Proc. Natl. Acad. Sci. USA
Vol. 84, pp. 4514–4518, July 1987
Genetics

Analysis of *nutR*, a site required for transcription antitermination in phage λ

(termination/*boxA*/NusA protein/λ N protein/translation)

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Communicated by Harold J. Evans, February 19, 1987

ABSTRACT **Deletions extending from the *cro* gene into *boxA* and *nutR* of the Rho-dependent t_{R1} terminator of bacteriophage λ have been generated and cloned between promoters and the *galK* gene of *Escherichia coli* on a multicopy plasmid. Terminators placed between the promoters and *galK* restrict transcription and expression of *galK* on these plasmids. However, when λ N protein is provided, and if a functional N interaction site, *nutR*, is intact, transcription antitermination occurs and *galK* expression increases. Deletions into the *nutR* region affect the ability to antiterminate. From the results obtained we conclude that: (*i*) *boxA*, a site believed to bind host factors (Nus), is not required for transcription antitermination in this system; (*ii*) the host NusA function is required even in the absence of *boxA*; (*iii*) *nutR* is required for N antitermination; (*iv*) translation across the *nutR* sequence prevents N-dependent antitermination.**

The *N* gene product of bacteriophage λ positively regulates phage early gene expression by antitermination of transcription at various terminator signals (see ref. 1). Salstrom and Szybalski (2) isolated a cis-acting mutation, *nutL*, in the P_L operon that impairs N protein-mediated antitermination activity. A similar site of protein N action in the right operon of phage λ, referred to as *nutR*, has also been identified on the basis of sequence homology to *nutL* (3), genetic deletion studies (4), and cloning experiments (5). In addition, an octamer sequence, CGCTCTTA, called *boxA*, located just promoter-proximal to *nutR*, has been implicated in *Escherichia coli* host protein NusA interaction (6, 7). The host proteins involved in antitermination are a complex of factors including at least the *nusA*, *nusB*, and *nusE* gene products [see review by Friedman *et al.* (8) and ref. 9]. To reveal the individual cis-acting components that participate in interaction with various phage- and host-encoded factors, deletions were generated *in vitro* in *boxA* and *nutR* and assayed for their effect on antitermination activity *in vivo*.

MATERIALS AND METHODS

Strains. Bacterial strains are listed in Table 1. Plasmids [pFW1 (11), pKG1800 and pKG100 (12), pMZ105 (13), and pMS3 (14)] were used to construct the plasmids used for the experiments summarized in Tables 2, 3, and 4 (see *Methods* below). Phages λ*bio*10 *c*I857, λ*imm*434, λ*imm*434 *N*am7 *N*am53, λ*imm*434 *nin*, and P1kc are from the National Institutes of Health collection.

Strain Constructions. MZ1 was constructed from N5271 (see Table 1). N5271 was lysogenized by λ*bio*10 *c*I857. The lysogen remains *bio*$^-$ but can grow on the biotin intermediate desthiobiotin; it is also temperature sensitive for cell growth at 42°C. Temperature sensitivity is caused by inactivation of the λ*c*I857 repressor and the induction of λ; growth on desthiobiotin is permitted by the product of the *bioB* gene of λ*bio*10. Homologous recombination can eliminate the λ*bio* phage. Such cells cured of the λ can be selected as the rare cells (<1%) that survive and form colonies on plates at 42°C. Some of these survivors remain *N*am7 *N*am53 like N5271, whereas others are N^+ (i.e., complement a λ*imm*434 N^- phage for growth at 42°C). One of these was saved as MZ1.

MZ2 was derived from MZ1 by P1 cotransduction of the *cya*$^-$ marker with *ilv*::Tn*10*. Tetracycline-resistant transductants were screened for a lactose-negative (*cya*$^-$) phenotype on MacConkey/lactose indicator plates.

DC1101 and DC1102 were made from MZ1 and N5271, respectively, by P1 cotransduction of the *nusA1* marker with *argG*::Tn*5*. The *nusA*$^-$ strain is characterized by the inability of λ*imm*434 to cause plaques at 42°C, whereas λ*imm*434 *nin* can cause plaque (15).

Generation of Deletions in pMZ105. Plasmid DNA (pMZ105) was linearized by cutting with *Hin*dIII (Fig. 1). The two 3′-OH ends of this DNA were resected with exonuclease III (by 100 to 300 nucleotides), and the resulting DNA with 5′ single-strand overhangs was digested with S1 nuclease (16). The flush ends were joined with T4 DNA ligase in the presence of phosphorylated *Hin*dIII linkers (CCCAAGCTTGGG). This DNA mixture was used to transform *E. coli* strain C600. Plasmid DNAs that had undergone the deletions were isolated and digested at the *Hin*dIII linker, as well as the *Pst* I site in the *bla* gene, with the respective enzymes, and the deletion fragment containing the t_{R1} terminator was purified by gel electrophoresis. The deletion end at *Hin*dIII was sequenced by following the Maxam and Gilbert (17) technique. Each *Hin*dIII–*Pst* I deletion segment was joined to the reciprocal *Hin*dIII–*Pst* I segment of pMZ105 containing the P_{gal} promoter to produce a set of *bla*$^+$ (ampicillin-resistant) plasmids with deletions originating at the *Hin*dIII site of pMZ105 and extending toward t_{R1} (Fig. 1).

Construction of pMZ215 and Its Deletion Derivatives. Terminator t_I on a DNA fragment that extends from 27,481 bp to 27,632 bp on the λ map (see ref. 18) was placed beyond the t_{R1} terminator of pMZ105. The joint between t_{R1} and the t_I clone was made at the *Nde* I site near *c*II (indicated in Fig. 1). The t_I segment came from plasmid pMS3 (14), a pKG1800 derivative in which *galK* follows t_I. Thus, a t_I *galK* segment between two *Nde* I sites was used to replace *c*II *galK* in pMZ105. In this way, the *c*II gene segment was replaced by t_I; one such construct, pMZ51, contains the t_I substitution beyond t_{R1} (Fig. 1). The P_{gal} promoter of pMZ51 was replaced with the P_{lac} promoter from pFW1 (11) to form the plasmid pMZ215 (Fig. 2). DNA from pMZ51 was first cut with *Eco*RI and repaired to a flush end with the Klenow fragment of DNA polymerase. After extraction with phenol, the DNA was cut with *Hin*dIII, and the large fragment containing *bla* and *galK*

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Table 1. Bacterial strains

Strain	Markers	Source
C600	*leu pro thr lacY tonA supE44*	NIH
N5271*	*his ilv rpsL galK*$_{am}$ *pgl*Δ8 (*bio–uvrB*)ΔH1	NIH
MZ1*	*his ilv rpsL galK*$_{am}$ *pgl*Δ8 (*bio–uvrB*)ΔH1	Our work
MZ2	MZ1 *cya*	Our work
DC1101	MZ1 *argG*::Tn*5 nusA1*	Our work
DC1102	N5271 *argG*::Tn*5 nusA1*	Our work
K1457	*galK argG*::Tn*5 nusA1 rpsL*	Friedman

NIH, National Institutes of Health bacterial stocks.
*N5271 and MZ1 carry a defective λ prophage. The genetic structure of the prophage is altered by two major deletion mutants. One is Δ*Bam* in the P_L operon (10); the other is ΔH1, which deletes the *cro* gene and all other λ prophage genes to the right of *cro*. Only three λ genes remain intact in this prophage: *N*, *rex*, and *c*I. In N5271, the *N* gene carries two amber mutations, *N*am7 and *N*am53, and the *c*I gene carries the temperature-sensitive mutation *c*I857. MZ1 is identical to N5271 except that the prophage is N^+.

was purified from an agarose gel. DNA from plasmid pFW1 was cut with *Pvu* II and *Hin*dIII. The small fragment containing P_{lac} was purified from a gel, mixed with the large *galK* fragment, and joined by DNA ligase. The correct plasmid, pMZ215, was detected by restriction analysis after transformation. The *Eco*RI site is restored at the *Eco*RI–*Pvu* II junction.

The other deletion isolates of pMZ105 were recombined in a similar way with the t_I terminator and the P_{lac} promoter to generate a set of deletion plasmids with t_{R1} and t_I between P_{lac} and *galK*.

The P_{gal} promoter is approximately 30–50% stronger than the P_{lac} promoter as measured in the *galK* vectors (ref. 12; M.Z. and D.L.C., unpublished data). Galactokinase levels from the P_{lac} promoter are dependent upon cAMP—i.e., *cya*$^-$ strains are defective for galactokinase, and addition of cAMP restores galactokinase to comparable levels in a *cya*$^+$ strain (data not shown; also see ref. 11).

Enzymes and Other Materials. Enzymes were obtained from New England Biolabs. [^{14}C]Galactose (58 mCi/mmol; 1

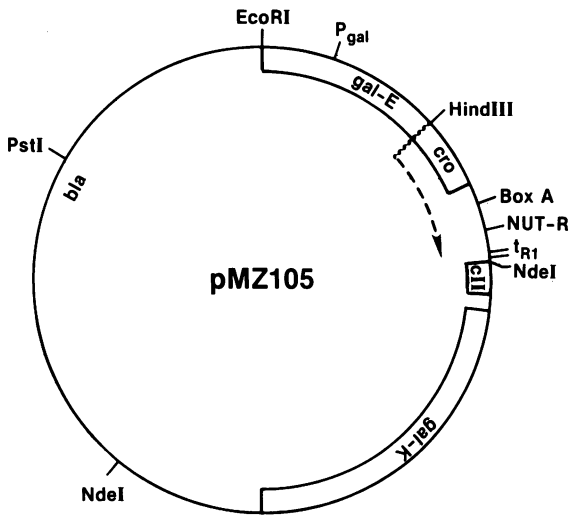

FIG. 1. Plasmid pMZ105 has the galactose promoter (P_{gal}), the *galE* structural gene to the *Hin*dIII site, and the *galK* structural gene of plasmid pKG1800 (12). Within the *Sma* I site of pKG1800 was inserted the *Hae* III–*Hin*cII fragment [399 base pairs (bp)] of λ that contains the distal part of the *cro* gene, the end of the *c*II gene that encodes the amino terminus of the protein, and the intercistronic region (*boxA*, *nutR*, t_{R1}). The broken line and arrow indicate the position and direction of the deletions produced. Restriction enzyme sites used in this work for other plasmid constructions are shown (*Eco*RI, *Hin*dIII, *Nde* I, *Pst* I).

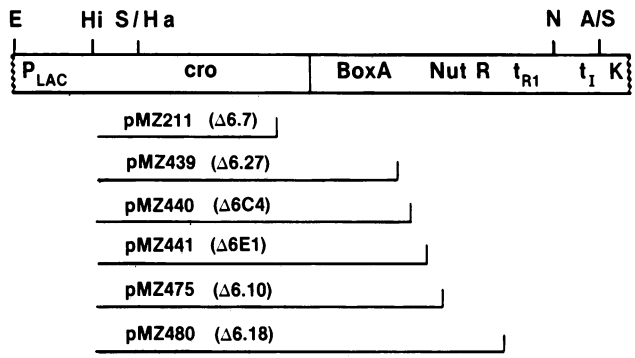

FIG. 2. Plasmid pMZ215 is developed to monitor N antitermination. The wild-type promoter P_{lac} is indicated. Transcription initiates at P_{lac} and extends rightward. This transcript lacks both a ribosome binding site and AUG initiation signal. The portion of the *cro* gene encoding the carboxyl end of the protein is present; the vertical bar represents the *cro* UAA codon. The terminators t_{R1} and t_I are positioned in tandem before *galK* and beyond *nutR*. Restriction sites: E, *Eco*RI; Hi, *Hin*dIII; Ha, *Hae* III; S, *Sma* I; N, *Nde* I; A, *Alu* I. The slashes represent hybrid sites joined by blunt-end ligation. Deletion endpoints are indicated below the map as nucleotide base pair position on the λ map (18): pMZ211 (38,231), pMZ439 (38,258), pMZ440 (38,260), pMZ441 (38,262), pMZ475 (38,268), and pMZ480 (38,320). At each deletion junction are the *Hin*dIII site and the sequence AAGCTTGGG followed by the λ nucleotide at the position indicated above (also see Fig. 3).

Ci = 37 GBq) is from Amersham. *Hin*dIII linkers are from New England Biolabs. Enzyme reaction conditions used are as specified by the supplier. Other DNA manipulations are taken from Maniatis *et al.* (19).

Galactokinase Enzyme Assays. Bacterial cells grown overnight in M56 minimal medium with fructose as carbon source were diluted 1:50 in fresh medium and were grown at 32°C to OD_{650} of about 0.2 (≈1 × 10^8 cells per ml) in preparation for the experiments. One milliliter of cells from each culture was treated by the method described by McKenney *et al.* (12). Galactokinase units were measured and expressed as nanomoles of galactose phosphorylated per minute per OD_{650}. In these strains, galactokinase levels are unaffected by the presence of fucose, an inducer of the *gal* operon (data not shown). The multicopy plasmid titrates *gal* repressor in this system (20). Plasmid copies per cell in different strains varied less than 2-fold as determined by quantitation of plasmid DNA yields.

RESULTS

λ N-dependent transcription antitermination has been reproduced on plasmids containing the *boxA* and *nut* sequences from λ (5, 7, 11, 21). In these plasmid systems, λ N function is required for antitermination and can be provided in trans from a prophage. In similar plasmids we will analyze the requirements for the *boxA nutR* segment of λ during antitermination by generating a set of deletions that dissect the *boxA nutR* segment and by determining each deletion's effect on antitermination. A transcription vector has been developed to specifically study this problem with the deletion mutants.

Transcription Antitermination Vector and Deletion Mutants. The vector pMZ215 (Fig. 2) is designed to allow transcription initiation at the normal *lac* promoter. However, the ribosome binding site and the AUG translation initiation signals have been eliminated to prevent translation of the transcript. Note that this same promoter region was used by Warren and Das (11) to show that upstream translation was not an essential component of N-dependent antitermination. The galactokinase gene, *galK*, from the galactose operon is located on the vector beyond the promoter. This is the same

galK construct that exists on the transcription vector pKG1800 (12). Between the *lac* promoter and *galK*, Rho-dependent (t_{R1}) and Rho-independent (t_I) terminators have been cloned in tandem. The dual terminator arrangement has been employed by others to reduce N^- galactokinase levels and increase the sensitivity of the assay for N function (6, 11). The *boxA nutR* region of λ is present in its natural location on the t_{R1} terminator DNA segment. Deletion mutants of pMZ215 were derived *in vitro*. These deletions removed DNA from the *Hin*dIII site into the *cro* t_{R1} region. The exact location of each deletion endpoint was determined by DNA sequence analysis (Fig. 2).

N-Dependent Antitermination. Two sites have been defined as being involved in N-dependent antitermination. The N-utilization site *nut* is believed to be specific for the N protein. The site has been defined by point mutations that prevent antitermination (2) and by the homology between *nutL* and *nutR* (3); 16 of 17 bases in these two regions are identical (see Fig. 3). Thus, *nutR* is defined as the 17-base segment from 38,265 bp to 38,281 bp on the λ map. Just 7 bases upstream of *nutL* and 8 bases upstream of *nutR* is a second conserved sequence in this region (see Fig. 3). It is 8 bases long and is called *boxA* (1). Specific point mutations in *boxA* can also affect N-dependent antitermination (22). We have analyzed the deletions that dissect this region (Figs. 2 and 3) for their effect on N-dependent galactokinase expression. Cells containing the plasmids can be monitored in either N^- or N^+ conditions. In experiment 1 of Table 2, N^- or N^+ conditions were achieved in the same strain by growth at 32°C or 42°C, respectively. At 32°C the λcI857 temperature-sensitive repressor protein in the cell (MZ1) represses the *N* gene of the prophage, whereas at 42°C the repressor is inactive and *N* is expressed. In experiment 2, N^- and N^+ conditions were both achieved under derepressed conditions at 42°C by using two strains, either an N^- prophage strain (N5271) or the N^+ prophage strain (MZ1). In both experiments, the results are similar. In the parental vector, N-dependent antitermination occurs, resulting in a high level of galactokinase in the N^+, as compared to the N^-, condition. Interestingly, deletions (Δ6.27 and Δ6C4) that remove *boxA* have little effect on the level of N-dependent antitermination, whereas deletions (Δ6.10 and Δ6.18) that remove *boxA* and *nutR* are defective for this antitermination property. One deletion (Δ6E1), which removes only *boxA* and extends two bases beyond 6C4, has also lost most of its N-dependent antitermination activity. Thus, *boxA* appears dispensable for N-dependent antitermination in this system, whereas *nutR*, and perhaps some additional signals between *boxA* and *nutR*, is essential.

Host NusA Requirement. If *boxA* is dispensable for N-dependent antitermination as suggested in the previous section, are the host Nus factors required for antitermination? This is a particularly important question for NusA, which is postulated to interact with *boxA* not only in λ antitermination but also at *boxA* sites in the *E. coli* genome to modulate transcription termination (1, 23). If NusA must interact with *boxA* to exert its effect, then we are led to the conclusion that NusA should also be dispensable for N antitermination. To test this, a set of isogenic strains, *nusA*$^+$ and *nusA1*, carrying each of the plasmids tested previously has been constructed and examined for antitermination activity. The result observed (Table 3) is that *nusA*$^+$ is required in all of the plasmids tested that showed N-dependent antitermination—i.e., pMZ215, pMZ211, pMZ439, and pMZ440. Thus, we are led to conclude that *nusA* can exert its effect independently of the presence of *boxA*.

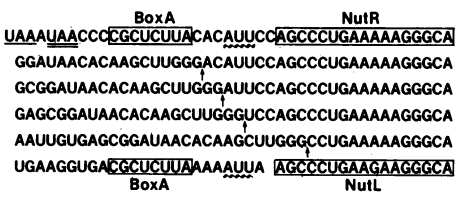

FIG. 3. Sequences of the *boxA* and *nut* regions of wild-type and deletion strains. Vertical arrows indicate the extent of the deletions shown. Deletion Δ6.7 has the wild-type (WT) sequence (top line). The underlined UAA is the *cro* gene stop signal in wild-type λ. The double-underlined UAA is the position at which terminating ribosomes have been shown to prevent N antitermination (7, 11). The *boxA* and *nut* sequences are in rectangles. The wavy lines indicate the AUU sequence common to all sequences active for antitermination. Note there are seven bases between *nutL* and *boxA*, but eight between the normal *nutR* and *boxA*. "Activity" refers to N antitermination.

Table 2. N-dependent antitermination: Effect of deletions

				Galactokinase			
				Exp. E1		Exp. E2	
Plasmid	Δ	*boxA*	*nutR*	N^+	N^-	N^+	N^-
pMZ215	—	+	+	58	3	99	3
pMZ211	6.7	+	+	66	7	84	6
pMZ439	6.27	−	+	63	14	69	20
pMZ440	6C4	−	+	48	4	69	8
pMZ441	6E1	−	+	22	9	37	13
pMZ475	6.10	−	−	18	12		
pMZ480	6.18	−	−	19	17		

Two experiments are presented, E1 and E2. In both experiments bacterial cultures were grown as described in *Materials and Methods*. In E1, the strain MZ1 was used. The N^+ condition was induced by cell growth at 42°C, whereas the N^- condition was maintained at 32°C. In E2, two strains were used: the N^+ condition was in MZ1 at 42°C as in E1, and the N^- condition was in N5271 at 42°C. The column Δ indicates the particular deletion allele number in this plasmid. In five experiments, the galactokinase levels under N^+ conditions varied (e.g., from 58 to 101 for pMZ215), however, the relative values within an experiment remained approximately the same as those in E1 and E2. In the N^- condition, there is a constant trend in all five sets of experiments. The larger deletions have greater N^- galactokinase levels because the t_{R1} terminator is inactive. Thus, in pMZ215 and pMZ211 there is always an additive effect of t_{R1} and t_I, whereas in the larger deletions only t_I is active (M.Z. and D.L.C., unpublished data).

Translation of *nutR* Inhibits Antitermination. Ribosome positioning has been found to influence antitermination at *nutR* (6, 7, 11). In phage λ DNA, the *cro* gene is located promoter proximal to the *boxA nutR* region. Its translation stops at a UAA codon seven bases before *boxA* (see Fig. 3). Frameshift mutants that cause ribosomes to move four bases beyond *cro* to a second UAA codon prevent N-dependent antitermination, whereas when ribosomes stop at the normal UAA codon four bases away, antitermination is unaffected. Ribosomes that stop within the *cro* gene also have no effect

Table 3. N-dependent antitermination: Effect of NusA*

				Galactokinase		
				N^+		N^-
Plasmid	Δ	*boxA*	*nutR*	*nusA*$^+$	*nusA1*	*nusA1*
pMZ215	−	+	+	72	8	1
pMZ211	6.7	+	+	60	6	5
pMZ439	6.27	−	+	55	15	10
pMZ440	6C4	−	+	64	18	4
pMZ441	6E1	−	+	24	13	11
pMZ475	6.10	−	−	22	15	16
pMZ480	6.18	−	−	20	25	22

*Conditions are as described for Table 2. N^+ is provided at 42°C by MZ1 (*nusA*$^+$) or DC1101 (*nusA1*). The N^- *nusA1* strain is DC1102 at 42°C.

on antitermination. To explain the inhibiting effect, it was suggested that ribosomes idling at the distal UAA nonsense codon might sterically block site(s) required for antitermination activity. Another possibility, not tested yet, is that ribosome translation through the RNA in the *nut* region may prevent this RNA from binding antitermination factors such as Nus, N, or even RNA polymerase. To test this, deletion mutants described above were joined downstream of the galactose operon promoter and fused to the first 140 codons of the structural gene *galE*. This allows translation from *galE* to enter the *cro* region.

Plasmid pMS3 is a control: it contains only the t_I terminator without the N recognition region of the *cro*–t_{R1} segment. As expected, it produces the same levels of galactokinase under N^+ or N^- conditions. Plasmid pMZ51 contains the *cro*–t_{R1} segment upstream of t_I. In this construct, translation from the *galE* gene stops within the *cro* gene message and, as others have shown under similar conditions (11, 22), N is able to antiterminate transcription. The N^- level of expression is reduced relative to pMS3 because of the additive effect of both terminators, t_{R1} and t_I (Table 4).

In plasmid pMZ49 the *galE* gene is fused to *cro* at the Δ6.7 deletion *Hin*dIII joint. In this case, unlike in pMZ51, translation from *galE* does not terminate within *cro* but passes beyond *cro* and through the t_{R1} terminator. Here no N-dependent antitermination occurs. The level of galactokinase is similar in N^+ and N^- conditions (Table 4). These levels are those found for pMS3 because translation through the t_{R1} terminator prevents Rho-dependent termination and only t_I is active (M.Z. and D.L.C., unpublished data).

Plasmid pMZ109 is identical to pMZ49 except that the *Hin*dIII joint between *galE* and *cro* has been modified by digesting with *Hin*dIII, resynthesis with the Klenow fragment of DNA polymerase, and ligation with T4 DNA ligase. This creates the sequence AAGCUAGCUU in the RNA. In this sequence, the UAG is the translation stop signal for *galE*, thereby preventing translation beyond the *cro* gene. In this construct, transcription antitermination by N occurs and yields higher levels of galactokinase than in the primary construct, pMZ49 (Table 4). In pMZ125 the *Hin*dIII site of pMZ49 was resected after *Hin*dIII treatment with S1 nuclease. In this fusion, *galE* and *cro* translation is in frame and stops at the normal *cro* UAA codon. Again, N is able to antiterminate in this condition (Table 4).

Table 4. N-dependent antitermination: Effect of translation

Plasmid	Galactokinase	
	N^+	N^-
pMS3	28	30
pMZ51	118	4
pMZ49	35	38
pMZ109	95	2
pMZ125	87	6

Plasmid pMS3 contains just the t_I terminator between P_{gal} and the *galK* gene (14). From pMS3, the units of galactokinase should be unaffected by *N* since no *nut* site is present. Plasmid pMZ51 is identical to pMZ215 except that it contains P_{gal} and part of the *galE* structural gene. In the same way, pMZ49 is analogous to pMZ211. Plasmids pMZ109 and pMZ125 are derived from pMZ49: pMZ49 was digested with *Hin*dIII and then, to make pMZ109, DNA polymerase was used to fill in the sticky ends before ligation. The plasmid pMZ109 has a new restriction site, *Nhe* I, created by this treatment. To make pMZ125, the sticky ends at the *Hin*dIII site were removed with S1 nuclease and joined with ligase. Translation from *galE* terminates upstream of the *cro* UAA stop codon (see Fig. 3) in plasmids pMZ51 and pMZ109 and at the *cro* UAA codon in pMZ125. In plasmid pMZ49 translation proceeds beyond *cro* and the *nutR* region to a stop codon between t_{R1} and t_I. The reason for higher levels of galactokinase in pMZ49 under N^- conditions is that the Rho-dependent t_{R1} terminator is not active because of translation into t_{R1} (M.Z. and D.L.C., unpublished data).

DISCUSSION

An analysis has been made of the *nutR* t_{R1} region of λ to determine the effect of deletion mutations and the act of translation on N-dependent antitermination. This analysis was carried out on plasmid vectors designed to analyze antitermination of transcripts by measuring changes in levels of galactokinase produced from the plasmids. Several deletions were isolated and characterized for their effect on antitermination.

The *boxA*, *nutR*, and NusA Requirements. Antitermination on the vector pMZ215 is N dependent. Deletions Δ6.27 and Δ6C4 remain active for antitermination despite the fact that *boxA* is deleted. Longer deletions, Δ6.10 and Δ6.18, are defective for antitermination, whereas the deletion Δ6E1 may retain some antitermination activity. Deletions Δ6.10 and Δ6.18 remove *nutR*, whereas Δ6E1 retains the 17 nucleotides conserved between *nutR* and *nutL* (Table 2). By comparing all of the deletion sequences with the wild-type *nutR* and *nutL* sequences, it appears that the 17-nucleotide *nut* sequence (2, 3), plus additional nucleotides between *boxA* and *nut*, are important. In this regard we note an AUU sequence common to all fully active sites but missing from the defective sites (Fig. 3). Thus, part or all of the AUU sequence may be required in common with the *nut* sequence for activity, but *boxA* itself is not required. Note that the deletion junction sequences of Δ6.27 and Δ6C4 do not recreate a *boxA* site. We also note that there is no other *boxA* site in the transcribed region between the promoters and the t_I terminator.

Host Nus factors are thought to interact with *boxA* to allow N antitermination; in particular, the factor NusA has been implicated in association with *boxA* (6, 7, 22). The fact that *boxA* is not required here provoked us to ask whether NusA is required. The result is that NusA is still required in the presence or absence of a *boxA* site (Table 3). This result allows us to suggest that NusA may recognize sites other than *boxA* itself or interacts directly with a protein component of the antitermination complex.

There is a discrepancy between the results here and those found in other laboratories; that is, the requirement for *boxA* in antitermination (21, 22, 24). The results of Olson *et al.* (22) are more easily compared with our results because the vector and *nutR* fragments examined were nearly the same. It is more difficult to compare results of Peltz *et al.* (21) and Brown and Szybalski (24). They used synthesized DNA cassettes for *boxA* and *nutR* and thereby changed the sequence between and at either end of *boxA* and *nutR* in their studies, sequences we believe may be important for antitermination activity. It should be mentioned, however, that Drahos *et al.* (25) and Peltz *et al.* (21) found that, in certain conditions, *boxA* could be deleted in their system, and N-dependent antitermination activity could be retained.

There are major differences between the system used by Olson *et al.* (22) and that used here: first, the promoter P_{gal} was used to test the requirement for *boxA* as opposed to P_{lac}; second, translation of the *galE* segment beyond P_{gal} occurs in their plasmid and not in the P_{lac} plasmid; third, although the t_{R1} region was identical between the two systems, the second terminator used here was the Rho-independent t_I terminator [Olson *et al.* (22) used the Rho-dependent terminator of the insertion element IS2]; last, Olson *et al.* (22) changed a single base in *boxA* in causing the defect, whereas our constructs are deletions of *boxA*. A careful analysis of each of these variables, as well as exchanging the systems, may be required to understand these differences and at the same time allow us to better understand the complex N-antiterminator system.

There is reason to believe that NusA protein interacts with RNA (23) and is required to interact with the *boxA* site at certain times for N antitermination and for RNA polymerase pausing (7, 22). However, a *boxA* interaction is not always required for NusA to exert its effect. NusA protein has been found to accentuate transcription termination *in vitro* at the rRNA terminator T1 and at the λ terminator t_{R2} in the absence of an upstream *boxA* signal (28). Finally, we note here that the Q antiterminator system appears to be much simpler than that for *N* (26). Q-dependent antitermination can proceed *in vitro* with a suitable DNA template and only RNA polymerase; NusA protein (without additional Nus factors) stimulates Q antitermination. However, deletion mutants lacking a normal *boxA* are also antiterminated.

Translation of the *nutR* Region. Translation of the *cro* gene of phage λ does not normally interfere with transcription antitermination by N at *nutR*. However, when ribosomes proceed beyond the *cro* UAA codon to a second UAA codon just 4 bases away (see Fig. 3), N-dependent antitermination is affected (7, 11). The model to explain this is that ribosomes idling at the distal UAA codon during translation termination sterically prevent a protein from binding at *boxA* on the RNA (22). Since we have suggested that the N binding site may be as close as 15 bases from this UAA codon, a distance easily encompassed by a ribosome (27), it is possible that these ribosomes may also block N binding at *nutR*.

We have shown that ribosomes translating through the *nutR* region prevent N antitermination (Table 4), as do ribosomes that terminate at a second UAA codon beyond the normal *cro* UAA codon (22). In the former case, the ribosome would not be idling at a stop codon, but actively translating the *nutR* RNA. Whether idling or actively translating, ribosomes may be exerting a similar steric hindrance for N (or Nus) binding to RNA.

We thank D. Friedman and A. Honigman for helpful discussions during this work, and we appreciate the excellent typing and editing of the manuscript by K. Cannon.

1. Friedman, D. I. & Gottesman, M. (1983) in *Lambda II*, eds. Hendrix, R. W., Roberts, J. W., Stahl, F. W. & Weisberg, R. A. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), pp. 21–51.
2. Salstrom, J. S. & Szybalski, W. (1978) *J. Mol. Biol.* **124,** 195–221.
3. Rosenberg, M., Court, D., Shimatake, H., Brady, C. & Wulff, D. L. (1978) *Nature (London)* **272,** 414–423.
4. Dambly-Chaudiere, C., Gottesman, M., Debouck, C. & Adhya, S. (1983) *J. Mol. Appl. Genet.* **2,** 45–56.
5. de Crombrugghe, B., Mudryj, M., DiLauro, R. & Gottesman, M. (1979) *Cell* **18,** 1145–1151.
6. Olson, E. R., Flamm, E. L. & Friedman, D. I. (1982) *Cell* **31,** 61–70.
7. Friedman, D. I. & Olson, E. R. (1983) *Cell* **34,** 143–149.
8. Friedman, D. I., Olson, E. R., Georgopoulos, C., Tilly, K., Herskowitz, I. & Banuett, F. (1984) *Microbiol. Rev.* **48,** 299–325.
9. Das, A. & Wolska, K. (1984) *Cell* **38,** 165–173.
10. Gottesman, M. E., Adhya, S. & Das, A. (1980) *J. Mol. Biol.* **140,** 57–75.
11. Warren, F. & Das, A. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 3612–3616.
12. McKenney, K., Shimatake, H., Court, D., Schmeissner, U., Brady, C. & Rosenberg, M. (1981) in *Gene Amplification and Analysis, Vol. II: Structural Analysis of Nucleic Acids*, eds. Chirikjian, J. G. & Papas, T. S. (Elsevier/North-Holland, New York), pp. 383–415.
13. Tsugawa, A., Kurihara, T., Zuber, M., Court, D. L. & Nakamura, Y. (1985) *EMBO J.* **4,** 2337–2342.
14. Montañez, C., Bueno, J., Schmeissner, U., Court, D. L. & Guarneros, G. (1986) *J. Mol. Biol.* **191,** 29–37.
15. Friedman, D. (1971) in *The Bacteriophage Lambda*, ed. Hershey, A. D. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), pp. 733–738.
16. Guo, L. & Wu, R. (1983) *Methods Enzymol.* **100,** 60–95.
17. Maxam, A. M. & Gilbert, W. (1980) *Methods Enzymol.* **65,** 499–560.
18. Daniels, D., Schroeder, J., Szybalski, W., Sanger, F., Coulson, A., Hong, G., Hill, D., Peterson, G. & Blattner, F. (1983) in *Lambda II*, eds. Hendrix, R. W., Roberts, J. W., Stahl, F. W. & Weisberg, R. A. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), p. 521.
19. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).
20. Irani, M. H., Orosz, L. & Adhya, S. (1983) *Cell* **32,** 783–788.
21. Peltz, S. W., Brown, A. L., Hasan, N., Podhajska, A. J. & Szybalski, W. (1985) *Science* **228,** 91–93.
22. Olson, E. R., Tomich, C.-S. C. & Friedman, D. I. (1984) *J. Mol. Biol.* **180,** 1053–1063.
23. Nakamura, Y., Mizusawa, S., Court, D. L. & Tsugawa, A. (1986) *J. Mol. Biol.* **189,** 103–111.
24. Brown, A. L. & Szybalski, W. (1986) *Gene* **42,** E125–E132.
25. Drahos, D., Galluppi, G. R., Caruthers, M. & Szybalski, W. (1982) *Gene* **18,** 343–354.
26. Grayhack, E. J., Yang, X., Lau, L. F. & Roberts, J. W. (1985) *Cell* **42,** 259–269.
27. Gold, L., Pribnow, D., Schneider, T., Shinedling, S., Singer, B. S. & Stormo, G. (1981) *Annu. Rev. Microbiol.* **35,** 365–403.
28. Schmidt, M. & Chamberlin, M. J. (1987) *J. Mol. Biol.* **195,** in press.