J. Mol. Biol. (1984) **180**, 1053–1063

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

ERIC R. OLSON[1]†, CHE-SHEN C. TOMICH[2] AND DAVID I. FRIEDMAN[1]

[1]*Department of Microbiology and Immunology*
The University of Michigan Medical School
Ann Arbor, Mich. 48109, U.S.A.

[2]*Molecular Biology Research*
Upjohn Company
Kalamazoo, Mich. 49001, U.S.A.

(Received 19 March 1984, and in revised form 19 June 1984)

The phage λ transcription antitermination protein, pN, acts with host factors, Nus, at sites on the phage genome, *nut*, to render RNA polymerase resistant to subsequent downstream termination signals. The NusA protein appears to recognize a seven to eight base-pair consensus sequence (5′Py-G-C-T-C-T-T(T)3′) called *boxA* that is found in the promoter-proximal part of the *nut* region.

Two types of change within or near the *boxA* sequence in the *nutR* region are shown to interfere with pN-mediated antitermination of transcription that has initiated at the upstream p_R promoter. (1) A change of one base-pair (from G to T at the second position) in the *boxA* sequence significantly reduces pN action. (2) We prove that a frameshift mutation, *cro*Δ62, at the end of the gene promoter-proximal to the λ *nutR* region, interferes with the pN antitermination reaction by allowing translation to proceed beyond *cro* into the *nutR* region. Using a series of plasmid constructions, we now show that the inhibition of antitermination caused by the *cro*Δ62 mutation can be suppressed when translation is terminated upstream from this mutation.

1. Introduction

Full transcription of bacteriophage lambda occurs only after RNA polymerase has acquired the ability to read through transcription termination signals, a process mediated, in part, by the product of the phage *N* gene (Friedman & Gottesman, 1983). Early λ transcription initiated at the promoters p_L and p_R (see Fig. 1), proceeds through the *N* gene in the p_L operon and *cro* in the p_R operon before stopping at the termination signals t_{L1} and t_{R1} (Roberts, 1969; Lozeron *et al.*, 1976; Salstrom & Szybalski, 1978*b*; Rosenberg *et al.*, 1978; Gottesman *et al.*, 1980).

† Present address: Biology Department, McGill University, Montreal, P.Q., Canada H3A LB1.

0022–2836/84/361053–11 $03.00/0

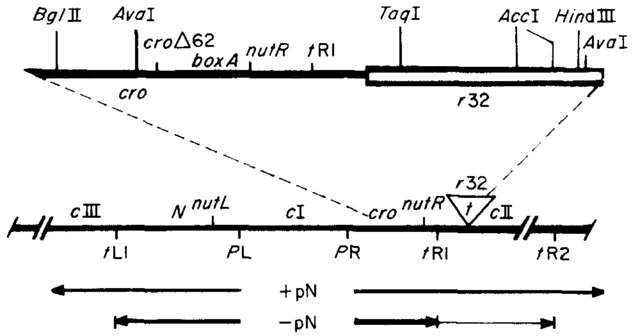

FIG. 1. Arrangement of genes and regulatory elements in the early control region of lambda. The second line shows the genetic arrangement of the region. The top line is an expansion of the region cloned to study the effect of *nutR* on pN-mediated transcription antitermination, including strategic restriction enzyme sites. The bottom lines show the pattern of early transcription in the presence and absence of pN.

The product of the *N* gene, pN, plus *Escherichia coli* functions, products of the *nus* genes, apparently modify RNA polymerase allowing subsequent transcription to proceed through these and other terminators located further downstream (i.e. t_{L2}, t_{L3}, t_{R2}) (Friedman *et al.*, 1973*b*; Adhya *et al.*, 1974; Franklin, 1974; Friedman & Gottesman, 1983).

Cis-acting mutations in the left operon that prevent transcription initiated at p_L from being modified by pN define a site necessary for N recognition (Salstrom & Szybalski, 1978*a*). These mutations are in a 17-bp† region of hyphenated dyad symmetry called the N-utilization site or *nutL*. A sequence homologous to *nutL* in 16 of the 17 bp was found in the right-hand operon, between *cro* and *c*II (Rosenberg *et al.*, 1978). This observation, combined with results from genetic studies, suggested that this 17-bp sequence is the site of N recognition on the right (*nutR*) (Rosenberg *et al.*, 1978; Reyes *et al.*, 1979). Cloning experiments have demonstrated that a 397-bp fragment extending from a site in *cro* to a site in *c*II, containing the *nutR* sequence, is sufficient for pN-mediated antitermination (de Crombrugghe *et al.*, 1979).

Cis-acting mutations in the right operon that prevent efficient N-modification have also been isolated (Olson *et al.*, 1982). Three independently selected mutations originally called *nutR*, because of their phenotypes and map locations, were subsequently shown to be deletions of an A·T bp in a run of seven A·T bp in the *cro* gene near the 3′ end. On the basis of this sequencing data, these mutations have been renamed *cro*Δ (e.g. *cro*Δ62).

Three hypotheses were presented to explain how such *cro* mutations result in the NutR$^-$ phenotype (Olson *et al.*, 1982). First, although complementation studies suggested otherwise, the altered Cro protein might interfere with pN action. Second, the deletion could alter a site utilized either by pN or one of the host factors. Third, since the deletion results in translation extending four bases into the *nutR* region, the ribosome terminating *cro* translation could interfere with one of the interactions necessary for pN action.

† Abbreviation used: bp, base-pair(s).

Consideration of this last possibility led to the identification of a sequence, 5′ Py-G-C-T-C-T-T-(T) 3′, called *boxA*, promoter-proximal to the *nut* dyad symmetry, which was postulated to be a recognition sequence for the *E. coli* NusA protein (Olson *et al.*, 1982). Moreover, isolation of a mutation in this sequence indicated that at least the 3′ terminus of *boxA* was required for NusA recognition as well as N-induced modification (Friedman & Olson, 1983).

In this paper we show the following. (1) When ribosomes translating the *cro* message stop upstream from the normal stop codon antitermination is not affected. (2) The *cro*Δ62 class of mutations cause a Nut^- phenotype because the ribosomes translating *cro* move an additional four bases into the *nutR* region. (3) A mutation in the promoter-proximal 5′ region of *boxA* blocks pN action.

2. Materials and Methods

(a) *Media*

Media not described elsewhere in this paper have been described by Friedman *et al.* (1973*a*).

(b) *Strains*

(i) *Bacteria*

The following *E. coli* strains were used: N5468 (*bio*$^-$, *ilv*$^-$, *his*$^-$, *galK am, pro* : : Tn*10*, λcI857ΔBamΔH1) was obtained from S. Adhya. DH1 (*recA1, endA1, gyrA96, thi-1, hsdRM, supE44*) was obtained from R. Schmickel. JM101, (F′, *lacZ*$^-$) was obtained from W. Dunnick. K2218 (N5468 *nusA1*) was constructed in this laboratory.

(ii) *Phages*

λcI857*r*32 was obtained from W. Szybalski. λcI857*cro*Δ62*r*32 (previously called λcI857*nutR*62*r*32) originated in this laboratory (Olson *et al.*, 1982). M13mp8 and M13mp9 were obtained from W. Dunnick.

(iii) *Plasmid*

pKG1800 was obtained from K. McKenney (Rosenberg *et al.*, 1983).

(c) *Plasmid construction*

(i) *Construction of pNPK-1, pNMK-62, pNPA-1 and pNMA-62*

Phage DNA, prepared as described by Yamamoto *et al.* (1970), was cleaved by the appropriate restriction enzymes. Where appropriate, DNA fragments were made blunt-ended and ligated into pKG1800 (Maniatis *et al.*, 1982). *E. coli* was made competent for transformation as described by Cohen *et al.* (1972). Colony hybridizations were performed according to Maniatis *et al.* (1982). The *nutR* probe was an M13mp9 derivative carrying the *nutR* region from λcI857*r*32 (unpublished results, this laboratory). Restriction fragments from pNPK-1, pNMK-62, pNPA-1 and pNMA-62 containing the *cro-nutR* region were cloned into M13 derivatives and sequenced by the method of Sanger *et al.* (1977). The sequence from the *Hin*dIII site in *cro* to the start of the *r*32 was determined and shown in Fig. 4.

(ii) *Construction of pBAGT*

Construction of pBAGT is shown diagrammatically in Fig. 2. A 13-base single-stranded oligonucleotide (whose synthesis is described below) was hybridized to a single-stranded

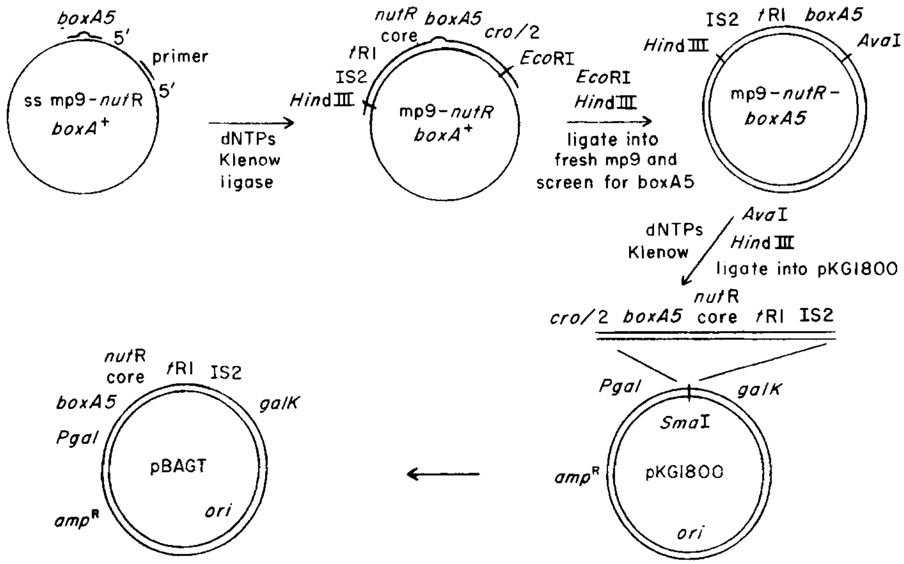

FIG. 2. Construction of pBAGT.

M13mp9 derivative carrying an insert with a wild-type *boxA* (3′ G-C-G-A-G-A-A-T). The insert extends from the *Bgl*II site in *cro* to the *Hin*dIII site in the IS2. The hybridization was done in a final volume of 100 μl and consisted of the following: 15 μg of single-stranded M13mp9-*nutR*-*boxA*$^+$ DNA, 35 ng of double-stranded 26 bp M13 universal primer (BRL, Gaithersburg, MD), 5 μg of the 13-base single-stranded oligonucleotide carrying the altered *boxA*, 7 mM-Tris·HCl (pH 7·4), 7 mM-$MgCl_2$, 50 mM-NaCl. The hybridization mixture was boiled for 3 min and allowed to cool to room temperature. The mixture was then added to 10 μl of solution B (20 mM-Tris·HCl (pH 7·4), 10 mM-$MgCl_2$, 10 mM-dithiothreitol, 1 mM of all 4 deoxynucleotide triphosphates, 1 mM-ATP) (Zoller & Smith, 1982) and 4 units of DNA polymerase I (Klenow fragment), and incubated overnight at 16°C. The mixture was then heated at 65°C for 10 min. After cooling the mixture on ice, *Eco*RI and *Hin*dIII were added and the mixture was incubated for 6 h at 37°C. The digested DNA was electrophoresed through a 1% (w/v) agarose gel containing 5 μg ethidium bromide/ml. The DNA fragment containing the hybrid *boxA* was electroeluted (Maniatis *et al.*, 1982) and purified over an eluptip-d column (Schleicher and Schuell). The purified fragment was then ligated into *Hin*dIII-*Eco*RI-digested M13mp9. JM101 was transfected with the ligated DNA and M13mp9 derivatives containing inserts were identified by plaque hybridization (not shown). Single-stranded DNA was purified from eight M13mp9 derivatives carrying inserts and sequenced by the method of Sanger *et al.* (1977). Of the 8 recombinants screened, one had a *boxA* with a T instead of a G residue at position 2 (data not shown). The replicative form of the M13mp9 derivative containing the altered *boxA* was digested with *Ava*I and *Hin*dIII, and the fragments containing the *nutR* region were ligated into pKG1800 as described for the construction of pNPK-1. This plasmid is called pBAGT. Sequence data revealed that except for the change in *boxA*, the sequence of the *nutR* region and the *galE*-*cro* junction from pBAGT are identical to that of pNPK-1 (data not shown).

(d) *Oligonucleotide synthesis*

The oligonucleotide 5′ A-A-C-C-C-C-T-C-T-C-T-T-A 3′ was synthesized by the phosphite coupling method on a silica-gel solid support according to Beaurage & Caruthers (1981), with slight modifications. After synthesis, the oligonucleotides were deprotected and then purified by preparative acrylamide gel electrophoresis.

The purified oligomer was identified by its size and sequence. Size analysis was done by acrylamide gel electrophoresis (20% (w/v) acrylamide, 7 M-urea) with commercial oligonucleotides (from New England BioLabs) of known sizes as standards. To detect the oligonucleotides by autoradiography, both the 13-base sequence and standards were end-labeled with ^{32}P by the action of the phage T4 polynucleotide kinase in the presence of [γ-^{32}P]ATP. Kinase reaction conditions were according to those given by the supplier, New England BioLabs.

Sequencing analysis was done by base-specific chemical cleavages of the end-labeled 13-base sequence, according to the procedures of Maxam & Gilbert (1980) with slight modifications. From the sizing and sequencing results (data not shown) the structure of the 13-base sequence was confirmed.

(e) *Galactokinase assay*

Bacteria were grown overnight in M56 minimal medium (Miller, 1972) containing (per ml) 0·01 μg biotin, 20 μg of histidine, valine, leucine and isoleucine. In addition, 25 μg of ampicillin per ml were included if the bacterium carried a plasmid. Cultures were diluted 1 : 50 in fresh medium and grown to a density of 10^8 per ml. A sample was removed for assaying galactokinase and the rest of the culture was shifted to 42°C for 20 min. Another sample was then removed for assay; 1 ml from each sample was treated with toluene and assayed as described by Adhya & Miller (1979). Galactokinase units are expressed as nanomoles of galactose phosphorylated per minute per O.D. unit of absorbance at 650 nm.

3. Results

(a) *Strategy of the experiments*

The *nutR* regions from λ derivatives, either *nut*$^+$ or *cro*Δ62, were cloned into the *galK* expression vector pKG1800 (Rosenberg *et al.*, 1983) so that the distance between *nutR* and in-frame translation stop codons upstream from *nutR* differed. This plasmid system was used also to test the effect of a single base change in *boxA*, *boxA5*, on N-mediated antitermination as well as a previously described base deletion in *cro*.

The vector pKG1800 is a pBR322 derivative with the following important features (Fig. 3). First, it contains a promoter from the *E. coli* galactose operon. P_G, plus the first 400 bases of the *galE* gene. Transcripts initiating from P_G will be translated by ribosomes starting at the *galE* AUG translation initiation codon. Since there are no in-frame translation stop codons between the end of the *galE* fragment and the cloning sites (e.g. *Ava*I), translation will continue into any inserted fragment, in this case into the end of the *cro* gene. Second, the *Ava*I restriction site between *galE* and *galK* is also recognized by *Sma*I. Since *Ava*I creates a staggered end while *Sma*I creates a blunt end, and since the cleavage sites for the two enzymes vary by two base-pairs, translation of fragments ligated into these two sites will be in different reading frames. The λ derivatives used in these studies carry an IS2 insertion, *r*32, downstream from the *nutR* region (Brachet *et al.*, 1970) (see Figs 1 and 3). The presence of the strong termination signal in the IS2 element in both the λ*r*32 and λ*r*32*cro*Δ62 in the right operon ensures that *galK* expression is dependent on termination-resistant transcription

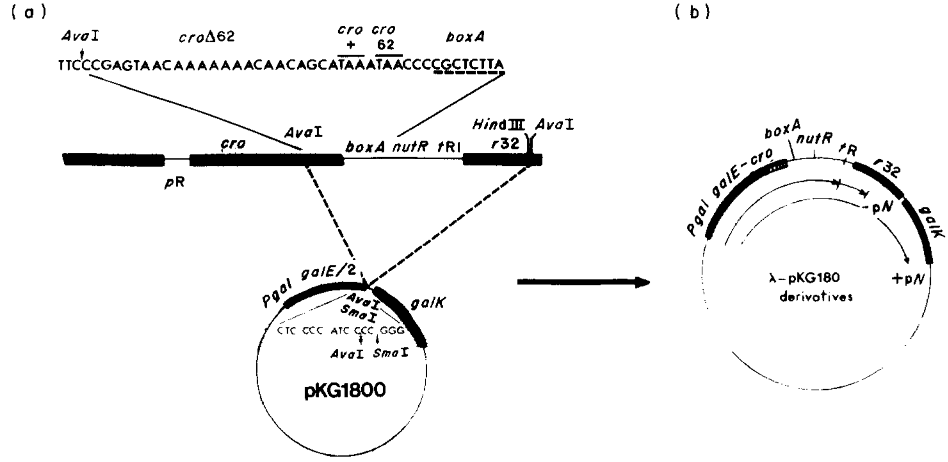

FIG. 3. Construction and structure of pNPK-1, pNMK-62, pNPA-1 and pNMA-62. (a) The sites and genes involved on the construction of the various plasmids are shown. The sequence (with hyphens omitted for clarity) from the *Ava*I site in *cro* to *boxA* is shown at the top. The *cro* stop codons that are in-frame in λcro^+ and $\lambda cro\Delta62$ are indicated by cro^+ and *cro*62. The *cro*Δ62 mutation is an A·T deletion in the run of 7 A·T bp at the end of *cro*. The essential features of pKG1800 are shown at the bottom. (b) The general structure of the completed plasmids and the patterns of *galK* transcription with and without pN are shown.

(Brachet *et al.*, 1970; Tomich & Friedman, 1977; de Crombrugghe *et al.*, 1973). The various pKG1800-λ*nutR*-derived plasmids were introduced into *E. coli* N5468 ($galK^-$, λ*c*I857ΔBamΔH1). The defective prophage, λ*c*I857ΔBamΔH1, has a temperature-sensitive repressor, so that at 32°C there is repression while at 40°C the *c*I857 repressor is inactivated and the only known phage function expressed is pN. When the *nutR* region and a terminator(s) are inserted between the *gal* promoter (*Pgal*) and the *galK* gene, and pN is supplied *in trans*, the level of *galK* expression reflects the efficiency of antitermination.

(b) *Plasmid construction and galactokinase values*

We will first discuss plasmids pNPK-1 and pNMK-62. The λ*r*32 and λ*r*32*cro*Δ62 *Ava*I-*Hin*dIII fragments containing the *cro-r*32 region were purified and made blunt-ended by adding dNTPs and Klenow fragment (Fig. 3). The fragments were then ligated into the *Sma*I site of pKG1800. The resulting plasmids, pNPK-1 and pNMK-62, contain the *nutR* regions from λ*r*32 and λ*r*32*cro*Δ62, respectively, in the orientations shown in Figures 3 and 4. The *galE–cro* junctions in these plasmids result in translation reading frames at the end of the *cro* 3′-terminal fragments identical to those of the parental phages. Thus, translation stops at the normal *cro* UAA in pNPK-1 and λ*r*32 while translation of pNML-62 and λ*r*32*cro*Δ62 stops at the UAA four bases further downstream (Fig. 4). At 42°C we would expect pNPK-1 to have a Nut^+ phenotype (manifested by a high level of *galK* expression in the presence of pN) and pNMK-62 should have a Nut^- phenotype (manifested by a low level of *galK* expression in the presence of pN).

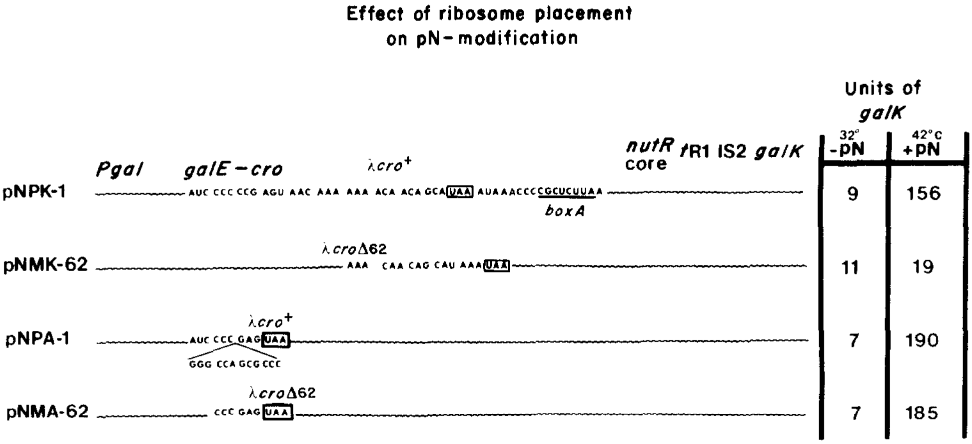

FIG. 4. Galactokinase expression from various *nutR*-containing plasmids. The genetic organization from the gal promoter (*Pgal*) to *galK* in each of these plasmids is indicated at the top. The UAA translation stop codons that are in-frame as a result of fusing the *galE* and *cro* genes are boxed. Except for the *cro*Δ62 mutation (T·A deletion, see Fig. 3) in pNMK-62 and pNMA-62, the wavy line indicates that the DNA sequence is identical to pNPK-1. Each plasmid sequence was determined from the *Hin*dIII site in *galE* (see Fig. 3) to the t_{R1}–IS2 junction. It should be noted that although pNPA-1, derived from λr32, has the reading frame expected for the *Ava*I–*Ava*I junction, it also has a 12-bp insertion at that junction (see Fig. 4). This insertion is probably a cloning artifact since the *Ava*I ends from the pKG1800 and the *cro* fragment differ by 1 base: 5′ C-C-C-G-G-G and 5′ C-C-C-G-A-G. There is no effect of the insertion on N modification since expression of galactokinase is still N-dependent and the levels are almost identical to those for pNPK-1.

Figure 4 shows the pattern of *galK* expression when these two plasmids were introduced into N5468. At 32°C, in the absence of pN, both plasmids directed the synthesis of only low levels of galactokinase. When, however, pN was supplied by thermoinduction of the prophage, *galK* expression by pNPK-1 was elevated 17-fold while expression by pNMK-62 was unaffected. Thus, the pattern of pN stimulation of gene expression in these plasmids corresponds to that for *nutR*-controlled gene expression observed in their respective parent phages.

The experiments discussed in the preceding paragraph suggest that *galK* expression in this system is N-dependent. We show that this is the case in experiments using K2218, a derivative of N5468 with the *nusA*1 allele. Phage λ grows poorly at higher temperatures (above 40°C) in *E. coli* with the *nusA*1 allele, because of insufficient pN activity (Friedman & Baron, 1974; Friedman, 1971). When pNPK-1 was inserted into K2218, only 17 units of galactokinase were made at 42°C. This ninefold reduction in galactokinase in the presence of the *nusA*1 allele in cells containing pNPK-1 offers strong corroborating evidence that *galK* expression from this plasmid is N-dependent.

The second set of plasmids, pNPA-1 and pNMA-62, was constructed by ligating the *Ava*I fragments from λr32 and λr32*cro*Δ62, respectively, into the *Ava*I site of pKG1800. Plasmids were isolated that contained the *Ava*I fragment with the *cro-r*32 region from each phage (Figs 3 and 4). The junction between *galE* and *cro* in these plasmids, which is different from that in pNPK-1 and pNMK-62, results in a shift of the translation reading frame at that junction (Fig. 4). The stop codon that is in-frame in both of these plasmids is upstream from the *cro*Δ62 mutation.

If the phenotype of the *cro*Δ62 mutation is caused by translation extending beyond the normal *cro* stop codon, the creation of an in-frame stop codon 15 bases upstream from the end of *cro* should prevent ribosomes from reaching the *nut* region and thus obviate the effect of the *cro*Δ62 frameshift mutation. Accordingly, in the presence of pN, cells containing pNMA-62 (translation is terminated upstream from *cro*Δ62) should make higher levels of galactokinase than cells containing pNMK-62 (translation terminates downstream from the normal *cro* UAA). On the other hand, if the *cro*Δ62 mutation exerts its effect on antitermination independently of the effect on translation, pNMA-62 should produce similar levels of galactokinase as pNMK-62. As shown in Figure 4, when pN was supplied, cells carrying pNMA-62 made high levels of galactokinase while cells carrying pNMK-62 made only low levels, even though both plasmids have the *cro*Δ62 mutation. These observations support the conclusion that ribosomes terminating translation four bases beyond the normal *cro* stop codon interfere with antitermination. Mechanistically, the terminating ribosomes probably act by interfering with the N-modification reaction. Furthermore, since pNPK-1 (*cro*$^+$ and translation terminates at the normal *cro* UAA), pNPA-1 (*cro*$^+$ and translation terminates upstream from normal *cro* UAA) and pNMA-62 (*cro*Δ62 and translation terminates upstream from the *cro*Δ62 mutation) make comparable levels of galactokinase at 42°C, normal translation to the end of *cro* must not be essential for N action.

In order to control for possible differences in plasmid copy number influencing the level of *galK* expression, we constructed phage vectors with the various P_G–*nut*–terminator–*galK* arrangements. Results from qualitative tests assaying for *galK* expression were completely consistent with the plasmid data presented above (data not shown).

(c) *Effect of changing a single base-pair in* boxA *on N-mediated antitermination*

Plasmid pBAGT, a derivative of pNPK-1, was used to assess the effect of changing what appears to be a highly conserved base-pair in the 5′ end of *boxA*. Details of the construction of pBAGT are outlined in Figure 2. The two plasmids differ only in their *boxA* sequences; pBAGT contains a T-A sequence at position 2; resulting in a 5′ C-T-C-T-C-T-T-A 3′ instead of the 5′ C-G-C-T-C-T-T-A 3′ wild-type *boxA*. This change is called *boxA*5.

The activity of the mutant *boxA* was tested using the Gal expression assay outlined above. Plasmid pBAGT was inserted into the *E. coli* host N5468. At low temperature (no pN synthesized), both plasmids expressed little galactokinase: pNPK-1, 9 units; and pBAGT, 15 units. Assays made 20 minutes after a shift to 42°C, however, revealed distinct differences between the two plasmids. Under these conditions, cells carrying pNPK-1 contained 156 units of galactokinase while those carrying pBAGT had only preshift levels, 11 units.

DNA sequence analysis of the cloned fragments revealed only the engineered

G·C to T·A change between pBAGT and pNPK-1. When all of the λ inserted material except for the last 122 bp of the IS2 was removed from pBAGT, the resulting plasmid expressed *galK* (data not shown), demonstrating that the P_G promoter and *galK* gene of pBAGT were functional. We conclude that the *boxA*5 mutation is responsible for the low level of galactokinase expressed by pBAGT, because the N-modification process is severely inhibited. Therefore, the experiments prove that the bases at the 5′ end of *boxA* are essential for this modification process.

4. Discussion

In this paper we have presented evidence substantiating two essentially unproved inferences made in previous reports concerning the mechanism of N-mediated antitermination. (1) Translation into the *nutR* region interferes with the N-modification process; and (2) the 5′ end of the *boxA* sequence is necessary for N modification.

Translation into the *nut* region was initially studied using the *cro*Δ62 mutation. This type of mutation was isolated on the basis of interference with N modification at *nutR* such that transcription initiating from the p_R promoter upstream from the mutation failed to become termination-resistant. Surprisingly, the *cro*Δ62 mutation was shown to be a single base-pair deletion in the *cro* gene near the 3′ end. We have previously argued by deduction that the Nut⁻ phenotype caused by *cro*Δ62 is due to the extension of translation beyond the normal *cro* UAA. The results presented here prove this contention. Two plasmids carrying the *cro*Δ62 mutation were constructed, pNMK-62 and pNMA-62. However, only one of them, pNMK-62, is defective in N-mediated antitermination. The difference in the *galE–cro* junction in these two plasmids results in different distances between in-frame translation stop codons and the *nutR* region. Translation of pNMK-62 (Nut^- phenotype) stops four bases beyond the normal *cro* stop codon while translation of pNMA-62 (Nut^+ phenotype) stops 15 bases further upstream (see Fig. 4).

The change in the position of the ribosome terminating translation with respect to the *nutR* region in pNMA-62 and pNMK-62 is 19 bases. Although both contain the *cro*Δ62 mutation, only pNMK-62 is defective in antitermination. The most likely explanation for this result is that ribosomes extending into the *nutR* region interfere with an RNA–RNA, RNA–DNA or RNA–protein interaction. One likely RNA–RNA interaction that could be interfered with is the potential stem–loop structure at *nutR*. We think this is unlikely for the following reason. Mutational analysis of the leader region of the histidine operon of *Salmonella* suggests that ribosomes can interfere with the formation of RNA secondary structure 14 bases downstream from a ribosome stop signal but not 20 bases downstream (Johnston & Roth, 1981). The translation stop codon that is in-frame in pNMK-62 (Nut^- phenotype) is 21 bases from the *nutR* stem–loop. Therefore, we feel it is unlikely that as a result of the *cro*Δ62 mutation the ribosomes terminating translation of the *cro* gene sterically interfere with secondary structure at the *nutR* region of hyphenated dyad symmetry.

We favor a second possible mechanism to explain how a four-base shift in ribosome position interferes with pN action. According to this model, the ribosome blocks an interaction between the RNA and a protein; e.g. pN or one of the Nus factors. The *boxA* sequence is located only eight bases from the end of *cro* (Figs 3 and 4) (Friedman & Olson, 1983) and in the case of $\lambda r32cro\Delta62$ and pNMK-62, *boxA* is only four bases from the terminating ribosome. Thus, the ribosome terminating *cro* translation in $\lambda cro\Delta62$ and pNMK-62 would be positioned to interfere with a NusA–*boxA* interaction. Since NusA is required for N activity, such an interference would be sufficient to explain the Nut^- phenotype of pNMK-62 and $\lambda cro\Delta62$. The fact that translation interferes with the modification process suggests that at least part of the reaction (NusA:*boxA*) takes place at the level of the messenger RNA. However, it is also conceivable that the initial recognition is between NusA and the DNA, in this case a ribosome tightly coupled to RNA polymerase might interfere with the transfer of NusA to the transcription complex. Moreover, we show that normal translation to the end of *cro* is not necessary for efficient N action. This conclusion was also reached by C. Debuck and M. Rosenberg as well as F. Warren and A. Das (personal communications) using similar plasmid systems.

We have also extended other studies on *boxA* by showing that the G·C base pair at position 2 in *boxA* is essential for N-determined antitermination. Plasmids pBAGT and pNPK-1 differ from each other only in their *boxA* sequences. When pN was supplied *in trans*, cells containing pNPK-1 produced more than ten times more galactokinase than those containing pBAGT. Since the only difference between these two plasmids is a substitution of an A·T bp for a G·C bp at position 2 of the *boxA* sequence, this G·C must be essential for N-mediated transcription antitermination. This conclusion is consistent with the observation that while there is heterogeneity at some of the positions in the *boxA*-like sequences identified so far, the G·C bp at position 2 is highly conserved (Olson *et al.*, 1982).

The results presented in this paper confirm and extend our previous studies (Olson *et al.*, 1982; Friedman & Olson, 1983) that identified *boxA* in the *nutR* region as an important sequence in the N-mediated transcription antitermination reaction. We have shown that a nucleotide at the 5′ end of *boxA* is essential for the N reaction. Moreover, the fact that this mutational interference can be duplicated by extending translation into *nutR* suggests that ribosomes can interfere with the modification reaction.

The authors thank Alan Schauer, Martin Smith and Sankar Adhya for helpful suggestions. Sankar Adhya is also thanked for supplying phage and bacterial strains. We thank Sankar Adhya, Drew Granston, Don Court, Wes Dunnick, Martin Smith and Fred Neidhardt for careful reading of the manuscript. Emma Williams and Patty Laird are thanked for help in preparing the manuscript and Lisa Olson for making the figures. These studies were supported in part by grants from the National Institutes of Health (to D.I.F.).

REFERENCES

Adhya, S. & Miller, W. (1979). *Nature (London)*, **279**, 492–494.

Adhya, S., Gottesman, M. & de Combrugghe, B. (1974). *Proc. Nat. Acad. Sci., U.S.A.* **71**, 2534–2538.

Beaurage, S. L. & Caruthers, M. H. (1981). *Tetrahedron Letters*, **22**, 1859.
Brachet, P., Eisen, H. & Rambach, A. (1970). *Mol. Gen. Genet.* **108**, 266–276.
Cohen, S. N., Chang, A. C. Y. & Hsu, C. L. (1972). *Proc. Nat. Acad. Sci., U.S.A.* **69**, 2110–2114.
de Crombrugghe, B., Adhya, S., Gottesman, M. & Pastan, I. (1973). *Nature New Biol.* **241**, 260–264.
de Crombrugghe, B., Mudryj, M., DiLaura, R. & Gottesman, M. (1979). *Cell*, **18**, 1145–1151.
Franklin, N. C. (1974). *J. Mol. Biol.* **89**, 33–48.
Friedman, D. I. (1971). In *The Bacteriophage Lambda* (Hershey, A. D., ed.), pp. 733–738, Cold Spring Harbor Laboratories, New York.
Friedman, D. I. & Baron, L. S. (1974). *Virology*, **58**, 141–148.
Friedman, D. I. & Gottesman, M. (1983). In *Lambda II* (Hendrix *et al.*, eds), pp. 21–51, Cold Spring Harbor Laboratories, New York.
Friedman, D. I. & Olson, E. R. (1983). *Cell*, **34**, 143–149.
Friedman, D. I., Jolly, C. T. & Mural, R. J. (1973*a*). *Virology*, **51**, 216–226.
Friedman, D. I., Wilgus, G. S. & Mural, R. J. (1973*b*). *J. Mol. Biol.* **81**, 505–516.
Gottesman, M. E., Adhya, S. & Das, A. (1980). *J. Mol. Biol.* **140**, 57–75.
Johnston, H. M. & Roth, J. R. (1981). *J. Mol. Biol.* **145**, 735–756.
Lozeron, H. A., Dahlberg, J. E. & Szybalski, W. (1976). *Virology*, **71**, 262–277.
Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982). *Molecular Cloning: A Laboratory Manual*, p. 371, Cold Spring Harbor Laboratory, New York.
Maxam, A. M. & Gilbert, W. (1980). *Methods in Enzymology* (Grossman, L. & Moldave, K., eds), vol. 65, pp. 499–560, Academic Press, New York.
Miller, J. (1972). In *Experiments in Molecular Biology*, p. 230, Cold Spring Harbor Laboratory, New York.
Olson, E. R., Flamm, E. L. & Friedman, D. I. (1982). *Cell*, **31**, 61–70.
Reyes, O., Gottesman, M. & Adhya, S. (1979). *Virology*, **94**, 400–408.
Roberts, J. W. (1969). *Nature (London)*, **224**, 1168–1174.
Rosenberg, M., Court, D., Shimatake, H., Brady, C. & Wulff, D. L. (1978). *Nature (London)*, **272**, 414–423.
Rosenberg, M., Chepelinsky, A. B. & McKenney, K. (1983). *Science*, **222**, 734–739.
Salstrom, J. S. & Szybalski, W. (1978*a*). *J. Mol. Biol.* **124**, 195–221.
Salstrom, J. S. & Szybalski, W. (1978*b*). *Virology*, **88**, 252–262.
Sanger, F., Nicklen, S. & Coulson, A. R. (1977). *Proc. Nat. Acad. Sci., U.S.A.* **74**, 5463–5467.
Tomich, P. K. & Friedman, D. I. (1977). In *DNA Insertion Elements, Plasmids and Episomes* (Adhya, A., Bukhari, A. & Shapiro, J., eds), pp. 99–107, Cold Spring Harbor Press, New York.
Yamamoto, K. R., Alberts, B. M., Benzinger, R., Lawhorne, L. & Treiber, G. (1970). *Virology*, **40**, 734–744.
Zoller, M. J. & Smith, M. (1982). *Nucl. Acids Res.* **10**, 6487–6500.

Edited by M. Gottesman