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Mutations of the Phage λ *nut*L Region That Prevent the Action of Nun, a Site-Specific Transcription Termination Factor

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Phage HK022 encodes a protein, Nun, that promotes transcription termination within the p_L and p_R operons of its relative, phage λ. The λ sequences required for termination had previously been shown to overlap the *nut* sites, which are essential for transcription antitermination during normal λ growth. To further specify the Nun target and to determine its relation to the *nut* sites, we constructed deletion and base substitution mutations of the λ *nut*L region and measured Nun-dependent reduction of the expression of a downstream reporter gene. The shortest construct that retained full Nun responsiveness was a 42-bp segment that included both *boxA* and *boxB*, sequences that have been implicated in λ antitermination. Deletion of *boxA* reduced Nun termination, and deletion of both sequences eliminated Nun termination. Base substitutions in *boxA* and the proximal portion of *boxB* impaired Nun termination, while base substitutions between *boxA* and *boxB*, in the distal portion of *boxB*, and immediately downstream from *boxB* had no appreciable effect. The termination defect of all of the base substitution mutations was relieved by increasing the level of Nun protein; in contrast, the deletions and a multiple-base substitution did not regain full Nun responsiveness at elevated Nun concentrations. We also asked if these mutant *nut* regions retained their ability to interact with N, the λ-encoded antitermination protein. A qualitative assay showed that mutations within *boxA* or *boxB* reduced interaction, while mutations outside *boxA* and *boxB* did not. These data show that (i) the recognition sites for N and Nun overlap to a very considerable extent but are probably not identical and (ii) a high concentration of Nun promotes its interaction with mutant *nut* sites, a behavior also reported to be characteristic of N.

Phage λ is unable to transcribe most of its early genes in cells lysogenic for the related phage HK022. Such lysogens contain Nun, an HK022-encoded protein that promotes termination of transcription at specific sites within the λ p_L and p_R operons (16). Nun-promoted termination requires the *Escherichia coli* NusA, NusB, NusG (1, 22), and NusE proteins and specific nucleotide sequences whose locations overlap those of the λ *nut*L and *nut*R sites (16). These sequences probably function in the form of RNA transcripts, since translation of the *nut* region blocks Nun action (16). The Nus factors and *nut* sites have the opposite role in normal λ infection: together with the λ N protein they change RNA polymerase to a terminator-resistant form and thereby promote transcription of early λ genes that are located downstream of transcription termination sites (6). When N and Nun are present in the same cell, the level of expression of a reporter gene located downstream of *nut*L or *nut*R depends on the N/Nun ratio, suggesting that the two proteins compete for a common target (16). Indeed, several base substitution mutations within the *nut* sites prevent both termination by Nun and antitermination by N (16, 18). Moreover, Nun, like λ N, acts poorly or not at all on lambdoid phages with different *nut* sites (16).

nut sites consist of two separate regions that are important for antitermination, a promoter-proximal segment called *boxA* and a promoter-distal segment called *boxB* (6, 7). The *boxA* sequence is moderately well conserved among several lambdoid phages, and the *boxB* sequence, although not conserved from one phage to another, usually contains two short inverted repeats (the stem) separated by a short spacer (the loop). The sequence requirements for Nun-mediated termination are less well understood. We previously reported the effects on Nun termination of two sets of deletions that removed sequences downstream of λ *nut*L or sequences upstream of λ *nut*R (16). The combined results suggested that a 60-bp region containing *boxA*, *boxB*, and some flanking sequence confers full Nun responsiveness. A deletion of part of *boxB* and base substitution mutations in *boxA* and the *boxB* loop abolished Nun termination (16, 18).

To define more precisely the sequence requirements for Nun termination and the relationship between the Nun target and the *nut* site, we synthesized a set of deletion and substitution mutations of the λ *nut*L site and characterized their ability to confer Nun or N responsiveness.

MATERIALS AND METHODS

E. coli. TAP106, constructed and kindly provided by T. Patterson, was used as a host for most plasmid experiments. The *lac* operon has been deleted, and this strain contains a defective λ prophage that lacks all phage genes except for *c*I857, which encodes a thermosensitive repressor, and *rex*A and *rex*B, which are irrelevant to this study. We also used TAP112 (25), which carries the same defective prophage in a different genetic background, as well as *nus*A1, *nus*B5, and *nus*E71 derivatives of this strain. N5947 (13) was used as a host for the multiple lysogen experiments. It is deleted for *lacZ* and contains a defective λ *c*I857 prophage with inactivating mutations in genes *N*, *int*, and *xis* and a deletion of gene *kil* and all phage genes to the right of *c*I (deletion H1).

Bacteriophages. B305 was constructed by crossing the immunity region of phage 21 into λ P118 of M. Berman and R. Zagursky (1a). λ P118 has the carboxyl-terminal coding

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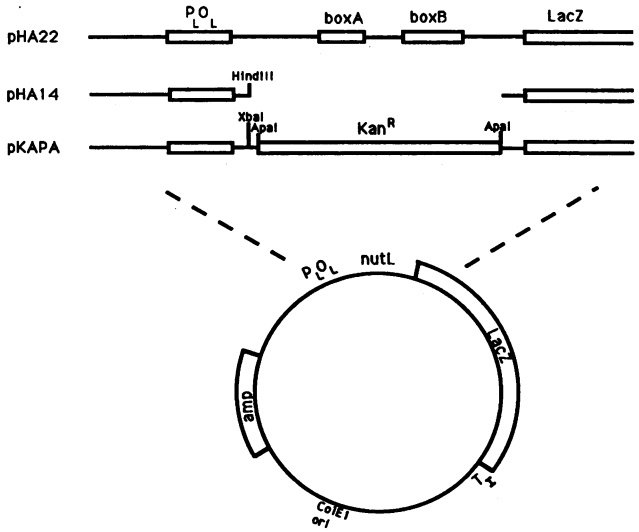

FIG. 1. Plasmids used to construct *nut*L region mutations. A map showing the common features of the plasmids is shown at the bottom. They all contain a λ p_L-*lacZ* transcriptional fusion followed by the λ T_I transcription terminator (4). The dashed lines indicate the boundaries of the p_L-*lacZ* segments. pHA22 (16) contains the wild-type λ left operon up to base 75 of the transcript. This includes the functionally important *boxA* and *boxB* sequences of *nut*L (see Fig. 2). pHA14 (16) contains the wild-type λ p_L promoter up to the transcription start site followed by a *Hin*dIII linker and the *lacZ* gene. The map has been opened at the *Hin*dIII site to allow alignment with the other p_L-*lacZ* regions. pKAPA (see Materials and Methods) is a derivative of pHA14 containing a segment encoding kanamycin resistance flanked by *Xba*I and *Apa*I restriction sites. Deletion and base substitution mutations of the *nut*L region were constructed by replacing the longer *Xba*I-*Apa*I fragment of pKAPA with synthetic oligonucleotides (see Materials and Methods).

sequences of *lacZ* and the pBR322 *bla* genes inserted into the λ *b* region. Rescue of p_L-*lacZ* fusions from plasmid to phage chromosome is signaled by the reconstitution of functional *lacZ* and *bla* genes. O245 and O246 are plaque-forming derivatives of HK022 that carry mini-Tn*10* insertions: the mini-Tn*10* within *nun* for O245 and elsewhere for O246 (16). The phage culture techniques used were described previously (11, 16).

Plasmids and mutant construction. To construct a plasmid containing restriction sites suitable for insertion of partially single-stranded oligonucleotides between λ p_L and a *lacZ* reporter gene, we proceeded as follows. pHA14 is a derivative of pBR322 bearing a *lacZ* gene that is fused to p_L via a unique *Hin*dIII linker (5′-CAAGCTTG) at the start point of transcription (16) (Fig. 1). We inserted a linker containing an *Apa*I and *Xba*I site (5′-TCAGCATCTAGACTCGAGGGCC) into the *Hin*dIII site after nuclease digestion of the *Hin*dIII overhang and then inserted a segment encoding kanamycin resistance flanked by *Apa*I sites into the plasmid *Apa*I site (pKAPA [Fig. 1]). To insert mutant sequences downstream of the p_L start point, we cut pKAPA with *Xba*I and then *Apa*I and gel purified the largest fragment. This fragment, which has one 5′ protruding end and one 3′ protruding end, was religated in the presence of a synthetic oligonucleotide which contained a mutant *nut*L site and ends complementary to the cleaved *Xba*I and *Apa*I sites. The oligonucleotides used were completely double stranded for constructing the deletion mutations and partially double stranded (nucleotides 9 to 33 of parent clone [Fig. 2]) for constructing the base substitutions. The sequence of the insert was always confirmed by dideoxy sequencing. pNAS200, used to supply N protein, is a low-copy-number, pBR322-compatible plasmid carrying a p_{Lac}-*N* fusion (19). pNUN and pNUNΔ were constructed as follows. J. Oberto (10) inserted an HK022 *Rsa*I-*Rsa*I fragment containing the *nun* coding sequence into the *Hin*cII site of pUC18 (24), downstream of p_{Lac}, to form pJO89. The p_{Lac}-*nun* segment was excised from pJO89 by cutting with *Pvu*II, gel purified, and inserted into the *Hpa*I site of plasmid pST34 to create pNUN. pST34 is a member of the ColE1 family but is compatible with pBR322 (20). The *Pvu*II-*Pvu*II segment (containing p_{Lac}) from pUC18 was similarly inserted into the *Hpa*I site of pST34 to create pNUNΔ. Thus, pNUN differs from pNUNΔ by the presence of the *nun* gene.

β-Galactosidase assay. The cells were grown overnight in a 96-well microtiter plate in tryptone broth (1% tryptone [Difco], 0.5% NaCl) at 32°C with shaking. In the morning, serial dilutions were made into wells containing fresh L broth (tryptone broth supplemented with 0.5% yeast extract). The cells were then grown again at 32°C until they were in logarithmic phase. They were then shifted to 41°C and shaken for an additional 100 min. Culture optical densities and β-galactosidase activities were measured by using a microtiter plate reader and associated Lotus123 software essentially as described previously (14, 15). We found that our β-galactosidase activities were linear with cell extract concentration down to activities of 0.1, but we have chosen an activity of 1.0 as the minimum reliable detection limit. The values shown represent a single experiment, but each determination was repeated at least once. The standard errors of the mean Nun^+/Nun^- ratios were less than 0.9 of the mean for ratios less than 0.1 and less than 0.6 of the mean for ratios greater than 0.1.

```
                +1        *         *         *         *         *         *         *         *         *
Wild type P_L   ATCAGCA   GGACGCACTGACCACCATGAAGGTGACGCTCTTAAAAATTAAGCCCTGAAGAAGGGCAGCATTCAAAGCAGAA
                                                    boxA                  boxB
Parent clone    .......TCTA.....................................................................GGCCC
 (9-21)         .......TCTA.                    ...............................................GGCCC
 (9-33)         .......TCTA.                              .....................................GGCCC
 (9-46)         .......TCTA.                                                  .................GGCCC
 (9-65)         .......TCTA.                                                                  .....GGCCC
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FIG. 2. Comparison of the wild-type p_L transcript to those produced by deletion mutants generated by insertion of synthetic oligonucleotides. The wild-type DNA sequence from bases 1 to 80 of the p_L transcript, with *boxA* and *boxB* of *nut*L underlined, and the sequences of the longest oligonucleotide insertion (Parent clone) and of a set of progressively shorter deletion mutants are shown. Dots (.) represent bases unchanged from the wild-type sequence. Spaces have been inserted to facilitate alignment of the sequences. The TCTA insertion and final GGCCC nucleotides are contributed by the cloning sites of the vector.

RESULTS

Effect of *nut* region mutations on Nun termination. To define more precisely the limits of the site required for Nun termination, we used a set of transcriptional fusions of the λ p_L promoter to a promoterless *E. coli lacZ* gene. The parental fusion contains a wild-type λ *nut*L site between p_L and *lacZ*. We constructed a set of overlapping deletion mutations extending from a point just downstream of p_L toward *lacZ* (see Materials and Methods). The wild-type and deleted p_L-*nut*L-*lacZ* segments were transferred by homologous recombination from the plasmid on which they were constructed to B305, a derivative of λ that carries the immunity (*imm*) region of the related phage 21, and segments of the *lacZ* and *bla* genes in its *b2* region. We then constructed a pair of double lysogens containing the λ imm^{21} p_L-*nut*L-*lacZ* recombinant and either HK022 nun^+ or HK022 *nun*::Tn*10* (O246 or O245, respectively) (16). A diagram of the source and target of the Nun protein in this experiment is shown in the top panel of Fig. 3. The early promoters of the HK022 and λ imm^{21} prophages were controlled by their respective prophage repressors and remained repressed throughout the experiment. The HK022 nun^+ prophage expressed a low constitutive level of Nun from p_M, the promoter used for transcribing the repressor gene (2). To control the expression of p_L-λ on the Nun target, the host contained the λ *c*I857 gene, which encodes thermosensitive λ repressor. The Nun responsiveness of each deletion was determined by comparing the level of β-galactosidase activity in the two members of each pair of double lysogens after thermal induction of p_L-λ, and these results are presented in Table 1, panel A. We found that a 42-bp segment extending from base 34 to base 75 of the p_L transcript, which includes only *boxA*, *boxB*, the intervening bases, and 9 bp of λ sequence downstream from *boxB*, was sufficient to confer Nun responsiveness. A fusion that lacked *boxA* and part of the spacer between *boxA* and *boxB* conferred partial Nun responsiveness, while a fusion that lacked both sequences was Nun insensitive. These results show that bases upstream of *boxA* are not required for Nun-promoted termination and thus reduce our previous estimate of the size of the Nun target. Some of these deletion mutations stimulate the expression of *lacZ* in the absence of Nun (Table 1). We speculate that they may have removed a transcription terminator or a site that decreases message stability.

We then synthesized single-base-pair substitution mutations in the *boxA-boxB* region of *nut*L and determined their effect on Nun termination in multiple lysogens as described above (Table 2). All of the substitutions were transversions that changed the composition of the base pair (e.g., A-T → C-G) and are named by the wild-type and mutant base and their position in the p_L transcript. Substitutions G35T, C38A, and T39G are all located in *boxA*, and all impaired Nun responsiveness. Substitutions A43C and A45C fall between *boxA* and *boxB* and did not appreciably alter Nun responsiveness. Substitutions C51A and C53A lie in the ascending stem of *boxB* and decreased Nun responsiveness. Substitutions G55T and A57C are both in the loop of *boxB*, yet only substitution G55T had an effect. Neither substitution A60C, which lies in the descending stem, nor substitution G66T, which lies downstream from *boxB*, had any effect. Substitutions C67A and T70G, located downstream of *boxB*, had the unexpected effect of reducing strongly β-galactosidase expression. We have no explanation of this phenotype.

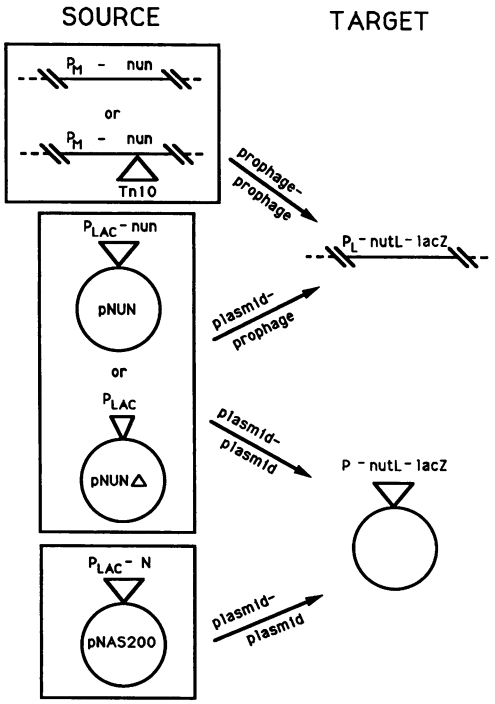

FIG. 3. Tests for responsiveness of a target *nut* site to Nun or N. In the prophage-prophage test (top arrow), the host strain was N5947, the source of Nun was a repressed HK022 prophage containing a wild-type *nun* gene driven by the phage p_M promoter, and the target of Nun was a second repressed prophage carrying a derepressed p_L-*nut*L-*lacZ* transcription fusion. A second double lysogen carrying an HK022 prophage with a disrupted *nun* gene served as a negative control. In the plasmid-prophage test (second arrow), the HK022 prophage was replaced by a high-copy-number plasmid containing a p_{Lac}-*nun* transcriptional fusion (pNUN) or the same plasmid lacking *nun* (pNUNΔ). No isopropyl-β-D-thiogalactopyranoside was added, and p_{Lac} may have been partially repressed. In the plasmid-plasmid test for Nun responsiveness (third arrow), the host strain was TAP106, which has the *lac* region deleted, and the prophage Nun target was replaced by a compatible high-copy-number plasmid carrying the same p_L-*lacZ* fusion. Finally, in the plasmid-plasmid test for N responsiveness (fourth arrow), the host was TAP106, TAP112, or a *nus* mutant derivative of TAP112, and the source of N was a low-copy-number plasmid carrying a p_{Lac}-*N* transcriptional fusion (pNAS200).

Effect of *nut* region mutations on N-*nut* interaction. To determine the effects of these mutations on interaction between *nut*L and the λ N antitermination factor, we used the following qualitative assay. When thermoinducible cells containing a plasmid with a p_L-*boxA-boxB-lacZ* fusion and an additional plasmid supplying N protein were incubated on antibiotic-containing plates at the inducing temperature, we observed no isolated colonies, only confluent patches where the initial inoculum was densest. This lethal effect required induction, N protein, a wild-type *boxA-boxB* region, and wild-type NusA, NusB, and NusE proteins (Table 3 and Table 1, panel C). To explain this observation, we propose that a functional *nut* site allows the N- and Nus-modified RNA polymerase to read through a terminator (T_I) located downstream of *lacZ* (Fig. 1) and that this read-through transcription prevents plasmid maintenance or the expression of plasmid-encoded antibiotic resistance. This proposal is consistent with the observations that transcription through the *ori* region or *rom* gene of ColE1-based plasmids reduces

TABLE 1. Nun and N responsiveness of deletion mutations in the *nut*L region

*nut*L region[a]	β-Galactosidase activity[b]						C (N responsiveness)[c]
	A			B			
	Nun⁻	Nun⁺	Nun⁻/Nun⁺ ratio	Nun⁻	Nun⁺	Nun⁻/Nun⁺ ratio	
Parental	64	<1	<0.02	550	17	0.03	+
Δ(9-21)	13	<1	<0.08	540	32	0.06	+
Δ(9-33)	25	<1	<0.04	830	28	0.03	+
Δ(9-46)	106	34	0.32	1,210	330	0.27	−
Δ(9-65)	158	133	0.84	2,380	1,450	0.61	−

[a] The parental and deleted *nut*L regions are those shown in Fig. 2.

[b] Panel A shows β-galactosidase activities in double lysogens in which *nun* or a *nun* disruption mutation was carried by one prophage (O246 or O245, respectively) and the indicated λ p_L-*nut*L-*lacZ* fusion was carried by the other prophage. *nun* was transcribed constitutively from the p_M promoter of its prophage, and the λ p_L promoter carried by the other prophage was thermally derepressed (see Materials and Methods). Panel B shows β-galactosidase activities in cells carrying two mutually compatible high-copy-number plasmids, one with the indicated λ p_L-*nut*L-*lacZ* fusion, the other with or without a *nun* insert (plasmid pNUN or pNUNΔ, respectively). In this case, *nun* was transcribed from a derepressed p_{Lac} promoter. See Fig. 3 for details.

[c] Panel C shows the response to N protein of cells carrying a plasmid copy of the indicated p_L-*lacZ* fusion as well as the N^+ plasmid pNAS200. N responsiveness (+) is indicated by the absence of colonies and N unresponsiveness (−) by their presence under the conditions described in the text and footnote *a* of Table 3.

copy number (21) and that cells carrying plasmids with a wild-type *boxA-boxB* region form colonies with high efficiency at the inducing temperature on plates lacking antibiotics, but these colonies consisted of cells lacking the plasmid (Table 3). In any event, the inability to form colonies at the inducing temperature must signal some aspect of the N-*nut*-Nus interaction, since it requires the integrity of all of these elements (see Discussion).

We therefore used this test to determine the ability of N to act on mutant *nut*L sites. We found that all of the deletions of and substitutions within *boxA* and *boxB* impaired N responsiveness, while those lying upstream of *boxA*, between *boxA* and *boxB*, or downstream from *boxB* had no apparent effect (Tables 1 and 2). These results suggest that the nucleotides required for Nun action overlap but do not entirely coincide with those required for N action. We note one unexpected finding with cells carrying mutation C53A, which failed to grow at the inducing temperature even in the absence of N protein (Table 2, footnote *b*). This mutation behaves as if it allows antitermination even in the absence of N.

Effect of increased Nun level on termination. To ascertain whether the termination defect of any of the *nut* region mutations could be suppressed by increasing the level of Nun, we determined the effect of supplying Nun from a high-copy-number plasmid containing a p_{Lac}-*nun* fusion (plasmid pNUN) instead of from a prophage. We assume that pNUN produces substantially more Nun than does a prophage, because the number of *nun* copies is higher and because p_{Lac} is probably stronger than p_M, the promoter for *nun* transcription in a repressed lysogen (2). With these conditions, the patterns of Nun responsiveness were not substantially changed for any of the deletion mutations, regardless of whether the target p_L-*nut*L-*lacZ* fusion was present on a prophage (data not shown) or on a compatible high-copy-number plasmid (Table 1, panel B). We obtained the same result for a Nun-resistant triple mutant (deletion of base 40 of *boxA* and the transversions G55T and G61T of the loop and descending stem, respectively, of *boxB*) (data not shown). In contrast, we found that all of the Nun-resistant single-base substitutions became responsive to Nun when the source of Nun was a high-copy-number plasmid and the target *nut* site was present in low copy number (Table 2), or when both the source and the target were present on compatible high-copy-number plasmids (data not shown). It therefore appears that the Nun-resistant phenotype of the single-base substitution mutations is suppressed by increasing the level of Nun, suggesting that the mutations hamper but do not totally block a step of termination that depends on Nun concentration. The *boxA-boxB* deletions and the triple mutant, all of which are resistant to even high levels of Nun, are either totally defective or interfere with another step of termination.

DISCUSSION

We have shown that a 42-bp segment of the λ p_L operon containing only *boxA*, *boxB*, the spacer between these two sequences, and 9 bp downstream of *boxB* is sufficient in the appropriate context to confer full Nun responsiveness. The phenotypes of base substitution mutations of this segment can be explained by postulating two regions of importance: the *boxA* sequence and a portion of *boxB* including the ascending stem and the proximal portion of the loop. These findings are consistent with and augment previous results involving changes in both *nut*R and *nut*L (16, 18).

We also tested our mutations for N responsiveness, using an assay in which N and Nus factors prevent maintenance of a plasmid with a transcribed, wild-type *nut*L site. It is very likely that our assay measures antitermination (i) because the requirements for N, *nut*, and Nus factors are highly characteristic of λ antitermination and (ii) because strong transcription across the *rom* gene or *ori* region of a ColE1-type plasmid has previously been found to interfere with plasmid replication (21). Indeed, the sequences found to be necessary for N action by our test are consistent with those previously found using a more direct antitermination assay (3, 12). Doelling and Franklin (3) have demonstrated the importance of each of the five bases in the loop of *boxB* as well as the two adjoining bases. In addition, several of their mutations within *boxA* were found to decrease N responsiveness. Our data confirm the importance of *boxA* and *boxB* for N responsiveness and the relative unimportance of the surrounding and intervening bases tested. In each case in which the same base pair substitution was made (G35T in *boxA* and G55T and A57C in *boxB*), our data agree with previous data obtained by direct assay for N antitermination (3, 12). In addition, a number of previously untested bases within *boxA* and *boxB* can now be identified as important for N action.

TABLE 2. Nun and N responsiveness of single-base-pair substitution mutations in the *nut*L region[a]

*Nut*L Muts.	β-galactosidase: Nun⁺/Nun⁻ Prophage-Prophage (Ratio)	Prophage-Prophage (Quotient)	Plasmid-Prophage	Response to: Nun	N
C^{34}					
B G→T	41/110	0.37	0.02	-	-
O C					
X T					
C→A	40/85	0.47	0.09	-	-
A T→G	35/47	0.75	<0.003	-	-
T^{40}					
A					
A					
A→C	<1/175	<0.006	N.D.	+	+
A					
A→C	<1/37	<0.03	N.D.	+	+
T					
T					
A					
A					
G^{50}					
C→A	80/541	0.15	<0.0005	-	+/-
C					
C→A	102/572	0.18	0.02	-	[b]
T					
B G^{c}→T	26/113	0.23	0.023	-	-
O A					
X A→C	<1/137	<0.007	N.D.	+	-
G					
B A					
A^{60}→C	<1/142	<0.007	N.D.	+	-
G					
G					
G					
C					
A					
G→T	<1/163	<0.006	N.D.	+	+
C→A		[d]	[d]		+
A					
T					
T^{70}→G		[d]	[d]		+

[a] The first column shows the sequence of the wild-type *nut*L region between base 34 and base 70 of the p_L transcript. *boxA* and *boxB* are indicated, and the vertical arrows show the inverted repeat of *boxB*. The mutations, shown by horizontal arrows, are transversions to base pairs of different composition. The second and third columns give the ratios and quotients, respectively, of β-galactosidase activities in the presence and in the absence of a prophage-borne *nun* gene, determined as described for panel A in footnote *b* of Table 1. The fourth column gives the quotients of β-galactosidase activities in the presence and absence of a p_{Lac}-*nun* fusion carried by a high-copy-number plasmid instead of an HK022 prophage (see text). N.D., not done. The fifth column summarizes the responses to Nun: − and + indicate that the β-galactosidase quotients of column 4 were greater than 0.1 or less than 0.05, respectively. The sixth column gives estimated responses of the mutant *nut* site to N protein, determined as described in the text and footnote *a* of Table 3.

[b] Cells carrying this mutant fusion, unlike the others, failed to form isolated colonies at 41°C on plates supplemented with ampicillin both in the presence and in the absence of N protein.

[c] This isolate had a 1-bp deletion at position 30 in addition to the indicated change. The data of Table 1 suggest that the phenotype is due entirely to the indicated substitution.

[d] The β-galactosidase activity was too low to determine a significant ratio.

The sequence requirements for Nun-mediated termination and N-mediated antitermination are similar but not identical. Within *boxA*, the requirements for N and Nun are indistinguishable. However, within *boxB*, the identity of two bases, position 57, within the loop, and position 60, immediately distal to the loop, appear to be important for N but not critical for Nun action. Although this divergence between the sequence requirements for N and Nun action might diminish if the mutant sites were tested at higher N or lower Nun levels, we doubt if it would disappear, since it has previously been shown that the antitermination-defective phenotype of substitution G57C is not completely suppressed by increasing the level of N (3). Thus, our data suggest recognition sites that overlap but are not congruent, a conclusion consistent with the observation that Nun and N act competitively (16). The unchanged Nun responsiveness of mutation A60C suggests that an A-U base pair between positions 54 and 60 of the wild-type *nut* transcript is not critical for termination. It remains possible that the remainder of the *boxB* stem, which consists entirely of G-C base pairs, is important.

Interestingly, when the source of Nun is a high-copy-number plasmid carrying a derepressed p_{Lac}-*nun* fusion, there is an apparent reduction of sequence specificity. In these circumstances, no single-base-pair substitution examined noticeably diminished Nun responsiveness. We suggest that Nun binds directly to the *nut* transcript and that a high concentration of Nun protein promotes its interaction with

TABLE 3. Test for N responsiveness[a]

nus	*N*	*nut*[b]	Growth of colonies		No. of Ap[r] colonies/total no. of colonies
			With ampicillin	Without ampicillin	
+	+	+	No[c]	Yes	0/40
+	−[d]	+	Yes	NT[e]	NT
+	+	G55T	Yes	Yes	40/40
A1	+	+	Yes	Yes	40/40
B5	+	+	Yes	Yes	37/40
E71	+	+	Yes	Yes	40/40

[a] Cultures of TAP112 or *nus* mutant derivatives carrying the indicated p_L-*nut*L-*lacZ* plasmids and with or without a second plasmid carrying a p_{Lac}-*N* fusion (pNAS200) were spread on L broth plates, incubated at 41°C overnight, and scored for the presence of individual colonies. The results were identical when the plates were supplemented with 2 mM isopropyl-β-D-thiogalactopyranoside. The plates were supplemented with ampicillin as indicated to select for retention of the p_L-*nut*L-*lacZ* plasmid and with spectinomycin where required to select for retention of pNAS200. Individual colonies growing on plates without ampicillin were replicated with toothpicks to plates containing ampicillin and incubated further to measure the frequency of Ap[r] colonies. All strains formed colonies on L broth plates containing ampicillin at the noninducing temperature of 32°C.

[b] The *nut*[+] parental site is shown in Fig. 2 and Table 1, and *nut*G55T is a base substitution mutation shown in Table 2.

[c] Quantitative plating showed that the efficiency of colony formation at 41°C on plates supplemented with ampicillin was less than 1% and the efficiency at 41°C on plates lacking ampicillin was 100% of that found at 32°C.

[d] This strain lacked pNAS200, the plasmid supplying N protein.

[e] NT, not tested.

mutant binding sites whose specific affinity has been reduced but not eliminated. A similar hypothesis can explain the observation that cells containing high-copy-number plasmids with a p_{Lac}-*nun* fusion grow more slowly than similar cells carrying the vector plasmid. High levels of Nun may interact with and terminate synthesis of cellular transcripts that contain *nut*-like sequences, leading to inappropriately low levels of expression of some important gene products. This apparent reduction in sequence specificity upon overexpression is also characteristic of N. High levels of λ N protein relieve the deficiency of *N* mutants in lambdoid phages 21 and P22 (5, 19), but no such complementation is observed with lower levels of N expression, presumably because of the differences in the sequence of the *nut* sites of these phages. Similarly, Doelling and Franklin (3) described mutant *nut*R sites that were nonfunctional at low levels of N and yet functional at higher levels of N.

Two groups (8, 23) have proposed models that describe the interaction of N, Nus factors, the *nut* site, and RNA polymerase during antitermination. In the model proposed by Whalen and Das (23), the *boxB* RNA stem loop serves to bind N and bring it into contact with a downstream RNA polymerase. *boxA* is thought to bind a host factor, possibly NusB, which then helps to bring N into the complex and to stabilize the N-polymerase interaction. The other Nus factors further stabilize the antitermination complex. The extensive similarities between N and Nun suggest that Nun physically replaces N in the antitermination complex and alters it so that it terminates transcription. The similarities include common requirements for at least four Nus factors and nearly identical nucleotide sequence requirements, which are recognized at the RNA level (1, 16) (Table 2). Recent genetic evidence suggests that Nun, like N, interacts functionally with RNA polymerase (17). The N and Nun proteins are of similar size, and both have a basic region near the amino-terminal end; this region appears to determine sequence specificity for N (9, 11).

This model explains why the *boxA* sequence requirements do not appear to differ between N-promoted antitermination and Nun-promoted termination and can easily accommodate the observation that the *boxB* target sites for the two processes are overlapping but not identical. One might expect the precise nucleotide sequence requirements for *boxB* region binding to differ, especially considering the relatively low degree of amino acid sequence similarity between the two proteins (11). However, the proximity of the two RNA binding sites might be required to allow the two proteins to occupy the same position in their respective complexes. If we assume that *boxB* base substitution mutations reduce but do not eliminate Nun binding, we can understand why these mutations are suppressed by increasing the level of Nun. However, additional assumptions are required to explain how increased Nun suppresses *boxA* mutations.

The molecular basis of the ability of N and Nun to function with similar families of sites and proteins is not apparent: the sequences of the two proteins are but distantly related (11). Also, the critical difference that results in divergent final effects is still unknown. It is possible that the difference in the *boxB* recognition sites could lead to a different configuration of the proposed complex, resulting in the very different final effects. Alternatively, the critical difference might not involve the difference in site recognition specificity but might instead involve other differences between the two proteins.

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