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# Evidence that a Nucleotide Sequence, "*boxA*," Is Involved in the Action of the NusA Protein

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## Summary

**We report the isolation of a mutation, *boxA*1, in the *nutR* region of the phage λ genome. The *nutR* region, located downstream of the $p_R$ promoter, includes the site *nutR* where the λ N protein is thought to act to render subsequent transcription termination-resistant. We have previously suggested that the *boxA* sequence, 5'CGCTCTTA3' (or its RNA analog), located 8 bp promoter-proximal to *nutR*, might be the recognition site for the E. coli host factor, NusA, which has been shown to be necessary for N action. The *boxA*1 mutation, an A:T to T:A transversion, results in a changed *boxA* sequence upstream of *nutR*, CGCTCTTT. This change is necessary for λ to effectively use the NusA of Salmonella typhimurium, a NusA function not normally active with the N product of λ. Other lambdoid phages with unique N functions and *nut* sites that are normally active with the NusA of Salmonella have *boxA* sequences with the terminal three Ts. Moreover, sequences closely resembling *boxA* have been found near transcription termination sequences in E. coli operons where NusA has been shown to be involved in termination. These findings identify *boxA* as an important recognition signal for the NusA protein.**

## Introduction

The product of the *nusA* gene of E. coli has been implicated in both transcription termination and antitermination reactions. The gene was initially identified and mapped based on the isolation of a mutation, *nusA*1, which reduced the bacterium's ability to support the activity of the *N* gene product of phage λ (Friedman, 1971; Friedman and Baron, 1974). Subsequent studies have shown that NusA (or L protein) plays a role in termination (Greenblatt et al., 1981; Kingston and Chamberlain, 1981; Farnham et al., 1982) and in stimulating β-galactosidase synthesis in a coupled transcription translation system (Kung et al., 1975).

Our studies have focused on the role of NusA protein in the action of the *N* gene product. The N protein regulates gene expression by permitting transcription to overcome many transcription termination signals (Roberts, 1969). A striking feature of the N reaction is its specificity; N protein primarily influences transcription from the early promoters $p_L$ and $p_R$ of λ and the closely related phage 434 (Friedman et al., 1973; Adhya et al., 1974; Franklin, 1974; see Figure 1). *Cis*-acting mutations downstream of the $p_L$ promoter were isolated that failed to allow transcription initiating from $p_L$ to become termination-resistant. This led to the identification of a site, *nutL*, where N protein apparently is recognized (Salstrom and Szybalski, 1978). Sequence analysis showed that the *nut* mutations are located in a 17 bp region of hyphenated dyad symmetry (Rosenberg et al., 1978). Analysis of sequences downstream of $p_R$ distal to the *cro* gene revealed a sequence nearly identical to *nutL*, homologous in 16 of 17 bp (Rosenberg et al., 1978). Cloning experiments confirm that this site is contained in a region essential for N action (de Crombrugghe et al., 1979).

Much of the knowledge about N function has derived from studies with hybrid phages constructed by crossing λ with other closely related phages; e.g., 434 (Kaiser and Jacob, 1957), 21 (Liedke-Kulke and Kaiser, 1967), and the Salmonella-phage P22 (Gemski et al., 1972; Botstein and Herskowitz, 1974). This family of phages, referred to as lambdoid, have nearly identically organized chromosomes but differ in the specificities of their analogous gene functions (reviewed by Friedman and Gottesman, 1983). Figure 1 shows the regions of nonhomology with λ of some of these hybrids. Each hybrid phage contains primarily λ DNA, but carries genes from the second phage for repressors, operators, and (except for 434) the *N* gene, *nut* regions and some adjacent DNA (Westmoreland et al., 1969). Because these regions encode both repressors and cognate operators, and are thus responsible for repression when the phage is in the prophage state, these regions are collectively referred to as the immunity regions. The hybrid phages are named according to the immunity region they carry, and the specific hybrids are entitled λ*imm*434, λ*imm*21, and λ*imm*P22. In the case of the latter two phages, these *N* gene products presumably only recognize their own *nut* sites. λ*imm*434, which shares the same *N* gene as λ, has the same *nut* sites.

In addition to the N protein, a number of bacterial factors, Nus (N-utilization substances), have been identified that appear to be needed for complete N action (Friedman et al., 1983). These factors, initially identified by mutations, have in some cases been biochemically characterized. The *nus* mutations, to a greater or lesser extent, result in the bacteria sharing a similar phenotype under restrictive conditions; N-dependent λ (e.g., wild type) will not grow in hosts with a *nus* mutation under restrictive conditions. However, λ derivatives that are N-independent, e.g., *nin* (Court and Sato, 1969), do grow in these hosts under the same conditions.

The best characterized *nus* gene is *nusA* (Friedman and Baron, 1974). The *nusA* gene encodes a 69 kd protein (Greenblatt and Li, 1981) that can act as a transcription termination factor. Our knowledge about the role of NusA in the action of N derives primarily from studies of E. coli carrying a mutation in the *nusA* gene, *nusA*1 (Friedman, 1971; Friedman and Baron, 1974), and of hybrid bacteria carrying the *nusA* gene from Salmonella typhimurium (Baron et al., 1970; Friedman and Baron, 1974). In bacteria with the *nusA*1 mutation or the Salmonella *nusA* gene, N-dependent λ fail to grow, while N-independent derivatives

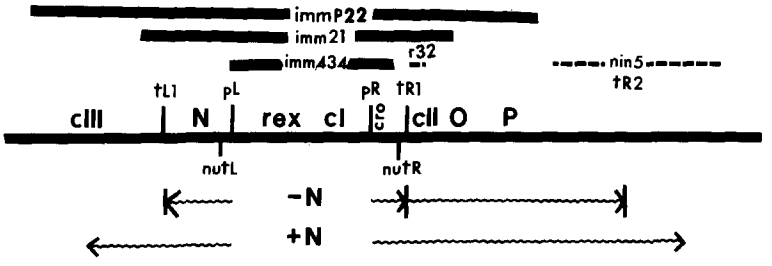


Figure 1. Genetic Arrangement and Gene Transcription in the "Early" Region of λ

The genetic arrangement in the early control region of λ is shown in the middle. The three upper lines show the extent of the substitutions in the hybrid phages formed by recombination between λ and the indicated phage. The lines under the map indicate the transcription patterns from the early promoters with and without N protein.

do grow. The growth patterns of hybrid phages differ in these bacteria. Although both the *N* gene products of λ*imm*21 (Friedman et al., 1973) and λ*imm*P22 (Hillicker et al., 1978) are less effective in *nusA*1 mutants, they are both active in hybrid bacteria with the *nusA* gene of Salmonella (Friedman and Baron, 1974).

We have identified a common sequence in the *nut* region located upstream of the 17 bp region of dyad symmetry characteristic of all *nut* region sequences that were available for our examination (Olson et al., 1982). This sequence, CGCTCTTA, or slight variations of it, is found upstream of the *nutR* and *nutL* of λ, *nutR* of 21, and *nutL* of P22. One of the variations observed was that this 8 bp sequence in the 21 and P22 *nut* regions ends in three Ts, $nutR_{21}$ having the sequence TGCTCTTT and $nutL_{22}$ having the sequence CGCTCTTT (see Olson et al., 1982). Although these phages have different N products, they all require NusA for maximal N activity. This led to the suggestion that the sequence CGCTCTTA might be a recognition site for NusA. Examination of bacterial sequences in regions where NusA is postulated to work revealed closely related sequences. In particular, the CGC and TT are usually conserved. Based on this information, the sequence was named *boxA* (Olson et al., 1982).

Affinity chromatography was used to demonstrate a direct interaction between NusA and N proteins (Greenblatt and Li, 1981). Moreover, a mutation in the *N* gene permits the phage to overcome the *nusA*1 block (Friedman et al., 1981). Thus one probable reason why λ fails to grow in the hybrid E. coli strain with the *nusA* gene from Salmonella is that its N product cannot interact with the NusA protein from Salmonella. According to this explanation, λ*imm*P22 and λ*imm*21, both of which can grow in the hybrid bacterium, should synthesize N products that can interact with NusA-Salmonella. These phages also share a similarity in the *boxA* sequence that differs from the *boxA* sequence found in λ. This difference might also be invoked to explain why λ fails to grow, but P22 and 21 grow in hosts with the Salmonella NusA.

If the three Ts play a role in the recognition of the NusA-Salmonella by the *boxA* sequence, then it is plausible to assume that a λ mutant that could utilize the NusA of Salmonella would have, in addition to an altered N, three Ts at the end of the *boxA* sequence in the *nutR* region. This assumes a tripartite interaction, N-NusA-*BoxA*.

We report the isolation and characterization of a mutant of λ that grows well in a hybrid E. coli containing the *nusA* gene from Salmonella typhimurium. As predicted, this phage has two types of mutations: one that maps in the region of the *N* gene and a second in the *nutR*-associated *boxA* sequence. The latter results in an A:T to T:A transversion, yielding a run of three Ts identical to the sequence found in the *boxA*s of 21 and P22.

## Results

### Isolation of Mutations Permitting λ Growth with NusA Salmonella

The strategy underlying the selection of a *boxA* mutation that would permit growth of λ in an E. coli-Salmonella hybrid that has the *nusA* gene of Salmonella was based on the following assumptions. One, the Salmonella NusA requires the $\frac{T}{C}$GCTCTTT sequence for optimal activity. Two, normal λ N protein interacts poorly with the Salmonella NusA. Three, only the *boxA* sequence in the *nutR* region would have to be altered to permit λ growth, because functions downstream of $t_{L1}$ are not essential for lytic phage growth (Manly et al., 1969). Four, the most common class of mutants of λ that will grow in the hybrid bacterium will be those that are N-independent; these would likely have acquired a *nin* deletion (Court and Sato, 1969).

In order to facilitate the search, we initiated the selection for *boxA* mutations with a λ derivative having two genetic changes from wild type. First, the phage has the *punA* mutation (Friedman et al., 1981) that maps in the *N gene.* As *punA* allows λ to grow in hosts carrying either the *nusA*1 or *nusE*71 mutation, we assumed that the *punA* change would facilitate, at least partially, the interaction between N and the Salmonella NusA product. Second, the phage has the *r*32 insertion (Brachet et al., 1970). This insertion sequence (*IS*2) is known to have a strong Rho-dependent termination signal (de Crombrugghe et al., 1973). A *nin* deletion, which must be promoter distal to the *r*32 insertion, would then not be expected to relieve the need for an active NusA product. This follows from the fact that N activity at *nutR* would be necessary to overcome the termination signal in the *IS*2 (Tomich and Friedman, 1977).

λ*punAr*32 was mutagenized by growth in the *mutD*-5 mutator strain (Fowler et al., 1974). An aliquot of this lysate was placed on the bacterial lawn K1102, an E. coli strain with the *nusA* gene from Salmonella typhimurium. The

plates were incubated overnight at 37°C, and a few extremely small plaques were found at a frequency of $\sim 10^{-7}$. One of the plaques was purified and used to grow a lysate. The mutation responsible for growth on K1102 was mapped according to the cross shown in Figure 2. The results of the mapping studies indicate that this mutation is to the left of the immunity region. The fact that 36 of 37 of the phages that crossed out the *N* amber mutations and did not have the *r*32 insertion could partially grow in the hybrid bacterium K1102 located the responsible mutation near or within the *N* gene. The mutation was preliminarily named *punA**. The isolation of λ*punA** was consistent with the considerations enumerated previously. Further study of the mutant showed that it failed to form plaques on K1102 at 42°C.

The poor growth of λ*punA*r*32 on K1102 was assumed to reflect the fact that the altered N was not sufficient and that a change giving a more effective NusA-*boxA* interaction was also necessary to permit effective growth of λ on K1102. Therefore, λ*punA** was subjected to a second round of mutagenesis by growth in the *mutD*-5 strain. Mutant phages were isolated that could form plaques on a lawn of K1102 at 42°C. Mapping studies identical to those used previously (see Figure 2) demonstrated that the mutation responsible for the improved growth on K1102 was located within or to the right of the *cI* gene. This mutation was preliminarily named *r*32*. All of the 76 recombinants that had crossed out both the *r*32 and the *N* amber mutations failed to grow in K1102 at 42°C. This is consistent with the change being in *boxA*.

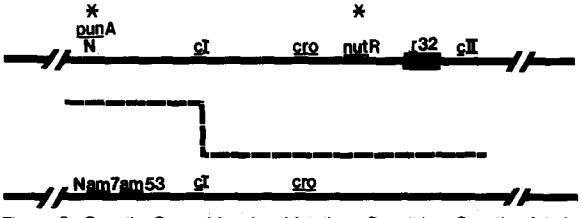


Figure 2. Genetic Cross Mapping Mutations Permitting Growth of λ in K1102

The *r*32 derivatives with the * mutations that permit good growth in K1102 (the hybrid E. coli with the *nusA* gene of Salmonella) were crossed with a λ derivative carrying two amber mutations in the *N* gene, *Nam*7,53. The host bacterium in this cross was the SuII containing strain, C600. A multiplicity of infection for each phage of 5 phages per bacterium was used. The infected bacteria were pelleted and resuspended in phosphate buffer at one tenth volume. They were then exposed to an ultraviolet irradiation dose of 300 ergs/mm² for 60 sec. Following irradiation, the bacteria were resuspended in LB broth and allowed to burst by incubating at 37°C. The phages produced by this joint infection were plated on a bacterial lawn of the Su° host K37 at 32°C. Because the *r*32-containing parent forms a clear plaque as a result of the *r*32 insertion (Brachet et al., 1970), recombinants that have crossed out the *r*32 can now form turbid plaques while those that have crossed out the *Nam* mutations can grow in the Su° host, K37.

When the phage obtained in the initial selection, λ*punA*r*32, was crossed with λ*cI*857*Nam*7,53 36 of 37 recombinants that formed turbid plaques carried the *punA** mutation. When the phage obtained in the second step, λ*punA*r*32*, was similarly crossed, 76 of 76 turbid-plaque formers did not exhibit the ability to form good plaques on K1102, the phenotype of the λ*punA*r*32* parent.

**Identification of *boxA* Mutation**

The *nutR* regions from the phages resulting from both the first and second rounds of the selection procedure were cloned into the M13 derivative mp9 (see Experimental Procedures). The single stranded DNAs from each of the resulting M13 clones were used as templates in dideoxy sequencing reactions (Sanger et al., 1977). The results of the sequencing studies are shown in Figure 3. The mutant resulting from the first selection has a wild-type *boxA* sequence. On the other hand, the mutant resulting from the second selection has an A:T to T:A transversion at the 3′ end of *boxA* and now has a *boxA* sequence, 5′CGCTCTTT3′. Thus it has the three Ts positioned as in the *boxA* sequences of phages 21 and P22. The prediction of this precise change was based on the assumption that the three Ts are important in the recognition of the Salmonella NusA.

In reading the *punA*r*32* sequence from the Bgl II site in *cro* to the *r*32 junction, no other deviations from the wild-type sequence (Rosenberg et al., 1978) were seen. Because the recombination experiments discussed in the next section demonstrate that the mutation responsible for good plating on K1102 maps between the Bgl II site in *cro* and the left-hand *r*32 junction, we conclude that the trans-

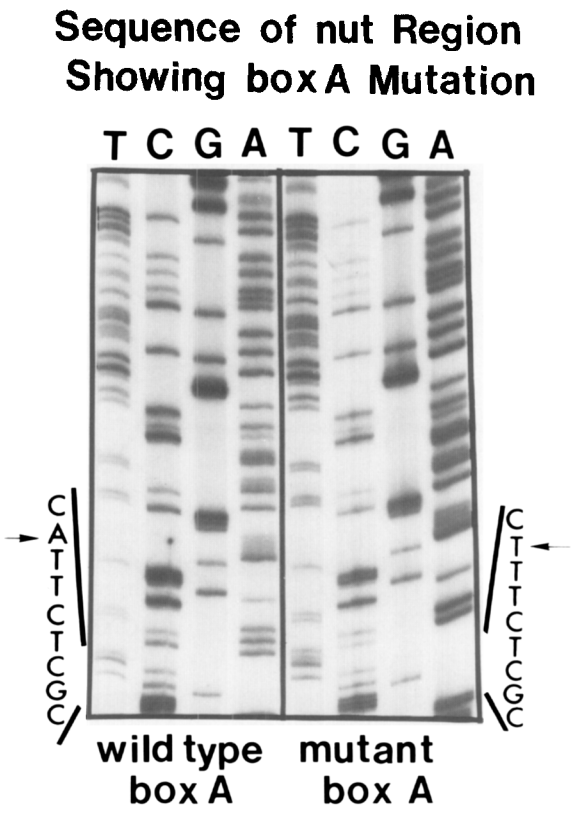


Figure 3. DNA Sequence of *boxA*1

The gel shows the sequences from the 3′ end of *cro* to the *r*32 junction. The left-hand sequence is from λ*punA*r*32 and the right-hand sequence is from λ*punA*r*32*. The *boxA* sequences are listed on the side. The *boxA*1 mutation is the AT–TA transversion indicated by the arrows.

version substituting the T for the A is responsible for this phenotype. The *boxA* mutation will now be referred to as *boxA*1.

### Rescue of *boxA*1 from a Cloned Fragment

We next confirmed biologically that the mutation identified in the cloned fragment is responsible for the added effectiveness of phage growth in the hybrid bacterium. The mutant isolated in the first selection, λ*punA*r32*, was crossed with an mp9 derivative carrying the cloned fragment with *boxA*1. As a control we crossed λ*punA*r32* with a wild-type *boxA* sequence similarily cloned into mp9. Figure 4A shows the details of the cross and lists the results. In the case of the cloned *boxA*1 sequence, recombinants were readily obtained; $4.8 \times 10^{-4}$ of the resulting phage plated well on K1102 at 42°C. However, in the cross with the cloned wild-type *boxA* sequence, no recombinants ($<10^{-9}$) were found.

In a second set of crosses a λ derivative with the *punA** mutation but lacking the *r32* was used. As shown in Figure 4B, the amount of homology between λ*punA** and the mp9 derivative is nearly 80% less than that available in the cross using λ*punA*r32*. Moreover, the elimination of the *r32* element means that mutations permitting growth on the hybrid bacterium should be isolated at a substantially higher frequency. The cross with the cloned *boxA*1 yielded a lower number ($\sim 10^{-6}$) of recombinants that grew at 42°C in the hybrid bacterium, a result expected from the reduction in homology. The cross with the cloned *boxA* wild-type sequence yielded phages that grew in the hybrid bacterium, but at 10 times lower frequency. We attribute these phages to mutations in the P–Q region that eliminate the effect of $t_{R2}$ and thus render the phages N-independent.

Because the second cross with *boxA*1 yields a significant number of recombinants above background that have acquired the ability to grow in the hybrid at high temperature, we conclude that the responsible mutation is between the Bgl II site in *cro* and the *r32* junction. Since the sequence analysis of the cloned λ material revealed only the one change from wild type, we further conclude that the A to T mutation is solely responsible for the added efficiency of growth in the hybrid bacterium.

An additional study suggests that the three Ts of the *boxA*1 mutant are necessary (if not sufficient) for growth

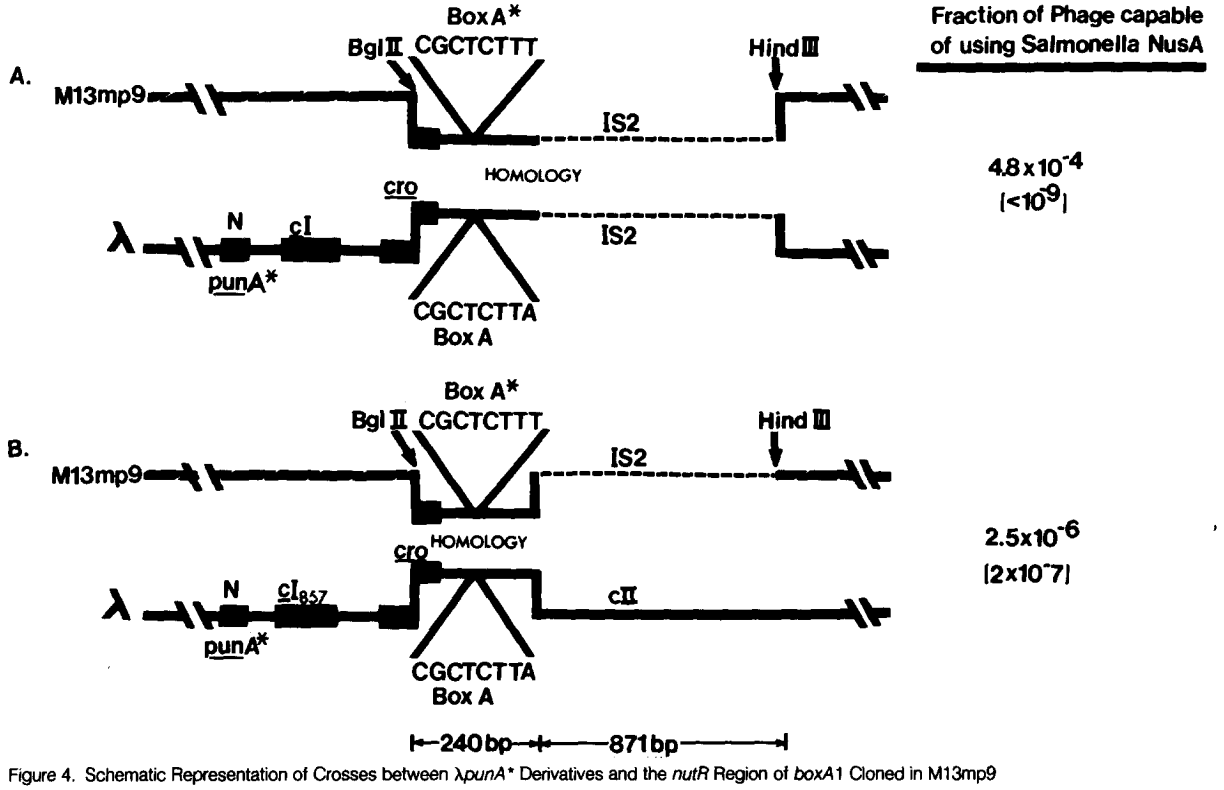


Figure 4. Schematic Representation of Crosses between λ*punA** Derivatives and the *nutR* Region of *boxA*1 Cloned in M13mp9

In (A) the *punA** phage carries the *r32* insertion. In (B), the phage does not carry the insertion.

Bacteria with the M13mp9 clones were grown to $\sim 10^8$/ml, infected with the appropriate phage at a multiplicity of 5 phages to 1 bacterium. Following a 15 min adsorption, bacteria were diluted into LB broth and grown at 37°C for 90 min. The cultures were then treated with chloroform to complete lysis. Lysates were plated for total phage titer using a bacterial lawn of K37 (contains the *nusA* of E. coli) and for *boxA*1 recombinants on K1102 (contains the *nusA* of Salmonella). Plates were incubated at 42°C.

The fraction of phages capable of using NusA-Salmonella was calculated by dividing the titer obtained on K1102 by that obtained on K37. The numbers in parenthesis are the results of similar experiments using an M13mp9 derivative with the cloned *boxA* region from the λ*punA*r32* phage. That cloned fragment carries the wild-type *boxA* sequence.

of λ in K1102. A second independently, but identically, selected λ*punA*r32** mutant was obtained starting with a different lysate of λ*punA*r32*. The second mutation also mapped within or to the right of the *cI* gene. The *nutR* region from this mutant was cloned and sequenced using the same procedures used in analyzing the *boxA*1 mutation. This second mutation, *boxA*2, also has the A to T transversion resulting in the run of three Ts.

## Discussion

Although a variety of studies led us to identify *boxA* as a possible NusA recognition sequence, this conclusion was an inference based on circumstantial evidence. Moreover, experiments using cloned *nutL* sequences that do not include *boxA* suggest that this *boxA* sequence is dispensable for N action (Drahos et al., 1982). The isolation of the *boxA*1 mutation offers the first direct evidence that the *boxA* sequence is important for the NusA contribution to the N reaction. While not addressing the question of whether the interaction is with the DNA or RNA, the argument is based on the following considerations. One, the hybrid bacterium, K1102, has the *nusA* region from Salmonella typhimurium. As P1 transduction was used to effect this substitution, not more than 2 min of the chromosome of the hybrid bacterium can be derived from Salmonella (Ikeda and Tomizawa, 1965). Because the only known E. coli gene involved in N action in this region is *nusA*, we have concluded that the substituted *nusA* gene must be responsible for the failure of the hybrid bacterium to support N action. Two, the fact that the NusA product of Salmonella can support the action of the N analogs of phages 21 and P22 correlates with a shared difference in *boxA*; three Ts at the 3′ end of *boxA* instead of two. Three, therefore the isolation of *boxA*1 demonstrates that the three Ts in *boxA* must facilitate the action of NusA of Salmonella.

We can conclude that, at a minimum, *boxA* is involved in N action. The fact that an alteration of *boxA* permits the foreign NusA to support λ-N action leads to a more substantial conclusion: namely, that *boxA* is directly involved in the action of NusA. However, alternative explanations for these observations are possible. For instance, NusA could control the synthesis or modification of a second host function. This hypothetical second function could interact at *boxA;* the NusA of Salmonella, according to this scenario, alters either the expression or modification of that product so that it now requires the three Ts for proper activity.

We are at a loss to explain the results of the Drahos experiments (Drahos et al., 1982) which appear to show that a functional *nut* sequence need not have *boxA*. Perhaps the nature of the plasmid constructions used in those experiments permits N to act with little or no NusA participation. These constructions have only one terminator between the promoter and the gene whose product is measured and this terminator is placed adjacent to the *nut* region. In contrast, in order for λ to be viable, polymerase modified at *nutR* must overcome distal termination signals ($t_{R2}$). The difference in the two sets of experiments might then be explained by a need for NusA participation only when a more complete modification, permitting polymerase to overcome termination signals at distal sites, is required.

Arguing in favor of the direct involvement of *boxA* in NusA action is the observation of Olson et al. (1982) that in each case where NusA has been shown to be active in vitro, a strategically placed *boxA*-like sequence can be identified. In the case of the leader region of the *rrnB* operon (Kingston and Chamberlin, 1982) the sequence TGCTCTTTA is found (Brosius et al., 1981). In the case of the *trp* operon terminators, t and t′ (Wu et al., 1981), the sequences found are CGCAGTTA and TGCGCTTA, respectively (Wu et al., 1980). Considering this information in light of the *boxA*1 mutation, we feel it leads to a compelling argument that the sequence $5'\frac{C}{T}$GCTCTT(T)A3′ is important for NusA action. Other studies from this laboratory (Olson and Friedman, unpublished data) suggest that the interaction between NusA and *boxA* occurs at the RNA level.

The fact that a two step procedure was necessary in isolating the *boxA*1 mutation indicates that another change is necessary in order for λ to utilize the NusA of Salmonella. Preliminary studies indicate that this change is in the *N* gene (A. T. Schauer and D. I. Friedman, unpublished data). This is not surprising as it has been shown that NusA and N can physically associate (Greenblatt and Li, 1981).

The study reported in this paper adds the necessary confirmatory evidence to our previously published identification of *boxA*. How N and the various Nus substances, in particular NusA, interact at *nut* to modify transcription is still not completely understood. For a discussion of possible models the reader is referred to the review of Friedman and Gottesman (1983).

### Experimental Procedures

#### Phage and Cell Growth

Media and methods for cell and phage growth have been described (Miller and Friedman, 1980).

#### Bacteria and Phages

The bacterial strains used, the genotype relevant to this study, and the sources are: K37, Su°, from M. Yarmolinsky; C600, SuII, from M. Yarmolinsky; JM101, Hfr, from W. Dunnick; K1617, *mutD*-5, from E. Flamm; K1102, *nusA* Salmonella typhimurium, from L. S. Baron.

Phages: M13mp9 was obtained from BRL. λ*punAr32* was constructed in this laboratory by crossing λ*punA* with λ*cI857Nam7,53r32* and selecting amber$^+$ phages that formed a clear plaque (Brachet et al., 1970). λ*cI857Nam7,53* was obtained from M. Gottesman. λ*punA* was isolated in this laboratory. λ*cI857Nam7,53r32* was obtained from W. Szybalski.

#### Mutagenesis and Phage Crosses

Phage mutants were obtained following growth under standard conditions in the *mutD*-5 (Fowler et al., 1974) strain, K1617. The methods employed in phage crosses are listed in the legends to Figures 2 and 4.

### Cloning and Sequencing

Lambda DNA was prepared from 5 ml lysates as described (Maniatis, 1982). M13mp9 RF DNA was prepared from JM101-infected cells by the method of Clewell and Helinski (1969).

All ligations were carried out at 16°C for 10–24 hr in 10 mM Tris-HCl (pH8), 5 mM $MgCl_2$, 1 mM dithiothreitol, 0.1 mM ATP and 1 U T4 DNA ligase. Bacteria were made competent for transformation as described by Cohen et al. (1972). Putative M13mp9 clones were screened either by dideoxy sequencing or by isolating the RF from 1 ml cultures (Birnboim and Doly, 1979) and analyzing their restriction patterns by agarose gel electrophoresis.

The *nutR* regions from λ*punA*r32* and λ*punA*r32** were cloned into M13mp9 by digesting the lambda DNA with Bgl II and Hind III and ligating the fragments into Bam HI, Hind III digested vector DNA. JM101 was transfected and clones containing an 871 bp piece from the Bgl II site in *cro* to the Hind III site in *r32* were identified and sequenced by the dideoxy method of Sanger et al. (1977).

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### References

Adhya, S., Gottesman, M., and de Crombrugghe, B. (1974). Release of polarity in *Escherichia coli* by gene N of phage λ: termination and antitermination of transcription. Proc. Nat. Acad. Sci. USA *71,* 2534–2538.

Baron, L. S., Penido, E., Ryman, I. R., and Falkow, S. (1970). Behavior of coliphage lambda in hybrids between *Escherichia coli* and *Salmonella.* J. Bact. *102,* 221–233.

Birnboim, H. C., and Doly, J. (1972). A rapid alkaline extraction procedure for screening recombinant plasmid DNA. Nucl. Acids Res. *7,* 1513–1523.

Botstein, D., and Herskowitz, I. (1974). Properties of hybrids between *Salmonella* phage P22 and coliphage λ. Nature *251,* 584–589.

Brachet, P., Eisen, H., and Rambach, A. (1970). Mutations of coliphage λ affecting the expression of replicative functions O and P. Mol. Gen. Genet. *108,* 266–276.

Brosius, J., Dulls, T. J., Sleeter, D. D., and Noller, H. (1981). Gene organization and primary structure of a ribosomal operon from *Escherichia coli.* J. Mol. Biol. *148,* 107–127.

Clewell, D., and Helinski, D. R. (1969). Supercoiled circular DNA-protein complex in *Escherichia coli:* purification and induced conversion to an open circular DNA form. Proc. Nat. Acad. Sci. USA *62,* 1159–1166.

Cohen, S. N., Chang, A. C. Y., and Hsu, C. L. (1972). Nonchromosomal antibiotic resistance in bacteria: genetic transformation of *Escherichia coli* by R factor DNA. Proc. Nat. Acad. Sci. USA *69,* 2110–2114.

Court, D., and Sato, K. (1969). Studies of novel transducing variants of lambda: dispensability of genes *N* and *Q.* Virology *39,* 348–352.

de Crombrugghe, B., Mudryj, M., DiLauro, R., and Gottesman, M. (1979). Specificity of the bacteriophage lambda *N* gene product (p*N*): *nut* sequences are necessary and sufficient for the antitermination by p*N.* Cell *18,* 1145–1151.

de Crombrugghe, B., Adhya, S., Gottesman, M., and Pastan, I. (1973). Effect of Rho on transcription of bacterial operons. Nature New Biology *241,* 260–264.

Drahos, P., Galluppi, G. R., Caruthers, M., and Szybalski, W. (1982). Synthesis of the *nut*L DNA segments and analysis of antitermination and termination functions of coliphage λ. Gene *18,* 343–354.

Farnham, P. J., Greenblatt, J., and Platt, T. (1982). Effects of nusA protein on transcription termination in the tryptophan operon of Escherichia coli. Cell *29,* 945–951.

Fowler, R. G., Degnen, G. E., and Cox, E. C. (1974). Mutational specificity of a conditional *Escherichia coli* mutator, *mut*D5. Mol. Gen. Genet. *133,* 179–191.

Franklin, N. C. (1974). Altered reading of genetic signals fused to the N operon of bacteriophage λ: genetic evidence for the modification of polymerase by the protein product of the N gene. J. Mol. Biol. *89,* 33–48.

Friedman, D. I., Schauer, A. T., Mashni, E. J., Olson, E. R., and Baumann, M. F. (1983). *Escherichia coli* factors involved in the action of the λ gene *N* antitermination function. In Microbiology 1983, D. Schlessinger, ed. (Amer. Soc. Micro.), pp. 39–42.

Friedman, D. I., and Gottesman, M. (1983). The lytic mode of λ development. In Lambda II, Hendrix et al., eds. (Cold Spring Harbor, New York: Cold Spring Harbor Laboratory), in press.

Friedman, D. I., Schauer, A. T., Baumann, M. R., Baron, L. S., and Adhya, S. L. (1981). Evidence that ribosomal protein S10 participates in the control of transcription termination. Proc. Nat. Acad. Sci. USA *78,* 1115–1119.

Friedman, D. I., and Baron, L. S. (1974). Genetic characterization of a bacterial locus involved in the activity of the N function of phage λ. Virology *58,* 141–148.

Friedman, D. I., Wilgus, G. S., and Mural, R. J. (1973). Gene N regulator function of phage λ*imm21*: evidence that a site of N action differs from a site of N recognition. J. Mol. Biol. *81,* 505–516.

Friedman, D. (1971). A bacterial mutant affecting λ development. In The Bacteriophage Lambda, A. D. Hershey, ed. (Cold Spring Harbor, New York: Cold Spring Harbor Laboratory), pp. 733–738.

Gemski, P., Jr., Baron, L. S., and Yamamoto, N. (1972). Formation of hybrids between coliphage λ and *Salmonella* phage P22 with a *Salmonella typhimurium* hybrid sensitive to these phages. Proc. Nat. Acad. Sci. USA *69,* 3110–3114.

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

Greenblatt, J., McLimont, M., and Hanly, S. (1981). Termination of transcription by the *nusA* gene protein of *Escherichia coli.* Nature *292,* 215–220.

Hilliker, S., Gottesmann, M., and Adhya, S. (1978). The activity of *Salmonella* phage P22 gene 24 product in *Escherichia coli.* Virology *86,* 37–47.

Ikeda, H., and Tomizawa, J. (1968). Prophage P1, an extrachromosomal replication unit. Cold Spring Harbor Symp. Quant. Biol. *33,* 791–798.

Kaiser, A. D., and Jacob, F. (1957). Recombination between related temperate bacteriophages and the genetic control of immunity and prophage localization. Virology *4,* 509–521.

Kingston, R. E., and Chamberlin, M. J. (1981). Pausing and attenuation of in vitro transcription in the *rrnB* operon of E. coli. Cell *27,* 523–531.

Kung, H., Spears, C., and Weissbach, H. (1975). Purification and properties of a soluble factor required for the deoxyribonucleic acid-directed *in vitro* synthesis of β-galactosidase. J. Biol. Chem. *250,* 1556–1562.

Liedke-Kulke, M., and Kaiser, A. D. (1967). Genetic control of prophage insertion specificity in bacteriophage λ and 21. Virology *32,* 465–474.

Maniatis, T., Fritsch, E. F., and Sambrook, J. (1982). Molecular cloning: a laboratory manual. (Cold Spring Harbor, New York: Cold Spring Harbor Laboratory), p. 371.

Manly, K., Signer, E. R., and Radding, C. M. (1969). Nonessential functions of bacteriophate λ. Virology *37,* 177–188.

Miller, H. I., and Friedman, D. I. (1980). An E. coli gene product required for λ site-specific recombination. Cell *20,* 711–719.

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

Roberts, J. W. (1969). Termination factor for RNA synthesis. Nature *224,* 1168–1174.

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

Salstrom, J. S., and Szybalski, W. (1978). Coliphage λ*nut*L: a unique class of mutants defective in the site of *N* utilization for antitermination of leftward transcription. J. Mol. Biol. *124,* 195–221.

Sanger, F., Nicklen, S., and Coulson, A. R. (1977). DNA sequencing with chain-terminating inhibitors. Proc. Nat. Acad. Sci. USA *74,* 5463–5467.

Tomich, P. K., and Friedman, D. I. (1977). Isolation of mutations in insertion sequences that relieve IS induced polarity. In Insertion Elements: Plasmids and Episomes, S. Adhya, A. Bukhari, and J. Shaprio, eds. (Cold Spring Harbor, New York: Cold Spring Harbor Laboratory), pp. 99–107.

Westmoreland, B. C., Szybalski, W., and Ris, H. (1969). Mapping of deletions and substitutions in heteroduplex DNA molecules of bacteriophage lambda by electron microscopy. Science *163,* 1343–1348.

Wu, A. M., Chapman, A. B., Platt, T., Guarente, L. P., and Beckwith, J. (1980). Deletions of distal sequences affect termination of transcription at the end of the tryptophan operon in E. coli. Cell *19,* 829–836.

Wu, A. M., Christie, G. E., and Platt, T. (1981). Tandem termination sites in the tryptophan operon of *Escherichia coli.* Proc. Nat. Acad. Sci. USA *78,* 2913–2917.