparison of fusions that either contain or lack the terminator or antiterminator sites, respectively, should be considered as approximations. In many cases we estimated efficiencies of termination and antitermination by comparing identical fusions in the presence and absence of a *trans*-acting protein, such as Nun, Psu, or wild-type RNAP, and these estimates are probably more reliable. For example, our estimate that unsuppressed Rho-mediated termination efficiency at $T_{R1}$ is 80% was based on comparison of different fusions (Fig. 2 and Table 4). Comparison of the amount of β-galactosidase produced by the fusion containing $T_{R1}$ in *rpoC*$^+$ and *rpoC-Y75N* cells (Fig. 2) suggested that the true termination efficiency in this fusion could be 90% or more.

## DISCUSSION

We have shown that *put* suppresses Nun- and Rho-dependent transcription terminators in vivo. Suppression was efficient when the termination factors were present at physiological levels, but increasing the concentration of Nun increased termination (on templates containing a *nut* site) both in the presence and absence of a functional *put* site. We previously demonstrated efficient suppression of several strong intrinsic terminators in vivo and in vitro. We also showed that the HK022 *put* sites could replace the function of the λ *N* gene and *nut* sites in λ-HK022 hybrid phages (10, 18, 28). The hybrid phages showed no obvious defect in lytic growth, lysogenization, or lysogenic induction. It therefore appears that the *put* sites suppress the numerous λ terminators in the early operons to the extent required for normal phage growth. *put*-mediated antitermination thus shows no demonstrable terminator specificity. The λ N/*nut* and Q/*qut* antitermination pathways also suppress intrinsic and Rho-dependent terminators, and N suppresses Nun-dependent termination by competing for binding to *nut* RNA (5, 8, 16, 33, 46). Q has not yet been tested on a Nun-dependent terminator. This apparent lack of terminator specificity suggests that *put* inhibits a step that is common to termination at the different types of terminator. What might this step be?

A short region of RNA-DNA hybrid at the 3′ end of the nascent RNA chain is believed to play a critical role in stabilizing the transcript elongation complex (27, 36). Current models propose that intrinsic and Rho-dependent termination are a consequence of hybrid disruption (15, 20, 40, 47). It is, therefore, tempting to suggest that *put* RNA acts by increasing the stability of the hybrid or by preventing a step that follows hybrid disruption. However, this suggestion fails to account for *put* suppression of Nun-dependent terminators. Nun binds to nascent *nut* RNA and is then delivered to the nearby elongation complex, where it arrests translocation. Arrest is believed to be the result of a Nun-DNA interaction that anchors the elongation complex to the template so that it can no longer translocate (17, 43). Since the arrested complex is stable and catalytically active in vitro, it is not obvious how further stabilization by *put* RNA could prevent arrest or restart translocation once arrest had occurred or, if it did, how increasing the concentration of Nun would overcome this effect.

We offer two models to explain suppression of Nun-dependent termination. First, the efficiency of Nun arrest in vitro is decreased by conditions that increase the rate of

translocation (16). It is possible that rapid translocation of RNAP away from the Nun binding site decreases the efficiency of transfer of Nun to the elongation complex. If so, *put*-mediated acceleration of the elongation complex through pause sites (18) could have the same effect on Nun transfer. Elevating the Nun concentration should quicken its binding to *nut* RNA and thus increase the probability of transfer. In the second model, *put* RNA impedes or delays binding of Nun to *nut* RNA. We have shown that nascent *put* RNA binds to elongating polymerase and that this complex persists as polymerase translocates (35). If the nascent *put* transcript binds close to the product RNA exit channel in RNAP, it might delay the binding of Nun to the nascent *nut* transcript until the elongation complex is too distant for efficient transfer of Nun to RNAP. This effect would be mitigated by increasing the Nun concentration. Either of these models can be adapted to explain the suppression of other classes of terminators by *put* RNA. However, it is possible that *put* suppresses different types of terminators in different ways and that no single mechanism suffices to account for its action.

## ACKNOWLEDGMENTS

We are grateful to Max Gottesman and Don Court for their comments on the manuscript. We also acknowledge Jennie Tang for constructing plasmid pJT6.

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