J. Mol. Biol. (1994) **236**, 217–228

# Bacteriophage Lambda N-Dependent Transcription Antitermination

## Competition for an RNA Site May Regulate Antitermination

**Thomas A. Patterson[1]†, Zhaoshan Zhang[1]‡, Teresa Baker[1], Linda L. Johnson[2]§ David I. Friedman[2] and Donald L. Court[1]**

[1]*Laboratory of Chromosome Biology, ABL-Basic Research Program NCI-Frederick Cancer Research and Development Center P.O. Box B, Frederick, MD 21702, U.S.A.*

[2]*University of Michigan Department of Microbiology and Immunology Ann Arbor, MI 48109-0620, U.S.A.*

Bacteriophage λ controls the expression of its early genes in a temporal manner by a series of transcription termination and antitermination events. This antitermination requires the λ N protein as well as host proteins called Nus, and *cis*-acting sites called *nut*. Following transcription of the *nut* site, N and Nus proteins bind to the *nut* RNA and modify the transcription complex to a termination-resistant form. The *nut* site is a composite of at least two components; one is the *boxB* hairpin structure which interacts with N. The other is *boxA*, a nine-nucleotide sequence upstream of *boxB*. To understand more about the formation of the antitermination complex, we have characterized the effect of point mutations in and deletions of *boxA* on antitermination. Point mutations in *boxA* were found to either enhance or reduce N-mediated antitermination. Several *boxA* deletions, on the other hand, had little effect on antitermination other than to eliminate the requirement for the NusB host protein. To explain these observations, we propose that at least two factors compete to interact with *boxA*, NusB and an inhibitor of the antitermination reaction. In addition, we propose that NusB is required to prevent the inhibitor from binding at *boxA*. The results with various *nusB* and *boxA* mutations can be explained by this model of competition between NusB and an inhibitor for *boxA* RNA.

*Keywords:* transcription elongation; termination; NusB; S10; N

## 1. Introduction

During bacteriophage λ development, the expression of most phage genes requires transcription antitermination by the phage-encoded N protein, host proteins called Nus (*N-utilization substances*), and *cis*-acting sequences called *nut* (*N-utilization*) sites (Friedman, 1988; Das, 1992; Roberts, 1993). Although N and Nus proteins form a complex with one another at the *nut* site, the specific interactions and signals required remain poorly defined.

The Nus factors have been identified primarily through the isolation of recessive mutations that cause a failure in λ development due to a defect in N-dependent antitermination (see Friedman *et al.*, 1984). NusA, NusC, NusD, and NusG are components associated with the normal RNA polymerase complex. NusC is the β-subunit of RNA polymerase (Georgopoulos, 1971). NusA interacts with the elongating RNA polymerase and enhances pausing and modulates transcription termination (Friedman *et al.*, 1973; Greenblatt & Li, 1981; Schmidt & Chamberlin, 1984). NusD is the transcription termination factor Rho (Das *et al.*, 1983), and NusG stimulates Rho-dependent transcription

† Present address: Dupont Merck Pharmaceutical, Experimental Station, Wilmington, DE 19898, U.S.A.

‡ Present address: Molecular Genetics Center, Beijing, 100850, Peoples Republic of China.

§ Present address: Department of Biochemistry, Parke-Davis Pharmaceutical, Warner-Lambert Company, 2800 Plymouth Road, Ann Arbor, MI 48105, U.S.A.



0022-2836/94/060217-12 $08.00/0

© 1994 Academic Press Limited

termination (Sullivan & Gottesman, 1992; Li *et al.*, 1993). Thus, both Rho and NusG also interact with elongating RNA polymerase. The NusE and NusB proteins are multifunctional, affecting transcription and translation (Keppel *et al.*, 1974; Friedman *et al.*, 1976, 1981). NusE is ribosomal protein S10 (Friedman *et al.*, 1981), and NusB appears to affect the rate of translation elongation (Shiba *et al.*, 1986; Taura *et al.*, 1992). It is noteworthy that the NusB and S10 proteins, which affect both transcription and translation, bind to each other (Mason *et al.*, 1992*a*).

The Nus proteins are essential for *Escherichia coli* growth. Most *nus* mutations that reduce N-dependent transcription antitermination are not null mutations, though null mutations do exist in NusB. Nonsense or insertion mutants in *nusB* grow poorly, if at all, at temperatures below 30°C (Swindle *et al.*, 1988; Taura *et al.*, 1992). At temperatures permissive for cell growth, the *nusB* null mutants fail to support λ development (Swindle *et al.*, 1988). All known *nus* mutants are most defective in supporting λ development at or above 42°C. Although mutations in NusG that block λ growth have not been isolated, a suppressor mutation in *nusG* can compensate for *nusA* and *nusE* mutations (Sullivan & Gottesman, 1992).

The sites *nutL* and *nutR*, the signals for transcription antitermination, are located in transcribed regions beyond the $p_L$ and $p_R$ promoters, respectively (Rosenberg *et al.*, 1978; Salstrom & Szybalski, 1978). Summarized below are several observations that lead to the conclusion that N and some of the Nus proteins bind to these sites on the RNA transcript, guiding them to form an antitermination complex with the transcribing RNA polymerase. RNA was first demonstrated to be important in the assembly of the complex by the observation that translation into or across the *nutR* region blocked transcription antitermination by N (Olson *et al.*, 1984; Warren & Das, 1984; Zuber *et al.*, 1987). Similarly, the synthesis of large quantities of mRNA from a plasmid containing the *nutR* site specifically prevents λ development by competing for a protein required in the antitermination complex (Friedman *et al.*, 1990). Indeed, *in vitro* studies show that the *nutR* sequence in the nascent RNA transcript serves as the binding site for N and Nus factors (Barik *et al.*, 1987; Horwitz *et al.*, 1987; Whalen & Das, 1990; Nodwell & Greenblatt, 1991, 1993; S. Chattopadhyay, J. Garcia-Mena, &. A. Das, personal communication).

Components of the *nutR* and *nutL* sequences of λ include the *boxB* RNA segment, capable of forming a stem-loop structure (Rosenberg *et al.*, 1978; Steege *et al.*, 1987), and the nine-nucleotide *boxA* RNA segment located upstream from the *boxB* stem-loop structure (Olson *et al.*, 1982). N-dependent antitermination can be blocked by mutations in either *boxB* (Salstrom & Szybalski, 1978; Doelling & Franklin, 1989; Baron & Weisberg, 1992) or *boxA* (Olson *et al.*, 1984; Robledo *et al.*, 1990). Interestingly, some *boxA* mutations, e.g. *boxA1*, improve transcription antitermination in *nusA* and *nusE* mutant hosts (Friedman & Olson, 1983; Schauer *et al.*, 1987). Thus, point mutations, depending on their location within *boxA*, can either inhibit or enhance N-dependent transcription antitermination. The spacing between *boxA* and *boxB* is seven and eight nucleotides for *nutL* and *nutR*, respectively, and the spacer sequences are not well conserved. Deletion analyses suggest that part of the spacer region must remain intact for transcription antitermination (Peltz *et al.*, 1985; Zuber *et al.*, 1987).

Lambdoid phages that encode different N proteins have similar *boxA* sequences, but differ from each other in their *boxB* sequences (Franklin, 1985). The *boxB* sequences are specific for their cognate N proteins (Lazinski *et al.*, 1989). The conservation of *boxA* sequence among the lambdoid phage *nut* sites (Friedman & Gottesman, 1983) and its presence in rRNA operons (Olson *et al.*, 1984; Sharrock *et al.*, 1985; Morgan, 1986; Berg *et al.*, 1989; Squires *et al.*, 1993) make it an ideal candidate for a site recognized by host Nus proteins. Studies have suggested that *boxA* is a possible recognition site for NusB or S10 (Friedman *et al.*, 1990; Nodwell & Greenblatt, 1993). We have undertaken an *in vivo* approach to analyze the role of *boxA* in transcription antitermination by examining point mutations in *boxA* as well as deletions that encompass *boxA* for their effect on N-dependent transcription antitermination. Our results suggest that *boxA* interacts with at least two competing factors; one of these is NusB and the other is an unidentified host factor that appears to inhibit the normal N-dependent transcription antitermination reaction.

## 2. Materials and Methods

### (a) *Strains*

Bacterial strains are described in Table 1. Plasmids pKG1805 (McKenney *et al.*, 1981), and pMZ215 (Zuber *et al.*, 1987) were used to construct the pMZ245 plasmids used in these experiments.

Phages $\lambda imm^{434}$, $\lambda imm^{434}$ *nin5*, $\lambda imm^{434}$ *Nam7 Nam53 nin3*, $\lambda imm^{434}$*cIts nusB5 nin3*, λ*cI60*, P1Cm*clr100*, and P1*vir* are from the National Institutes of Health Collection. λ*bio69 cI857 boxA5 nin3* was provided by Dr Max Gottesman.

### (b) *Strain constructions*

In the following descriptions, $Nus^-$ has a functional definition referring to the phenotype of a strain that permits plaque formation at 42°C by $\lambda imm^{434}$*nin* but not $\lambda imm^{434}$ phage.

The origins of DC556 and TAP98 are detailed in another publication (Patterson *et al.*, 1993). TAP67 is a $nad^+$ $galK^-$ transductant with λ immunity from a cross between P1 · DC556 and TAP66. TAP112 was constructed by transducing TAP67 to $Kan^R$ using P1Cm grown on TAP98. The isogenic *nus* mutant strains described in Table 1 were made by standard P1 transduction, except that TAP92 was constructed by a cycle of integration and excision $\lambda imm^{434}$ *cIts nusB5 nin3* at the *nusB* gene on the

**Table 1**
*Bacterial strains*

| Strain | Genotype | Source |
|---|---|---|
| TAP66 | *his ilv rpsL nadA* :: Tn*10* | T. Patterson |
| TAP67 | *his ilv rpsL galK*(am) [λ*kil*⁻ *N*⁺*cI857 (cro-bioA)*Δ] | This work |
| TAP92 | TAP67 *nusB5* | This work |
| TAP98 | *recD1903* :: ΔTn*10*Δ16Δ17 [λ*kil*⁻ *N* :: *kan cI857 (cro-bioA)*Δ] | Patterson *et al.* (1993) |
| TAP112 | TAP67 *N* :: *kan* | This work |
| DC556 | *galK*(am) *rpsL [λkil N*⁺*cI857 (cro-bioA)*Δ] | Patterson *et al.* (1993) |
| DC1161 | TAP67 *argG* :: Tn5 *nusA1* | This work |
| DC1162 | TAP67 *nusE71 zhb511* :: Tn*10* | This work |
| DC1163 | TAP67 *nusD026 (rho026) ilv*⁺ | This work |
| DC1165 | TAP67 *nusA1* | This work |
| DC1166 | TAP112 *nusB5* | This work |
| DC1167 | TAP112 *nusE71 zhb511* :: Tn*10* | This work |
| DC1168 | TAP112 *nusD026 (rho026) ilv*⁺ | This work |
| DC1170 | TAP112 *nusA1* | This work |
| DC1198 | TAP67 *nusB* :: IS10 *zba-525* :: Tn*10* | This work |
| DC1199 | TAP112 *nusB* :: IS10 *zba-525* :: Tn*10* | This work |
| IQ577 | W3110 *nusB* :: IS10 *(ssyB63) zba-525* :: Tn*10* | Taura *et al.* (1992) |
| K95 | *nusA1 galK2 rpsL* | D. Friedman |
| K450 | *nusB5 galK2 rpsL* | D. Friedman |
| K1457 | *argG* :: Tn5 *nusA1 galK2 rpsL* | D. Friedman |
| K2016 | *zhb511* :: Tn*10 nusE71 galK2 rpsL* | D. Friedman |
| N5117 | *his argX* :: Tn*10 rpsL nusD026 (rho026)* | Das *et al.* (1983) |

*E. coli* chromosome (Ward *et al.*, 1983). The excised, λ cured derivatives were selected at 42°C on LB agar and screened for the presence of the *nusB5* mutation (Nus⁻). Approximately 50% of the cured cells were *nusB5*.

### (c) *Construction of pMZ245 derivatives with altered* boxA *regions*

pMZ245 and pMZ278 are described in Fig. 1. The *boxA5* mutation was placed into pMZ245 from λ*bio69 cI857 boxA5 nin5* DNA (Robledo *et al.*, 1990). The *boxA5* change is present between an *Ava*I site at 38,214 bp in the λ chromosome and an *Nsi*I site at 38,311 bp. This fragment was cut from phage DNA and isolated from an agarose gel, and then was used to replace the corresponding *Ava*I to *Nsi*I fragment in pMZ245. The resulting plasmid, pDLC128, was shown to contain the *boxA5* change by sequence analysis.

Plasmid pMZ245 *boxA*Δ*37* was constructed by digesting pMZ245 with *Ava*I, treating with calf intestinal phosphatase, and purifying the linear DNA from an agarose gel. A double-stranded oligonucleotide fragment was synthesized with the appropriate complementary ends to be ligated between *Ava*I and *Nsi*I cleaved pMZ245; the remaining nucleotides in the fragment are those corresponding to λ DNA sequence 38,219 to 38,307 inclusive except that *boxA* and the 29 nucleotides upstream of *boxA* were deleted as shown in Fig. 2. The oligonucleotide fragment was phosphorylated with polynucleotide kinase and ligated with the *Ava*I-digested, dephosphorylated pMZ245 DNA. The ligase was inactivated and the plasmid DNA digested with *Nsi*I. After inactivating the restriction enzyme, ligase was added again and a transformant was isolated. The structure of the resulting plasmid, pMZ245 *boxA*Δ*37*, was verified by DNA sequence analysis.

All other pMZ245 derivatives used here were made from pTAP27, a derivative of pMZ245. pTAP27 was made by the protocol used to make (pMZ245 *boxA*Δ*37*, except that the oligonucleotide used for the construction was changed for the base sequence between *Ava*I and *Nsi*I indicated in Fig. 2 (see ΔR28). This deletion joined 3 cytosine residues to the left of *boxA* with 3 guanosine residues in *boxB* to create a unique *Sma*I site at the novel joint.

The *boxA* substitution mutations shown in Fig. 2 were made by creating a 28-bp double-strand oligonucleotide carrying a mutation in *boxA* and inserting it at the *Sma*I

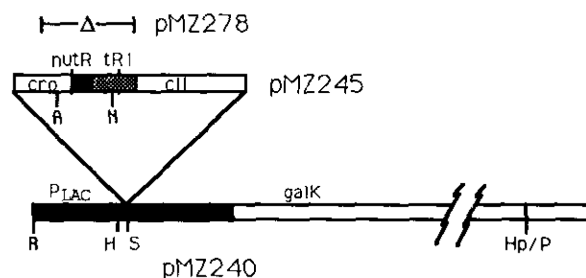


**Figure 1.** Structure of pMZ240, pMZ245, and pMZ278. pMZ240 was made by substituting a *Pvu*II to *Hin*dIII fragment containing the $p_{LAC}$ promoter from pFW1 (Warren & Das, 1984) for the *Eco*RI to *Hin*dIII fragment of pKG1800 (KcKenney *et al.*, 1981). The *Eco*RI site was repaired to a blunt end with Klenow enzyme before ligation. The resulting *Eco*RI-*Pvu*II joint recreated an *Eco*RI site. In short, the *Eco*RI (R) to *Hpa*I (Hp) fragment of pMZ240 replaces the *tet* gene segment of pBR322 from *Eco*RI to *Pvu*II (P). pMZ245 contains the *tR1* segment of λ inserted at the *Sma*I site (S) of pMZ240. The *Hin*dIII site (H) in pMZ245 was used to generate the deletion in pMZ278 that removes *tR1* (Zuber *et al.*, 1987). The *Ava*I (A) and *Nsi*I (N) sites used in making the *boxA* mutants of pMZ245 are indicated. Previous studies had shown that galactokinase expression from pMZ240 is completely dependent upon the *lac* promoter (Zuber *et al.*, 1987). The λ*cII* gene segment and the galactokinase gene, *galK*, are located beyond *tR1*.

| Mutation | Sequence (*boxA*, *boxB*) | % Antitermination *nus+* | *nusA1* | *nusB5* |
|---|---|---|---|---|
| WT | CCCGAGTAACAAAAAAACAACAGCATAAATAACCCCGCTCTTACACATTCCAGCCCTGAAAAAGGGC | 70 | 15 | 24 |
| A1 | CCCGAGTAACAAAAAAACAACAGCATAAATAACCCCGCTCTTTCACATTCCAGCCCTGAAAAAGGGC | 84 | 26 | 25 |
| A5 | CCCGAGTAACAAAAAAACAACAGCATAAATAACCCCTCTCTTACACATTCCAGCCCTGAAAAAGGGC | 11 | 2 | 18 |
| A16 | CCCGAGTAACAAAAAAACAACAGCATAAATAACCCCGCTATTACACATTCCAGCCCTGAAAAAGGGC | 2 | 0 | NT |
| A69 | CCCGAGTAACAAAAAAACAACAGCATAAATAACCCATAGAGGCCACATTCCAGCCCTGAAAAAGGGC | 42 | 7 | 31 |
| WT | CCCGAGTAACAAAAAAACAACAGCATAAATAACCCCGCTCTTACACATTCCAGCCCTGAAAAAGGGC | 75 | 15 | 26 |
| ΔR28 | CCCGAGTAACAAAAAAACAACAGCATAAATAACCC GGGC | 1 | 0 | 4 |
| ΔA37 | CCCGAG CACATTCCAGCCCTGAAAAAGGGC | 74 | 28 | 78 |
| ΔA30 | CCCGAGTAACAAA CACATTCCAGCCCTGAAAAAGGGC | 82 | 16 | 68 |
| ΔA26 | CCCGAGTAACAAAAAAA CACATTCCAGCCCTGAAAAAGGGC | 65 | 12 | 49 |
| ΔA9 | CCCGAGTAACAAAAAAACAACAGCATAAATAACC CACATTCCAGCCCTGAAAAAGGGC | 32 | 5 | 22 |
| ΔA40 | CCCGAGTAAC ATTCCAGCCCTGAAAAAGGGC | 65 | 2 | 50 |
| ΔA11 | CCCGAGTAACAAAAAAACAACAGCATAAATAACCC ATTCCAGCCCTGAAAAAGGGC | 23 | 4 | 21 |
| Δ10 | CCCGAGTAACAAAAAAACAACAGCA CGCTCTTACACATTCCAGCCCTGAAAAAGGGC | 99 | 35 | 32 |

**Figure 2.** BoxA mutations and N-dependent transcription antitermination. The wild-type (WT) sequence of *nutR* is shown with the location of *boxA* and *boxB* indicated by underlining the base. In all of the diagrams, sequences downstream to the *Ava*I site (CCCGAG) of pMZ245 are shown. The TAA sequence 10 bp upstream of *boxA* is the stop codon for the $\lambda$ *cro* gene. The name of the mutant allele is shown (R = *nutR*, A = *boxA*, and Δ indicates deletion with the number of bases missing). The mutational changes are indicated in the sequence. Deletions are indicated by the omission of the deleted bases and substitution mutations are indicated by stippling of the changed bases. N-mediated transcription antitermination values were determined as described in Table 2. Transcription termination efficiencies for the deletion mutants tested here range between 0·85 and 0·98 for $N^-$ and $nus^+$ conditions except for *boxA16* which had a termination efficiency of ~0·80. As indicated in Materials and Methods, the relative termination efficiency of each mutant is taken into account in determining the antitermination values. The isogenic *nus* strains used are described in Tables 1 and 3. Because experiments with plasmids carrying *boxA* substitutions and deletions were run separately, data for the wild-type controls are shown twice.

site of pTAP27. The changes in the resultant plasmids were confirmed by sequence analysis.

The *boxA* and *cro* deletions were made by inserting the appropriate synthetic double-strand oligonucleotide between the *Ava*I site (in *cro*) and the *Sma*I site of pTAP27. All resultant plasmids were sequenced from *tR1* through the *Ava*I site.

### (d) *Enzymes and other materials*

All enzymes were purchased from New England Biolabs except T4 ligase, which was purchased from Bethesda Research Laboratories, and reverse transcriptase and calf intestinal phosphatase, which were purchased from Boehringer-Mannheim Biochemicals. Oligonucleotides were purchased from Midland Certified Reagents, Midland, Texas. [$^{14}$C]Galactose (58 mCi/mmol) is from Amersham. Enzymes were used according to manufacturer's recommendations. DNA sequencing from double-strand templates utilizing reverse transcriptase or Sequenase was performed according to our published procedures (Takiff *et al.*, 1989).

### (e) *Galactokinase enzyme assays to measure termination and antitermination*

Galactokinase enzyme assays were performed as described (Adhya & Miller, 1979; McKenney *et al.*, 1981). Termination activities are calculated from the galactokinase values measured at 42°C with pMZ245 and pMZ278 in strains lacking the phage $\lambda$ N protein. Plasmid pMZ245 carries the *tR1* terminator while plasmid pMZ278 lacks *tR1*.

N-dependent transcription antitermination activities are expressed as percentage of terminated transcripts which are antiterminated. To determine the transcription antitermination frequency, it is necessary to subtract the number of transcripts which escape termination in the $N^-$ conditions, i.e. the $N^-$ level of galactokinase through pMZ245. This number is subtracted from the total transcription initiation events for pMZ278 and from the $N^+$ readthrough levels of pMZ245.

Termination frequency is determined in the *N*-defective strains and is calculated according to the following formula:

$$\frac{galK \text{ units from pMZ278} - galK \text{ units from pMZ245}}{galK \text{ units from pMZ278}}$$

Transcription antitermination frequency is calculated according to the following formula:

$$\frac{galK \text{ units from pMZ245 (N}^+\text{ strain)} - galK \text{ units from pMZ245 (N}^-\text{ strain)}}{galK \text{ units from pMZ278 (N}^+\text{ strain)} - galK \text{ units from pMZ245 (N}^-\text{ strain)}}$$

Although the results presented in Tables and Figures represent data from a single experiment, similar results were obtained from at least 3 independent experiments with the variability in the data being less than 15%.

#### (f) *Competition assay*

The method employed was essentially that described by Friedman *et al.* (1990). Briefly, a log phase culture of *E. coli* strain K450, carrying the indicated plasmid, was infected at a multiplicity of 0·1 with *λcI60*. The pKK223 3 plasmid derivatives have *nutR* regions either with *boxA*$^+$ or *boxA5* cloned downstream of $p_{TAC}$. Each of the infected bacterial cultures was divided into 2 parts, 1 of which was made 1 mM in isopropyl-β-D-Thiogalactoside (IPTG) to induce maximal transcription of the cloned *nut* site. The cultures were grown at 40°C and at the indicated times portions were removed and lysed with chloroform. The lysate was assayed for its phage titer.

## 3. Results

#### (a) *N-dependent transcription antitermination*

Plasmid pMZ245 provides a system to quantify transcription antitermination at the Rho-dependent *λ tR1* terminator. The starting vector, pMZ240, contains the wild-type *lac* promoter directing transcription of the structural gene for galactokinase, *galK* (Fig. 1). A contiguous 400-base pair (bp) fragment of phage λ DNA, containing the distal end of the *cro* gene, *nutR*, *tR1*, and the proximal part of the *cII* gene, was inserted between the promoter and *galK* reporter gene (Fig. 1). To measure transcription termination free of the effects of ribosomes translating the RNA, we constructed the vector such that transcripts initiated at the *lac* promoter do not contain ribosome binding sites upstream of *tR1* (Warren & Das, 1984; Zuber *et al.*, 1987). To measure *galK* expression from the *lac* promoter unimpeded by a terminator, and thus to determine the value of 100% read-through, we made pMZ278 a derivative of pMZ245 deleted for *tR1* (Fig. 1).

The efficiency of termination at *tR1* under N$^-$ or N$^+$ conditions has been calculated from the measurements of galactokinase expression from pMZ245 and pMZ278 (Table 2). In the absence of N protein, *tR1* termination reduces galactokinase levels more than 25-fold (Table 2). In the presence of N, the galactokinase level expressed from pMZ245 approaches the level expressed from the control plasmid pMZ278. When *nutR* is deleted from pTAP27(*nutRΔ28*), termination at *tR1* still occurs but N-dependent transcription antitermination is eliminated, demonstrating the central importance of *nutR* in N-dependent antitermination (Table 2).

#### (b) *The dispensability of* boxA *in N-dependent transcription antitermination*

In a previous study, we found that pMZ245-derived plasmids, having *nut* regions from which all of *boxA* and over 100 nucleotides upstream had been deleted, maintained the ability to direct N-dependent transcription antitermination at the Rho-independent terminator *tI* (Zuber *et al.*, 1987). In the current study, we examine the role of *boxA* in N-mediated transcription read-through of Rho-dependent terminator *tR1* employing altered *boxA* sequences that include both nucleotide substitutions as well as a set of nested deletions (Fig. 2).

To assess the role of *boxA* in our tester plasmid system, we first determined that *boxA* mutations (*boxA1*, *boxA5* and *boxA16*), known to alter N antitermination in the λ genome (Friedman & Olson, 1983; Olson *et al.*, 1984; Robledo *et al.*, 1990;

**Table 2**
*N-dependent transcription antitermination*

| Plasmid | *tR1*§ | *nutR*§ | Galactokinase units† | | Percentage‡ | |
|---|---|---|---|---|---|---|
| | | | *N*$^-$ | *N*$^+$ | (T) | (A) |
| pMZ278 | Δ | Δ | 2594 | 2610 | 0 | · |
| pMZ245 | + | + | 102 | 1963 | 96 | 74 |
| pTAP27 | + | Δ | 235 | 248 | 91 | 1 |

† *E. coli* derivatives TAP67 (*N*$^+$) and TAP112 (*N*$^-$) with a defective *λcI857* prophage. These strains carry the indicated plasmid and were grown at 32°C to log phase and then shifted to 42°C for 60 min to induce N expression before measuring galactokinase.

‡ Percentage transcription termination (T) and antitermination (A) are calculated from the galactokinase units (see Materials and Methods for rationale):

$$\text{Termination for pMZ245: } \frac{2594-102}{2594}\times 100 = 96\%$$

$$\text{Antitermination for pMZ245: } \frac{1963-102}{2610-102}\times 100 = 74\%$$

§ A + indicates *tR1* or *nutR* is intact; Δ, indicates *tR1* or *nutR* is deleted. Plasmid pTAP27 contains the deletion *nutRΔ28* of Fig. 2.

**Table 3**
*Effects of* nus *mutations on transcription antitermination at* tR1 *when the* nut *region has the* boxAΔ37 *mutation*

| *boxA* | % Antitermination† | | | | |
|---|---|---|---|---|---|
| | *nus*$^+$ | *nusA1* | *nusB5* | *nusD026* | *nusE71* |
| *boxA*$^+$ | 76 | 16 | 25 | 27 | 16 |
| *boxAΔ37* | 77 | 15 | 70 | 18 | 39 |

† Plasmids pMZ245 (*boxA*$^+$) and pZSZ37 (pMZ245*boxAΔ37*) were transformed into isogenic strains that either express N (TAP67) or fail to express N (TAP112) and have *nus* alleles indicated (see Table 1). Galactokinase levels were measured and transcription termination and antitermination percentages were determined as in Table 2. Percent termination under $N^-$ conditions for the *boxA*$^+$ construct in *nus*$^+$ was 96%; in *nusA1* was 95%; in *nusB5* was 93%; in *nusD026* was 81%; in *nusE71* was 95%. For the *boxAΔ37* mutant, percent termination values were slightly reduced (less than 10%) relative to *boxA*$^+$ for each strain. As described in Materials and Methods and in Table 2, the differences in termination levels are taken into account in determining percent antitermination.

N. Costantino, Z. Zhang, & D. Court, unpublished results), have similar effects in the tester plasmid. The *boxA1* point mutation exhibits slightly enhanced transcription antitermination levels over the levels seen with *boxA*$^+$ in both *nus*$^+$ and *nusA1* hosts, whereas, the *boxA5* and *boxA16* mutations greatly reduce antitermination in *nus*$^+$ (Fig. 2).

The deletions of *boxA* can be divided into two functional classes. One class includes the shortest deletions *boxAΔ9* and *boxAΔ11* which, like the *boxA69* substitution (Fig. 2), partially reduce N-mediated transcription antitermination. The second class, the longer deletions (Δ40, ΔA37, ΔA30, ΔA26), extend further upstream of *boxA* (Fig. 2). Note that this second class allows levels of N-dependent antitermination comparable to those observed with the wild-type *boxA* sequence (Fig. 2).

### (c) *Role of Nus factors in N-mediated transcription antitermination*

It appeared paradoxical that a *nut* region with the *boxA5* point mutation fails to support N-mediated antitermination, while *boxA* deletions, like *boxAΔ37*, support the process. One explanation for these findings could be that changes in *boxA* associated sequences modify the host factor requirements for effective N action. We tested this hypothesis using pMZ245 derivatives with the deletion *boxAΔ37* or wild-typed *boxA* to compare N-mediated antitermination in hosts with different *nus* mutations. This analysis showed that N-mediated transcription antitermination from pMZ245 (*boxA*$^+$) is reduced as expected in hosts with the *nus* mutations indicated in Table 3, showing that wild-type NusA, NusB, NusD (Rho), and NusE (S10) all are required for effective antitermination at *tR1* when the *nut* region contains a wild-type *boxA* sequence. The effects of *nus* mutations are very different when the *nut* region in pMZ245 has the *boxAΔ37* deletion. N-mediated transcription antitermination was significantly reduced in hosts with either the *nusA1* or *nusD026* mutations, less reduced in a host with the *nusE71* mutation, and not significantly reduced in a host with the *nusB5* mutation. Thus, with a *boxAΔ37* deletion in *nutR*, wild-type NusA, Rho, and, to a lesser extent, S10 are required, but surprisingly, NusB is not required for effective antitermination.

It was possible that the *boxA* deletion lowered the level of NusB required for antitermination, since there is evidence that a residual level of functional NusB is contained in *nusB5* mutants. In fact, a high-copy plasmid containing a *nusB5* mutant gene complements a *nusB5* mutant cell for λ growth (T. Patterson & D. Court, unpublished results). Furthermore, the *nusB5* mutant, unlike amber and insertion mutants of *nusB*, grows at temperatures below 30 °C. Therefore, we re-examined the requirement of NusB for antitermination in a host with an IS10 insertion within the *nusB* gene (Taura *et al.*, 1992). As with the *nusB5* mutant, the insertion mutant prevented efficient antitermination when a wild-type *nutR* region was used (18% antitermination) but had little, if any, effect on N-mediated antitermination when a *boxA* deletion was used (73% antitermination). Thus, deleting *boxA* bypasses the requirement for NusB.

These apparently contradictory observations led us to extend our analysis of the role of *boxA* in N-mediated transcription antitermination to include a variety of *boxA* mutations (Fig. 2). Again, the pMZ245 vector was employed and the role of Nus factors was assessed using hosts with either the *nusA1* or *nusB5* mutations. If *boxA* is deleted from the *nut* region, the level of transcription antitermination varies little in hosts with the *nusB5* mutation from that seen in hosts that are wild type at the *nus* loci (Fig. 2). In fact, pMZ245 variants with deletions of *boxA* that retain full transcription antitermination activity in a *nus*$^+$ host are as functional in a *nusB5* host. Moreover, those derivatives of pMZ245 with deletions of *boxA* that impose significant reductions in N-mediated transcription antitermination (e.g. *boxAΔ9*) exhibit no further reduction in the *nusB5* mutant host (Fig. 2).

In contrast to the above results, derivatives of pMZ245 containing *nut* regions with long or short

*boxA* deletions exhibit a further reduction in transcription antitermination in the *nusA1* mutant host (Fig. 2). Similarly, a pMZ245 derivative containing a *nut* region with the *boxA5* point mutation exhibits a low level of antitermination in a $nus^+$ host that is lowered more in a *nusA1* host (Fig. 2). Collectively, these latter results suggest that NusA acts independently of *boxA*, in confirmation of our previous results (Zuber *et al.*, 1987).

Deletion of 10 bases (Δ10) between *cro* and *boxA* (leaving *boxA* intact) causes a small but reproducible stimulation of N-mediated antitermination in $nus^+$ (Fig. 2). As indicated, this antitermination is dependent upon both NusA and NusB.

(d) *NusB competition with a transcription antitermination inhibitor*

To explain our results, we suggest that the *boxA* region can inhibit antitermination, and this inhibition is counteracted by binding of NusB protein to *boxA*. The inhibition by *boxA* may be direct or mediated through another protein that binds *boxA*. In either case, deletion of *boxA* would obviate the requirement for NusB. By either model, the antitermination phenotype of base substitution mutations in *boxA* would depend on whether they alter the inhibitor function or prevent the action of the NusB anti-inhibitor.

We tested the specific hypothesis that an inhibitory factor can bind to *boxA* using a previously developed *in vivo* competition assay (Friedman *et al.*, 1990). The competition assay takes place in a cell with two added components: (1) A plasmid with a cloned *nut* region downstream of the inducible $p_{TAC}$ promoter to allow regulated transcription of the *nut* region. (2) An infecting λ to determine the effectiveness of N-mediated antitermination as measured by phage burst. If there is an inhibitory factor in the cell that binds to the *boxA* region, the model predicts that competition between NusB and the putative inhibitory factor for *boxA* could regulate the outcome of N-mediated transcription antitermination and phage development. By using a *nusB5* mutant to host the competition experiment, we limit the functional levels of NusB in the cell and restrict the burst of the infecting λ, presumably because the inhibitor competes effectively with the limiting NusB for the λ *boxA* sites, thereby preventing N antitermination. We predict that transcripts of the wild-type *boxA* sequence would bind NusB preferentially. In the *nusB5* mutant host, this binding would further limit the level of NusB. Transcripts of the mutant *boxA5* sequences, on the other hand, would be expected to select and bind the inhibitor instead of NusB.

The yields of λ following infection of the *nusB5* host in the competition assay (Fig. 3) are consistent with the proposal that an inhibitor binds *boxA* and binds the *boxA5* mutant more avidly. In the presence of the fully transcribed (induced with IPTG) plasmid-based *nut* region with *boxA5*, there is substantially greater phage production than in the

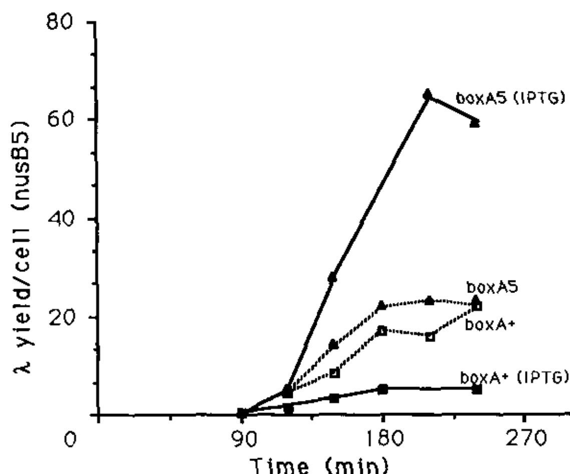


**Figure 3.** Suppression of the *nusB5* mutation by transcription of a plasmid-based *nut* region with the *boxA5* mutation. For experimental protocol see Materials and Methods. The yield of λ per infected bacterium (carrying the *nusB5* mutation) is shown at various times after infection. Data are shown for 1 of 3 independent experiments in which similar results were found. Maximum phage yields from the same bacterium but containing the vector plasmid pKK223-3 were ~20 per cell with or without the addition of IPTG (kinetic data not shown.)

non-induced control. In contrast, in the presence of the fully transcribed wild-type *boxA* region, there is reduced phage production relative to the uninduced control. In the controls where transcription of the *nut* regions is not induced (no ITPG), neither the presence of wild-type nor the *boxA5* mutant plasmid suppresses the *nusB5* mutation; the phage bursts are low and similar to those found in *nusB5* cells without the plasmid (data not shown).

The increased λ burst caused by transcription (induced with IPTG) of the plasmid based *nut* region containing *boxA5* supports the idea that there is titration of an inhibitory function. Likewise, the decreased λ burst caused by transcription of the wild-type *nut* region ($boxA^+$) is consistent with its RNA competing for the limited amount of NusB activity in the *nusB5* mutant cell. Neither of these results is easily explained by the alternative model in which *boxA* RNA directly inhibits the antitermination complex.

## 4. Discussion

Genetic and biochemical studies have shown that N-mediated antitermination requires, in addition to N protein, host Nus proteins and *cis*- acting sites called *nut*. These studies show that N and Nus proteins bind to the RNA transcript of *nut* and modify RNA polymerase (Das, 1992; Roberts 1993). The *nut* segment is composed of at least two components: *boxB* which interacts with N, and *boxA*, a nine-nucleotide sequence upstream of *boxB*, which interacts with host factors (Friedman & Gottesman,

1983). We have analyzed several deletion and substitution mutants of the *nutR* site for their effect on transcription antitermination at the *tR1* terminator. As expected, deletions of the *nutR* site including *boxA* and *boxB* eliminate antitermination at *tR1* completely. Interestingly other deletion mutations that encompass *boxA* alone are not defective for N-mediated transcription antitermination at either the Rho-dependent terminator *tR1* (Fig. 2) or the Rho-independent terminator *tI* (Zuber *et al.*, 1987). Thus, surprisingly, deletions that remove all of the *boxA* sequence exert minimal effects on N-mediated antitermination, whereas single base substitution mutations, *boxA5* and *boxA16*, severly reduce antitermination (Olson *et al.*, 1984; Robledo *et al.*, 1990). Adding to the complexity, the *boxA* deletions that fail to interfere with efficient N-mediated antitermination nevertheless change the requirements for host Nus factors. When the *nut* region is intact, antitermination is significantly reduced if the bacterium lacks any one of the functional Nus proteins. If, however, the *nut* region contains a deletion of the *boxA* segment (e.g. *boxA*Δ*37*), antitermination shows reduced dependence on NusE and no dependence on NusB. Antitermination observed in the *nusB5* mutant is not dependent upon residual NusB activity since a similar level of *boxA*-independent antitermination is also observed in a strain with an IS10 insertion mutation in *nusB*. These results are consistent with previous studies suggesting that *boxA* is the site at which NusB interacts to carry out its role in N-dependent transcription antitermination (Friedman *et al.*, 1990), and they also suggest an additional role for *boxA*.

Point mutations in *boxA* could be more defective for antitermination than deletions if the *boxA* region of λ has the added potential to interfere with the transcription antitermination process. The *boxA* RNA itself may bind to and inhibit some component of the transcription antitermination complex. Alternatively, a host factor may bind to *boxA* and inhibit the transcription antitermination process. Considering the results of a competition assay, we favor the latter explanation and propose that the function of NusB protein, at least in part, is to prevent this interference and allow transcription antitermination. In the competition experiments, transcription of a plasmid-based *nutR* region containing the *boxA5* mutation suppressed the defect in λ development caused by the host *nusB5* mutation. This suggests that the mutant *boxA5* RNA removes a *trans*-acting inhibitor allowing more effective expression of phage functions. Further, this would imply that the *boxA5* RNA has a relatively greater affinity for the inhibitor than does the analogous wild-type *nutR* RNA and/or that NusB does not compete effectively with the inhibitor for binding the *boxA5* mutant RNA (Fig. 4). Accordingly, when λ carries the *boxA5* mutation in *nutR* , the inhibitor would be selectively bound resulting in a failure of N-dependent transcription antitermination of rightward transcription and poor expression of functions essential for lytic growth (Olson *et al.*, 1984; Robledo *et al.*, 1990; N. Costantino, Z. Zhang & D. Court, unpublished results). In an analogous manner, when the *nut* region located on the plasmid used in the competition assay has the *boxA5* mutation, it would selectively bind the inhibitory factor, lowering the effective cellular concentration of this factor thereby reducing the requirement for NusB. When *boxA* is deleted, NusB would not bind *nut* RNA, but, if the inhibitor also cannot bind, NusB is not needed. The relative concentrations of inhibitor and NusB in the cell should influence the function of N and resultant phage development.

Surprisingly *nut* regions with short deletions removing just *boxA* are quite defective for antitermination, while those with longer deletions that remove upstream sequences in addition to *boxA*, are not defective. However, both *nut* regions with small or large deletions are NusB-independent for their N-mediated antitermination. Presumably the inhibitor site remains at least partly functional in the shorter deletions. The recognition site for the inhibitor may only partially overlap that of NusB (Fig. 4B); it is also possible that there is more than one type of inhibitor and site. The longer deletions of *boxA* include the translation termination site for the *cro* gene of λ (Fig. 4); such a site is suspected to bind translation termination factors (Craigen & Caskey, 1987). We find that a deletion of the ten bases between *cro* and *boxA* had a small but reproducible stimulatory effect on N-mediated antitermination that is NusB-dependent. Since NusB has been previously implicated in translation control (Taura *et al.*, 1992), we speculate that other translation factors may be able to compete with NusB and regulate the activity of the N-mediated transcription antitermination complex by binding to this segment upstream of *boxA*.

We have demonstrated previously (Friedman *et al.*, 1990) that variants of the *boxA* sequence, both natural and mutant, observed in lambdoid phages can be ranked in a hierarchical order on the basis of function. The functionally most effective *boxA* sequence, $boxA_{con}$, is also the consensus sequence derived by a comparison of the naturally occurring *boxA* sequences. When $boxA_{con}$ (CGCTCTTTA) is substituted for the natural sequence (CGCTCTTAC) in the λ *nutR* region, defects in λ growth caused by *nus* mutations are suppressed (Friedman *et al.*, 1990). High-level transcription of the plasmid-based *nut* region containing $boxA_{con}$ impedes λ growth, suggesting that $boxA_{con}$ RNA competes for cell factor(s) limiting to N-dependent transcription antitermination. Competition for λ growth is reversed by supplying excess NusB from a plasmid. Thus, NusB appears to be the limiting component that is titrated by $boxA_{con}$ RNA, either by interacting directly with $boxA_{con}$ or by interacting with another factor bound to $boxA_{con}$. This interpretation is supported by *in vitro* studies (Mason *et al.*, 1992*a*; Nodwell & Greenblatt, 1993) that shows NusB and S10 form a complex with each other and bind to the

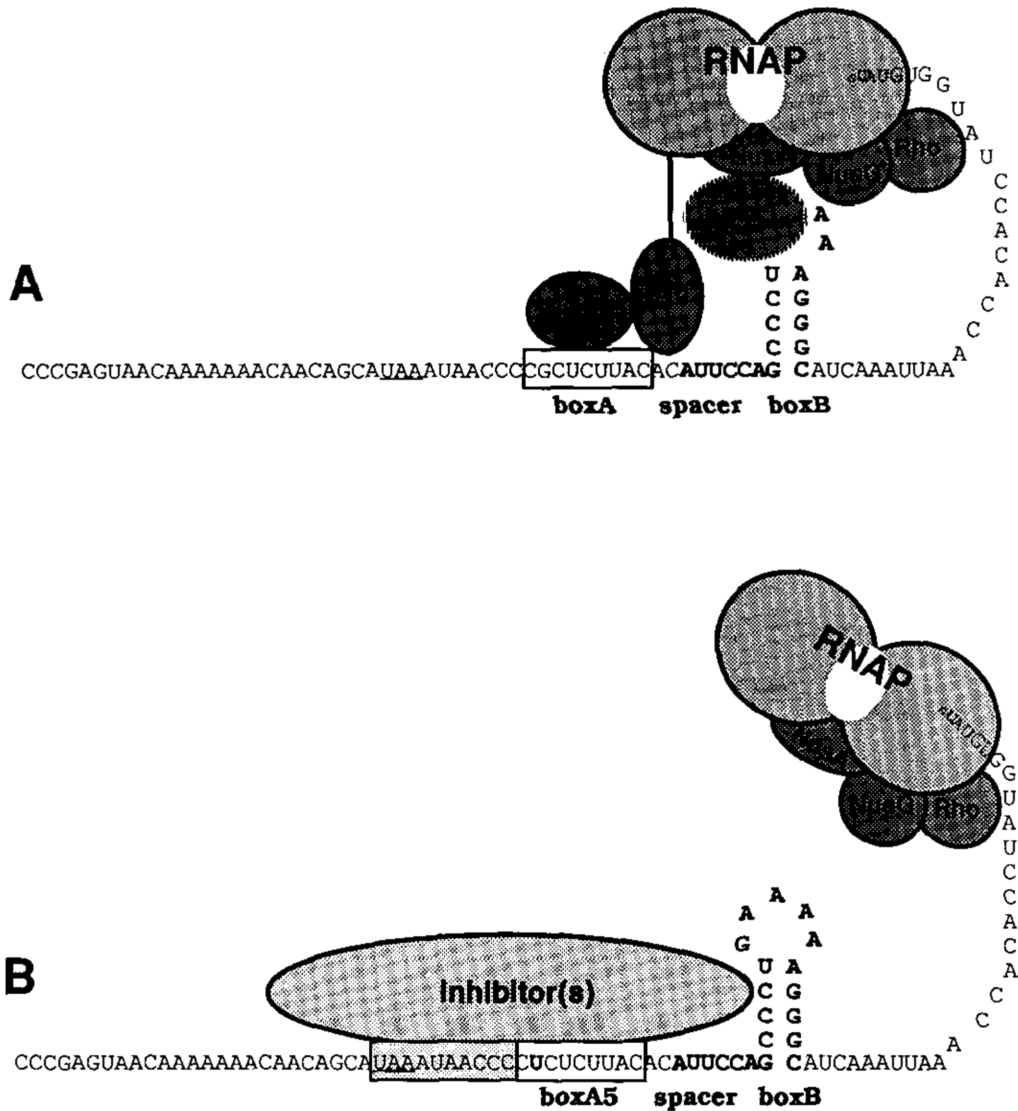


**Figure 4.** Model for NusB regulation at *boxA*. A, The sequence of the RNA segment from the *cro* gene of $\lambda$ through the *nutR* signal is shown with a representation of how RNA polymerase (RNAP) and the Nus proteins might interact at the *nut* site. The *boxA* sequence is enclosed in the open rectangle, and the *boxB* sequence is defined by the stem-loop. The spacer sequence includes the 8 bases between *boxA* and *boxB*. The bases of the spacer and *boxB* that appear in bold indicate the minimal *nutR* sequence that is required for N-dependent transcription antitermination (Zuber *et al.*, 1987). The underlined UAA sequence is the translation stop codon for the $\lambda$ *cro* gene. NusA, S10, and NusG are shown as contacting RNA polymerase; connections between any 2 proteins indicate binding has been demonstrated (Greenblatt & Li, 1981; Mason & Greenblatt, 1991; Li *et al.*, 1992). NusB is placed above the *boxA* RNA to which it is presumed to bind, and N is shown at the loop of *boxB* to which it binds (Das, 1992). RNA beyond the *nutR* region remains attached to the transcribing RNA polymerase. B, The same RNA segment but containing the *boxA5* mutation (G→U change in bold type face) is shown with the representation of how RNA polymerase and Nus proteins might interact when inhibitor binds, preventing an antitermination complex. The 10 bases upstream of *boxA* are shaded to indicate a sequence that with *boxA* may be involved in binding inhibitor protein(s). This complex will terminate at the nearby Rho-dependent *tR1* terminator.

$boxA_{con}$ sequence found in the leader regions of *rrn* operons. This RNA binding requires a mixture of both NusB and S10; individually, neither binds. Thus, *in vitro*, Nodwell & Greenblatt (1993) could not demonstrate which component of the NusB-S10 complex bound directly to $boxA_{con}$ nor could they demonstrate binding to the wild-type *boxA* sequence of $\lambda$ *nut*. They determined that the functional hierarchy of *boxA* sites reflects an affinity for the NusB-S10 complex; the most efficient site being $boxA_{con}$. Our results indicate that *in vivo* it is NusB that binds to the *boxA* portion of $\lambda$ *nutR*. This binding presumably requires the NusB-S10 complex. We do not understand why binding to the $\lambda$ *boxA* RNA could not be demonstrated *in vitro* (Nodwell & Greenblatt, 1993) unless a component(s) active *in vivo* was missing in the *in vitro* reactions, or as suggested by Roberts (1993), the *boxA* sequence in $\lambda$ functions differently from *boxA* of the *rrn* operons used by Nodwell & Greenblatt (1993).

For example, the adjacent λ *boxB* signal may alter the *boxA* requirements.

According to the model proposed here, we suggest that NusB-S10 binds *boxA* and prevents inhibitor(s) from binding. By binding to *boxA*, NusB may also facilitate the entry of S10 into the antitermination complex (Fig. 4A). In the absence of NusB (or with the *boxA5* mutant) inhibitor(s) bound at *boxA* might prevent S10 from interacting with RNA polymerase to form a stable complex (Fig. 4B). The *boxA5* mutant that we postulate to bind the putative inhibitor, for example, is as defective (11% antitermination in Fig. 2) as a *nusE71* mutant (16% antitermination in Table 3). It remains to be determined how the requirement for S10 is satisfied in the *boxA*Δ*37* deletion mutant. Note that in the deletion mutant, $nusE^+$ cells were some twofold more active for antitermination than the *nusE71* cells (Table 3). This suggests that S10 is still required for full activity and therefore might enter the antitermination complex by an alternative pathway, i.e. one that is independent of binding with NusB to *boxA*. In this regard, *in vitro* experiments by Mason & Greenblatt (1991) have indicated that S10 may bind to RNA polymerase independently of NusB.

The regulatory role for NusB and the *boxA* RNA raises another question. Do NusB and *boxA* also partake in the actual process of transcription antitermination by the N system, or do they only act in a regulatory role by modulating the binding of the inhibitory factor and bringing S10 efficiently to the antitermination complex? N-dependent transcription antitermination *in vitro* can proceed with only N, NusA, and *nut* components present (Whalen *et al.*, 1988). This minimal system is able to function in the absence of *boxA*; however, the efficiency of the minimal system decreases significantly as the distance between *nut* and the terminator is increased (W. Whalen & A. Das, personal communication). It is postulated that other Nus proteins are required to stabilize the N-NusA minimal complex in order to maintain efficient transcription antitermination over long distances (Mason *et al.*, 1992*b*). In this regard, it is possible that in the absence of *boxA* and NusB, the *in vivo* efficiency of transcription antitermination will be high only for terminators, like *tR1*, near to *nut*; distant terminators may require NusB as part of the antiterminator complex. We should note, however, that the minimal NusB- and boxA-independent transcription antitermination observed here still requires the other Nus factors for full antitermination at *tR1*, while the minimal system observed *in vitro* requires only N and NusA (Whalen *et al.*, 1988; Mason *et al.*, 1992*b*). Thus, there may be reason to question whether the *in vitro* reaction fully mimics what occurs *in vivo*.

We thank Asis Das, Robert Weisberg, Nina Costantino, Stanley Brown, Santanu Dasgupta, and Helen Wilson for helpful comments on the manuscript and critical discussions. Julie Ratliff provided excellent technical and editorial help. Research was sponsored in part by the National Cancer Institute, DHHS, under contract no. NO1-CO74101 with ABL and NIH grant AI1459-10. The contents of this publication do not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products, or organizations imply endorsement by the U.S. Government.

## References

Adhya, S. & Miller, W. (1979). Modulation of the two promoters of the galactose operon of *Esherichia coli*. *Nature (London)*, **279**, 492–494.

Barik, S., Ghosh, W., Lazinski, D. & Das, A. (1987). An antitermination protein engages the elongating transcription apparatus at a promoter-proximal recognition site. *Cell*, **50**, 885–899.

Baron, J. & Weisberg, R. A. (1992). Mutations of the phage λ *nutL* region that prevent the action of Nun, a site-specific transcription termination factor. *J. Bacteriol.* **174**, 1983–1989.

Berg, K. L., Squires, C. & Squires, C. L. (1989). Ribosomal RNA operon antitermination: function of the leader and spacer region *boxB-boxA* sequences and their conservation in diverse micro-organisms. *J. Mol. Biol.* **209**, 345–358.

Craigen, W. J. & Caskey, C. T. (1987). The function, structure and regulation of *E. coli* peptide chain release factors. *Biochimie*, **69**, 1031–1041.

Das, A. (1992). How the phage lambda *N* gene product suppresses transcription termination: communication of RNA polymerase with regulatory proteins mediated by signals in nascent RNA. *J. Bacteriol.* **174**, 6711–6716.

Das, A., Gottesman, M. E., Wardwell, J., Trisler, P. & Gottesman, S. (1983). A mutation in the *Escherichia coli rho* gene that inhibits the N protein activity of phage λ. *Proc. Nat. Acad. Sci., U.S.A.* **80**, 5530–5534.

Doelling, J. H. & Franklin, N. C. (1989). Effect of all single base substitutions in the loop of boxB on antitermination of transcription by bacteriophage λ's N protein. *Nucl. Acids Res.* **17**, 5565–5577.

Franklin, N. C. (1985). Conservation of genome form but not sequence in the transcription antitermination determinants of bacteriophages λ, φ21, and P22. *J. Mol. Biol.* **181**, 75–84.

Friedman, D. I. (1988). Regulation of phage gene expression by termination and antitermination of transcription. In *The Bacteriophages* (Calendar, R., ed.), vol. 2, pp. 263–319, Plenum Publishing Corp., New York.

Friedman, D. I. & Gottesman, M. (1983). Lytic mode of lambda development. In *Lambda II* (Hendrix, R., *et al.*, eds), pp. 21–51, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Friedman, D. I. & Olson, E. R. (1983). Evidence that a nucleotide sequence, "*boxA*", is involved in the action of the NusA protein. *Cell*, **34**, 143–149.

Friedman, D. I., Wilgus, G. S. & Mural, R. J. (1973). Gene N regulator function of phage λimm21: evidence that a site of N action differs from a site of N recognition. *J. Mol. Biol.* **81**, 505–516.

Friedman, D. I., Baumann, M. & Baron, L. S. (1976). Cooperative effects of bacterial mutations affecting lambda *N* gene expression. I. Isolation and characterization of a *nusB* mutant. *Virology*, **73**, 119–127.

Friedman, D. I., Schauer, A. T., Baumann, M. R., Baron, L. S. & Adhya, S. L. (1981). Evidence that ribosomal protein S10 participates in the control of transcription termination. *Proc. Nat. Acad. Sci., U.S.A.* **78**, 1115–1118.

Friedman, D. I., Olson, E. R., Georgopoulos, C., Tilly, K., Herskowitz, I. & Banuett, F. (1984). Interactions of bacteriophage and host macromolecules in the growth of bacteriophage lambda. *Microbiol. Rev.* **48**, 299–325.

Friedman, D. I., Olson, E. R., Johnson, L. L., Alessi, D. & Craven, M. (1990). Transcription-dependent competition for a host factor: the function and optimal sequence of the phage $\lambda$ *boxA* transcription antitermination signal. *Genes Develop.* **4**, 2210–2222.

Georgopoulos, C. P. (1971). Bacterial mutants in which the gene *N* function of bacteriophage lambda is blocked have an altered RNA polymerase. *Proc. Nat. Acad. Sci., U.S.A.* **68**, 2977–2981.

Greenblatt, J. & Li, J. (1981). Interaction of the sigma factor and the *nusA* gene protein of *E. coli* with RNA polymerase in the initiation-termination cycle of transcription. *Cell*, **24**, 421–428.

Horwitz, R. J., Li, J. & Greenblatt, J. (1987). An elongation control particle containing the *N* gene transcriptional antitermination protein of bacteriophage lambda. *Cell*, **51**, 631–641.

Keppel, F., Georgopoulos, C. P. & Eisen, H. (1974). Host interference with expression of the $\lambda$ *N* gene product. *Biochimie*, **56**, 1505–1509.

Lazinski, D., Grzadzielska, E. & Das, A. (1989). Sequence-specific recognition of RNA hairpins by bacteriophage antiterminators requires a conserved arginine-rich motif. *Cell*, **57**, 207–218.

Li, J., Mason, S. W. & Greenblatt, J. (1993). Elongation factor NusG interacts with termination factor rho to regulate termination and antitermination of transcription. *Genes Develop.* **7**, 161–172.

Mason, S. W. & Greenblatt, J. (1991). Assembly of transcription elongation complexes containing the N protein of phage lambda and the *Escherichia coli* elongation factors NusA, NusB, NusG, and S10. *Genes Develop.* **5**, 1504–1512.

Mason, S. W., Li, J. & Greenblatt, J. (1992*a*). A direct interaction between two *Escherichia coli* transcription antitermination factors, NusB and ribosomal protein S10. *J. Mol. Biol.* **2223**, 55–56.

Mason, S. W., Li, J. & Greenblatt, J. (1992*b*). Host factor requirements for processive antitermination of transcription and suppression of pausing by the N protein of bacteriophage lambda. *J. Biol. Chem.* **267**, 19418–19426.

McKenney, K., Shimatake, H., Court, D., Schmeissner, U., Brady, C. & Rosenberg, J. (1981). A system to study promoter and terminator signals recognized by *Escherichia coli* RNA polymerase. In *Gene Amplification and Analysis. Vol II. Structural Analysis of Nucleic Acids* (Chirikjian, J. G. & Papas, T. S., eds), pp. 383–415, Elsevier/North-Holland, New York.

Morgan, E. A. (1986). Antitermination mechanisms in rRNA operons of *Escherichia coli*. *J. Bacteriol.* **168**, 1–5.

Nodwell, J. R. & Greenblatt, J. (1991). The *nut* site of bacteriophage $\lambda$ is made of RNA and is bound by transcription antitermination factors on the surface of RNA polymerase. *Genes Develop.* **5**, 2141–2151.

Nodwell, J. R. & Greenblatt, J. (1993). Recognition of *boxA* antiterminator RNA by the *E. coli* antitermination factors NusB and ribosomal protein S10. *Cell*, **72**, 261–268.

Olson, E. R., Flamm, E. L. & Friedman, D. I. (1982). Analysis of *nutR*: a region of phage lambda required for antitermination of transcription. *Cell*, **31**, 61–70.

Olson, E. R., Tomich, C. S. & Friedman, D. I. (1984). The NusA recognition site. Alteration in its sequence or position relative to upstream translation interferes with the action of the N antitermination function of phage lambda. *J. Mol. Biol.* **180**, 1053–1063.

Patterson, T. A., Costantino, N., Dasgupta, S. & Court, D. L. (1993). Improved bacterial hosts for regulated expression of genes from $\lambda p_L$ plasmid vectors. *Gene*, **132**, 83–87.

Peltz, S. W., Brown, A. L., Hasan, N., Podhajska, A. J. & Szybalski, W. (1985). Thermosensitivity of a DNA recognition site: activity of a truncated *nutL* antiterminator of coliphage lambda. *Science*, **228**, 91–93.

Roberts, J. W. (1993). RNA and protein elements of *E. coli* and $\lambda$ transcription antitermination complexes. *Cell*, **72**, 653–655.

Robledo, R., Gottesman, M. E. & Weisberg, R. A. (1990). $\lambda$ *nutR* mutations convert HK022 Nun protein from a transcription termination factor to a suppressor of termination. *J. Mol. Biol.* **212**, 635–643.

Rosenberg, M., Court, D., Shimatake, H., Brady, C. & Wulff, D. L. (1978). The relationship between function and DNA sequence in an intercistronic regulatory region in phage lambda. *Nature (London)*, **272**, 414–423.

Salstrom, J. S. & Szybalski, W. (1978). Coliphage lambda *nutL*$^-$: a unique class of mutants defective in the site of gene *N* product utilization for antitermination of leftward transcription. *J. Mol. Biol.* **124**, 195–221.

Schauer, A. T., Carver, D. L., Bigelow, B., Baron, L. S. & Friedman, D. I. (1987). Lambda N antitermination system. Functional analysis of phage interactions with the host NusA protein. *J. Mol. Biol.* **194**, 679–690.

Schmidt, M. C. & Chamberlin, M. J. (1984). Amplification and isolation of *Escherichia coli* nusA protein and studies of its effects on *in vitro* RNA chain elongation. *Biochemistry*, **23**, 197–203.

Sharrock, R. A., Gourse, R. L. & Nomura, M. (1985). Inhibitory effect of high-level transcription of the bacteriophage lambda *nutL* region on transcription of rRNA in *Escherichia coli*. *J. Bacteriol.* **163**, 704–708.

Shiba, K., Ito, K. & Yura, T. (1986). Suppressors of the *secY24* mutation: identification and characterization of additional *ssy* genes in *Escherichia coli*. *J. Bacteriol.* **166**, 849–856.

Squires, C. L., Greenblatt, J., Li, J., Condon, C. & Squires, C. L. (1993). Ribosomal RNA antitermination *in vitro*: requirement for Nus factors and one or more unidentified cellular components. *Proc. Nat. Acad. Sci., U.S.A.* **90**, 970–974.

Steege, D. A., Cone, K. C., Queen, C. & Rosenberg, M. (1987). Bacteriophage lambda *N* gene leader RNA: RNA processing and translational initiation signals. *J. Biol. Chem.* **262**, 17651–17658.

Sullivan, S. S. & Gottesman, M. E. (1992). Requirement for the *E. coli* NusG protein in factor-dependent transcription termination. *Cell*, **68**, 989–994.

Swindle, J., Zylicz, M., Georgopoulos, C., Li, J. & Greenblatt, J. (1988). Purification and properties of the NusB protein of *Escherichia coli*. *Nature (London)*, **292**, 10229–10235.

Takiff, H., Chen, S. & Court, D. L. (1989). Genetic analy-

sis of the *rnc* operon of *Escherichia coli*. *J. Bacteriol.* **171**, 2581–2590.

Taura, T., Ueguchi, C., Shiba, K. & Ito, K. (1992). Insertional disruption of the *nusB* (*ssyB*) gene leads to cold-sensitive growth of *Escherichia coli* and suppression of the *secY24* mutation. *Mol. Gen. Genet.* **234**, 429.

Ward, D. F., DeLong, A. & Gottesman, M. E. (1983). *Escherichia coli NusB* mutations that suppress *nusA1* exhibit λ N specificity. *J. Mol. Biol.* **168**, 73–85.

Warren, F. & Das, A. (1984). Formation of a termination-resistant transcription complex at phage λ *nut* locus: effects of altered translation and a ribosomal mutation. *Proc. Nat. Acad. Sci., U.S.A.* **81**, 3612–3616.

Whalen, W. & Dass, A. (1990). Action of an RNA site at a distance: role of the *nut* genetic signal in transcription antitermination by phage-λ *N* gene product. *New Biologist*, **2**, 975–991.

Whalen, W., Ghosh, B. & Das, A. (1988). NusA protein is necessary and sufficient *in vitro* for phage λ *N* gene product to suppress a Rho-dependent terminator placed downstream of *nutL*. *Proc. Nat. Acad. Sci., U.S.A.* **85**, 2494–2498.

Zuber, M., Patterson, T. A. & Court, D. L. (1987). Analysis of *nutR*, a site required for transcription antitermination in phage λ. *Proc. Nat. Acad. Sci., U.S.A.* **84**, 4514–4518.

*Edited by M. Gottesman*

*(Received 16 July 1993; accepted 20 October 1993)*