Structure and Mechanism in Transcriptional Antitermination by the Bacteriophage λ N Protein

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Each of the two early operons and the late operon of bacteriophage λ contain transcriptional terminators (Fig. 1a). As a consequence, the pattern of transcription during a lytic infection by λ can be viewed as a cascade of transcriptional antitermination mechanisms (for review, see Das 1993; Greenblatt et al. 1993; Friedman and Court 1995; Richardson and Greenblatt 1996). These antitermination mechanisms are "processive" in the sense that they can influence termination through multiple terminators over many kilobases of DNA and many minutes of transcription time. The *N* gene is the first gene to be transcribed in the leftward early p_L operon and encodes an antitermination factor. Once N protein is made, it modifies *Escherichia coli* RNA polymerase transcribing the early p_L and p_R operons so that the RNA polymerase transcribes efficiently through all the intrinsic and Rho-dependent terminators of both operons. This leads to the expression of genes involved in DNA replication (*O*, *P*) and genetic recombination (*exo*, *int*, *xis*). Some time later, the *Q* gene, which is located far downstream from the rightward early promoter p_R, is transcribed as a consequence of antitermination by N. Its product, the Q protein, modifies the *E. coli* RNA polymerase molecules initiating constitutively at the late promoter p'_R so that the RNA polymerase passes through the downstream terminator t'_R and transcribes all of the late lysis and morphogenesis genes of the phage. The N and Q proteins are operon-specific activator proteins and recognize their target operons because they have specific sites, *nut* ("N utilization") in the case of N and *qut* ("Q utilization") in the case of Q. Similar antitermination mechanisms exist in related lambdoid bacteriophages like phage 21 and the *Salmonella* phage P22, but the λ N protein is unable to function with the *nut* sites of phages 21 and P22.

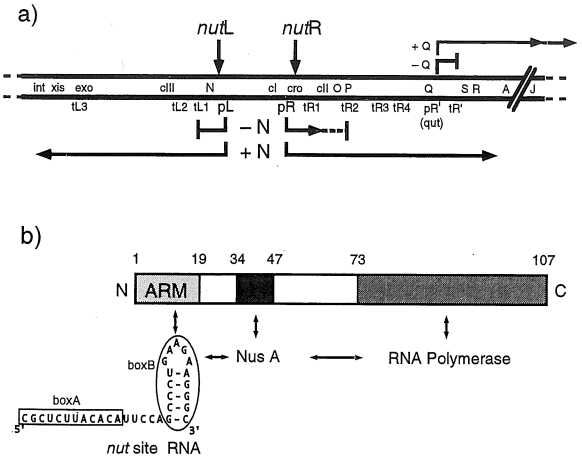

Figure 1. Transcriptional regulation by the N protein of bacteriophage λ. (*a*) Partial genetic and transcriptional map of λ showing the early promoters p_L and p_R, the late promoter p'_R, various terminators (t_{R1}, t_{R2}, ...), the N utilization sites *nutL* and *nutR*, the Q utilization site *qutR*, and the effects of the N and Q antiterminator proteins on transcription. The *int*, *xis*, and *exo* genes are involved in genetic recombination, the *c*I, *c*II, and *c*III genes in the control of lysogeny, the *O* and *P* genes in DNA replication, the *S* and *R* genes in bacterial cell lysis, and the genes *A* through *J* in phage particle morphogenesis. (*b*) Functional domains of the N protein and their interactions with the *nut* site RNA, NusA, and RNA polymerase. Amino acids 1–19 constitute an arginine-rich motif (ARM).

RNA LOOPING

The *nutL* and *nutR* sites are located in transcribed regions downstream from p_L and p_R, respectively. Observations made initially by Friedman and colleagues that mutations allowing ribosomes to translate across a *nut* site prevent antitermination by N (Olson et al. 1982, 1984; Warren and Das 1984; Zuber et al. 1987) provided the first evidence that *nut* sites might function as RNA rather than DNA. This evidence led us to suggest that N and bacterial antitermination cofactors might remain associated with the *nut* site RNA and RNA polymerase during chain elongation, leading to the formation of an RNA loop (see Fig. 2) (Greenblatt 1984). This RNA looping model for antitermination was supported subsequently by several observations: First, we showed that the *nut* site in the nascent transcript is protected from chemical modification and ribonuclease digestion by the presence of N and its bacterial cofactors during transcription in vitro (Nodwell and Greenblatt 1991); second, we found that protection of the *nut* site RNA is weakened by mutations in RNA polymerase that interfere with antitermination by N (Nodwell and Greenblatt 1991); third, Whalen and Das (1990) showed that removal of the *nut*-site-containing DNA fragment from a transcription template by digestion with a restriction enzyme once RNA polymerase had moved past the *nut* site does not interfere with antitermi-

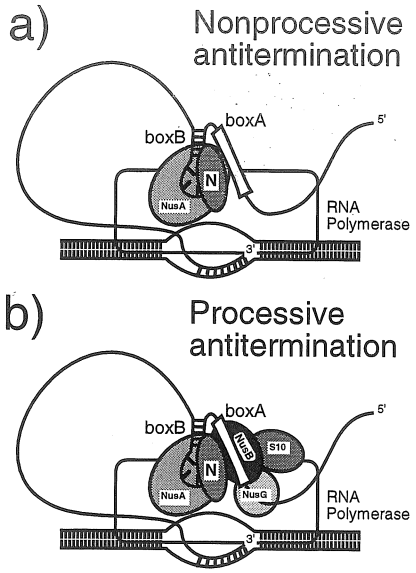

Figure 2. RNA looping models for (*a*) nonprocessive antitermination at a terminator located just downstream from a *nut* site and (*b*) processive antitermination mediated by N over kilobases of DNA. See text for details.

nation by N. According to this model, the *nut* site is effectively a mobile RNA enhancer. A similar RNA looping model involving a mobile RNA enhancer was proposed later to account for transcriptional antitermination by the Tat *trans*-activator proteins of the immunodeficiency viruses (Sharp and Marciniak 1989).

ASSEMBLY OF THE N-MODIFIED ELONGATION COMPLEX

The analysis of mutations in *E. coli* genes, the *nus* genes, that prevent the growth of λ because they interfere with antitermination by N (for review, see Friedman et al. 1984) led to the identification of three *E. coli* proteins, NusA, NusB, and ribosomal protein S10 (NusE), that participate in antitermination by N (Friedman and Baron 1974; Keppel et al. 1974; Friedman et al. 1976, 1981; Das and Wolska 1984; Goda and Greenblatt 1985). Subsequently, we reconstituted processive antitermination by N in a fully purified system containing seven proteins (RNA polymerase, N, Rho factor, NusA, NusB, S10, and NusG), and this led to the identification of NusG as a fourth *E. coli* protein involved in antitermination by N (Li et al. 1992). NusG also interacts with termination factor Rho (Li et al. 1993) and is important for termination mediated by Rho in some circumstances (Sullivan and Gottesman 1992; Li et al. 1993; Nehrke and Platt 1994; Burns and Richardson 1995). Although a mutation in the *nusG* gene suppresses the deleterious effects of the *nusA1* and *nusE71* mutations on antitermination by N (Sullivan et al. 1992), NusG appears to be nonessential for antitermination by N in vivo (Sullivan and Gottesman 1992). Therefore, yet another unidentified bacterial protein may participate in antitermination by N and make NusG redundant for antitermination in vivo.

Purification of elongation complexes from transciption reactions containing N, RNA polymerase, and all four *E. coli* Nus proteins enabled us to show that N and the four Nus factors become associated stably with the elongation complex if the DNA template contains a *nut* site (see Fig. 2b) (Horwitz et al. 1987; Mason and Greenblatt 1991). This entire assembly process is apparently DNA-independent: We used gel mobility shift assays to show that N, RNA polymerase, and the four Nus factors can also be made to associate stably with *nut* site RNA in the absence of DNA (Mogridge et al. 1995, 1998a). Stable association of RNA polymerase with *nut* site RNA requires N and all four Nus proteins (Mogridge et al. 1998a), which probably explains why all four Nus factors are also required for processive antitermination by N in vitro (Mason et al. 1992a; DeVito and Das 1994). Among the many protein-protein interactions in this complex, three of the Nus factors, NusA, NusG, and S10, interact directly with RNA polymerase (Greenblatt and Li 1981b; Mason and Greenblatt 1991; Li et al. 1992). In the absence of NusB, NusG, and S10, we found that association of RNA polymerase with the N-NusA-*nut* complex is unstable (Mogridge et al. 1995, 1998a). In this situation (see Fig. 2a), antitermination by N is nonprocessive but still occurs at a terminator located just downstream from a *nut* site (Whalen et al. 1988; Mason et al. 1992a). In these circumstances, it is likely that antitermination fails further downstream because the weakly bound N-NusA-*nut* complex dissociates from RNA polymerase as the RNA loop grows large and its localizing effect is lost (Nodwell and Greenblatt 1991; Van Gilst et al. 1997). In the nonprocessive N-modified transcription complex shown in Figure 2a, N and NusA interact with each other (Greenblatt and Li 1981a), with RNA polymerase (Greenblatt and Li 1981b; Mogridge et al. 1998b), and with the *nut* site RNA (Chattopadhyay et al. 1995; Mogridge et al. 1995; Tan and Frankel 1995; Cilley and Williamson 1997; Su et al. 1997b; Van Gilst et al. 1997). In the stable, processive complex shown in Figure 2b, there are additional protein-protein (Mason and Greenblatt 1991; Li et al. 1992; Mason et al. 1992b) and protein-RNA (Nodwell and Greenblatt 1993; Mogridge et al. 1998a) interactions that are likely to account for the increased stability of the complex.

The ability of N to antiterminate at intrinsic and Rho-dependent terminators in the absence of NusB, NusG, and S10 (Whalen et al. 1988; Mason et al. 1992a) implies that the basic antitermination mechanism resides in N and NusA. On its own, NusA increases the termination efficiency at intrinsic terminators (Greenblatt et al. 1981; Grayhack and Roberts 1982; Schmidt and Chamberlin 1987). Moreover, high concentrations of N can suppress termination in the absence of NusA or a *nut* site (Rees et al. 1996). This and other observations (Mogridge et al. 1998b) have implied that N can antiterminate by directly contacting RNA polymerase. The *nut* site RNA and bacterial cofactors may participate in antitermination mostly because they localize N in the vicinity of the RNA polymerase molecules that are transcribing the λ p_L and p_R operons.

RECOGNITION OF *NUT* (*BOXA+BOXB*) BY N AND BACTERIAL PROTEINS

The *nut* sites have at least two important elements, *boxA* (Friedman and Olson 1983) and *boxB* (Fig. 1b) (Salstrom and Szybalski 1978; Doelling and Franklin 1990; Chattopadhyay et al. 1995). The *boxA* elements, located upstream of *boxB*, are very similar in the *nut* sites of various lambdoid phages and are closely related to *boxA* antiterminator elements in the *E. coli rrn* operons, which encode ribosomal RNA (see Fig. 3d) (Li et al. 1984). We used gel mobility shift assays to show that the *rrn* and λ *boxA* elements are recognized by NusB and S10 (Nodwell and Greenblatt 1993; Mogridge et al. 1998a), which themselves form a heterodimer (Mason et al. 1992b). Moreover, there is evidence that NusB is required for *rrn boxA*-dependent antitermination in vitro (Squires et al. 1993) and is titrated by overproduction of *boxA*-containing RNA in vivo (Friedman et al. 1990). One possibility is that *boxA* is recognized by NusB and that the interaction of NusB with *boxA* is somehow stabilized by S10.

In the case of *rrn boxA*, NusB and S10 are sufficient for weak but detectable binding in a gel mobility shift assay (Nodwell and Greenblatt 1993). This is not the case for λ *boxA*, for which *boxA*-dependent protein binding, dependent on the sequence GCUCUU in *boxA*, requires NusB, S10, and NusG as well as the preassembly on a complete *nut* site of N, NusA, and RNA polymerase (Mogridge et al. 1998a). This implies that λ *boxA* is a mutant version of *rrn boxA* in which the binding of NusB and S10 depends on N and *boxB*. Stabilization of the N-modified complex (Fig. 2) by NusB, NusG, and S10 requires the *boxA* sequence (Mogridge et al. 1998a).

The *boxB* elements located downstream from *boxA* differ considerably in their nucleotide sequences among the various lambdoid phages, but they are all, at least theoretically, capable of forming small RNA hairpins (Franklin 1985). The construction and assay in vivo of chimeric N proteins revealed that their phage specificity resides in short arginine-rich motifs (ARMs) located near the amino termini of the various N proteins (Lazinski et al. 1989). Gel mobility shift assays and ribonuclease protection assays were then used to show that the N proteins bind specifically to *boxB* RNA in vitro (Chattopadhyay et al. 1995; Mogridge et al. 1995; Tan and Frankel 1995). Moreover, the in vitro binding specificity of the various ARMs could explain the *nut* site specificity of the N proteins in vivo: For example, the λ N protein binds to λ *boxB* but not phage 21 or P22 *boxB*, whereas the ARM of

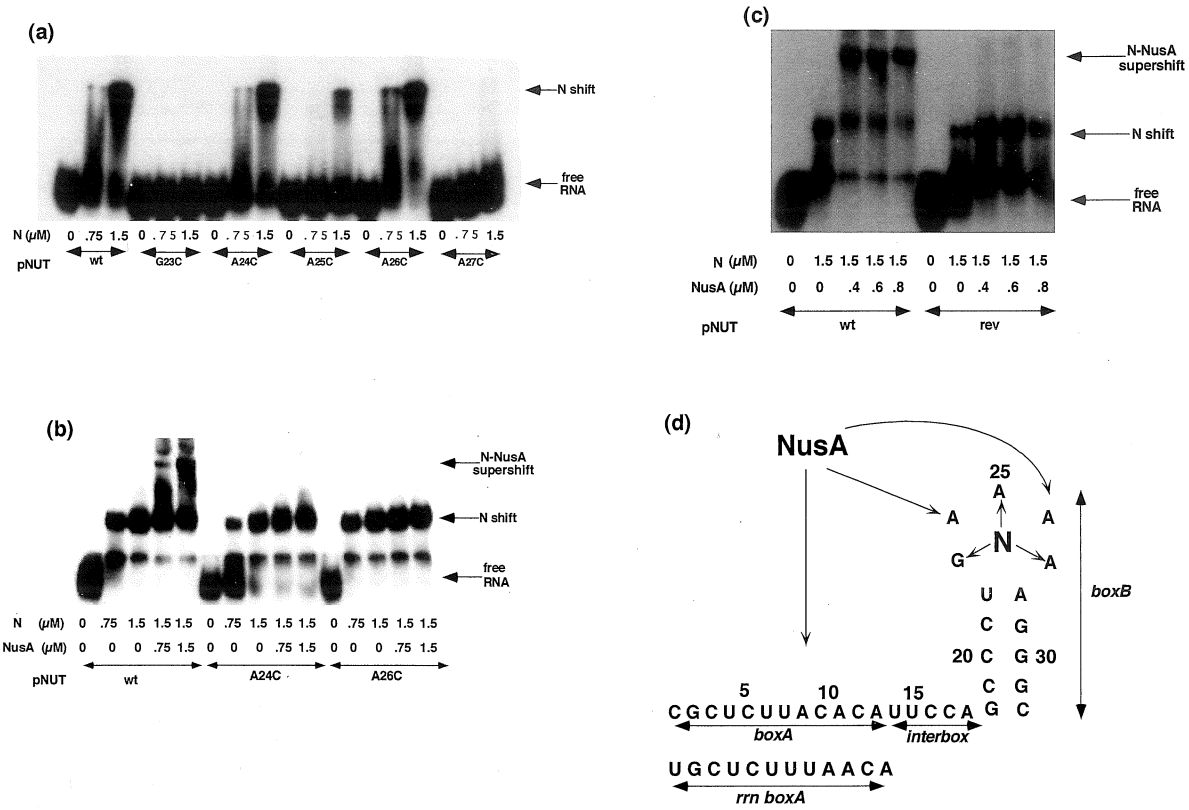

Figure 3. Effects of *boxA* and *boxB* loop mutations on the binding of N and NusA to *nutR* site RNA in gel mobility shift assays. (*a*) Binding of the indicated concentrations of N to wild-type *nut* site RNA and to *nut* site RNAs containing the indicated mutations in the *boxB* loop. (*b*) Effects of the indicated *boxB* loop mutations on the binding of NusA to the N-*nut* site complex. (*c*) Effect of reversing the *boxA-interbox* sequence upstream of *boxB* on the binding of NusA to the N-*nut* site complex. (*d*) Sequence of the *nutR* RNA, including a comparison of the λ *boxA* sequence with the *boxA* sequences found in the *E. coli rrn* operons. (Data in panels *a–c* are reprinted, with permission, from Mogridge et al. 1995.)

phage 22 N binds equally well to *boxB* RNA from phage λ or phage P22 (Tan and Frankel 1995). This corresponds to observations that the λ N protein cannot function with the *nut* sites of phages 21 and P22 (Friedman et al. 1973; Hilliker and Botstein 1976) unless N is overproduced (Schauer et al. 1987; Franklin and Doelling 1989), whereas the P22 N protein has specificity but can function to some extent with the *nut* sites of heterologous phages (Hilliker and Botstein 1976; Lazinski et al. 1989).

The λ *boxB* element is a 15-nucleotide hairpin with a 5-bp stem and a five nucleotide loop (Fig. 1b). The analysis of mutations in *boxB* has revealed the nucleotide sequence requirements for binding and antitermination by N. Although base pairing in the stem is important for antitermination, the identities of all but the top U-A base pair seem to have little importance. In contrast, the identities of all five loop nucleotides are critical for antitermination in vivo and in vitro (Doelling and Franklin 1990; Chattopadhyay et al. 1995). It was therefore of great interest when most studies found that only the identities of nucleotides 1, 3, and 5 of the loop, and not the identities of nucleotides 2 and 4, were important for the binding of N (Fig. 3a) (Chattopadhyay et al. 1995; Mogridge et al. 1995; Tan and Frankel 1995). This implied that nucleotides 2 and 4 are required for some other aspect of antitermination by N.

STRUCTURE OF A COMPLEX CONTAINING THE ARGININE-RICH MOTIF OF N AND *BOXB* RNA

High-resolution structures have now been determined for four ARMs bound to their target RNAs. Although ARMs all consist of a high density of arginine residues in a short stretch of only 10–20 amino acids, these structures have revealed that there is substantial flexibility in the ways in which ARMs recognize RNA. The ARMs of human immunodeficiency virus type-1 (HIV-1) Rev and bovine immunodeficiency virus (BIV) Tat make nonspecific and base-specific contacts with both RNA strands in widened major grooves of double-stranded regions of the HIV-1 Rev response element (RRE) and BIV TAR element, respectively (Puglisi et al. 1995; Ye et al. 1995, 1996; Battiste et al. 1996). Nevertheless, Rev recognizes the RNA as a regular α-helix, whereas BIV Tat forms a β-hairpin.

The N proteins of phages λ and P22 recognize their cognate *boxB* RNA hairpins in yet another way (Fig. 4a,b). We (Legault et al. 1998) and Cai et al. (1998) used heteronuclear magnetic resonance to produce structures of the ARMs of these N proteins bound to their cognate *boxB* RNAs. Both bind on the major groove face, but neither N protein penetrates deeply into the major groove. Rather, each N peptide forms a bent α-helix that interacts almost entirely with the loop and only one strand, the 5′ strand, of the regular A-form hairpin stem. These structures are consistent with the observation that λ N protein protects only the loop and the 5′ strand of the stem from ribonuclease digestion (Chattopadhyay et al. 1995). The different modes of RNA recognition by the ARMs of Tat, Rev, and the N proteins may be partly explained by the flexibility of RNA structure and the ability of arginine side chains to form a variety of hydrophobic, hydrogen-bonding and electrostatic interactions with RNA.

The λ and P22 N peptides interact in very similar ways with their *boxB* hairpin stems (Fig. 4). In each case, for example, the methyl group of an alanine residue (Ala-3 in λ N protein) makes hydrophobic contacts with riboses and bases of the first two nucleotides of the 5′ stem, and an arginine side chain (Arg-7 in λ N protein) enters the major groove, where it makes hydrophobic contacts with nucleotides in the stem and probably forms a hydrogen bond with the first nucleotide of the loop. This may explain why all tested mutations at Arg-7 of the λ N protein abolish antitermination (Franklin 1993). However, the striking bend in the α-helix of the λ N peptide (Su et al. 1997a; Legault et al. 1998) is displaced toward the amino terminus by about one α-helical turn in the P22 peptide (Cai et al. 1998), and the two N peptides interact very differently with their hairpin loops (Fig. 4).

Interestingly, the five nucleotide loops of the λ and P22 *boxB* elements adopt a fold identical to that of the GAAA

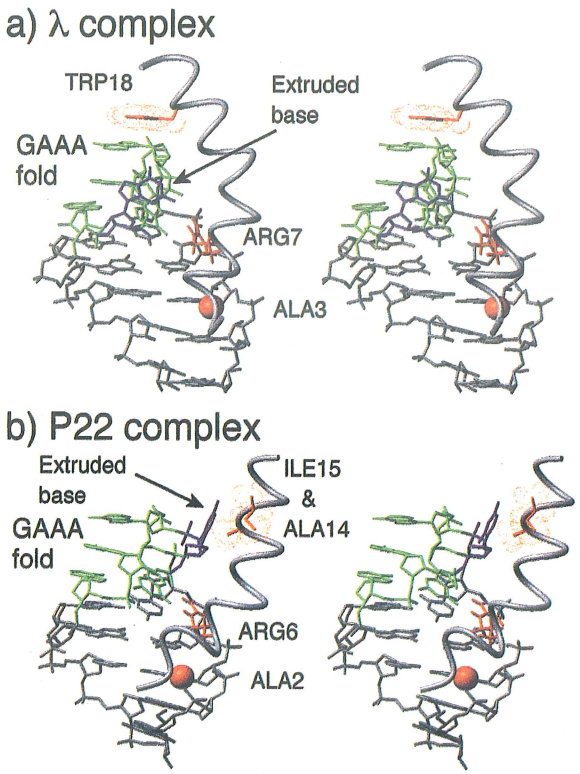

Figure 4. Stereoviews comparing the structures of (*a*) the phage λ ARM peptide (amino acids 1–22) and (*b*) the phage P22 ARM peptide (amino acids 14–28; pdb file 1A4T) bound to their cognate *boxB* RNAs (Cai et al. 1998; Legault et al. 1998). (*Green*) Stacked nucleotides of the GNRA folds of the *boxB* loops; (*purple*) nucleotides that extrude from the GNRA folds, loop nucleotide 4 in λ, and loop nucleotide 3 in P22; (*gray*) *boxB* hairpin stems and the α-helical peptides. Individual representative structures were generated using the program MOLMOL (Koradi et al. 1996) and were oriented relative to each other by superimposing the bases of the GAAA folds and the U-A closing base pairs. See text for other details.

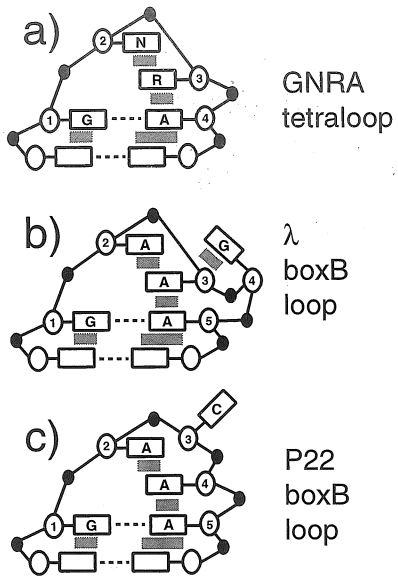

Figure 5. GNRA folds in the λ and P22 *boxB* loops. (*a*) Structural features of the GAAA tetraloop (Heus and Pardi 1991) and summarization of the base requirements for a GNRA fold; (*b*) structural features of the λ *boxB* GAAGA pentaloop (Legault et al. 1998). Nucleotide 4 is extruded from the GNRA fold and is required for the interaction of NusA with the complex. The base of extruded nucleotide 4 interacts with the ribose of loop nucleotide 3. (*c*) Structural features of the P22 *boxB* GACAA pentaloop (Cai et al. 1998). Nucleotide 3 is extruded from the GNRA fold and interacts with the P22 N peptide.

tetraloop, a member of the family of GNRA tetraloops (Heus and Pardi 1991). In this type of GNRA fold (Fig. 5a), the first and last nucleotides of the loop form a sheared G-A base pair, there is a large change in direction of the phosphate backbone between the first and second nucleotides, and the second, third, and fourth nucleotides stack sequentially on the 3′ stem.

Quite remarkably, the five nucleotide loops of the λ and P22 *boxB* elements use different means to create four nucleotide GNRA folds in five nucleotide loops (Fig. 5b,c). In the case of the GAAGA *boxB* loop of λ *nutL*, nucleotides 1, 2, 3, and 5 form a GAAA fold which excludes nucleotide 4 (Fig. 5b). Moreover, nucleotides 1, 3, and 5, which are critical for the formation of the GNRA fold, are precisely those residues in the loop which are critical for the binding of the λ N protein (see Fig. 3a) (Mogridge et al. 1995). This suggests that the λ N protein recognizes the shape of the GNRA fold, and indeed, the indole ring of Trp-18 of λ N is stacked on the top nucleotide of the fold (i.e., nucleotide 2 of the loop) (Figs. 4a and 5b) (Su et al. 1997b; Legault et al. 1998). The excluded nucleotide 4 does not interact with N and can be deleted without the loss of binding (Legault et al. 1998). Therefore, the λ N protein recognizes a genuine GNRA tetraloop.

In striking contrast, nucleotides 1, 2, 4, and 5 of the *boxB* loop of P22 form a GAAA fold which excludes nucleotide 3 (Cai et al. 1998) (Fig. 5c). In this case, the excluded nucleotide 3, a cytidine, makes hydrophobic contacts with several residues of the P22 N peptide (Fig. 4b). Since P22 N binds equally well to the P22 *boxB* element, whose loop is GACAA, and the *boxB* element of λ *nutL*, whose loop is GAAGA (Tan and Frankel 1995), the λ *boxB* loop most likely can form two different kinds of GAAA tetraloops, excluding nucleotide 4 when λ N is bound and nucleotide 3 when P22 N is bound. Viewed this way, it is easy to understand how the P22 N protein can function to some extent with either *boxB* of P22 or *boxB* of λ (Hilliker and Botstein 1976). More generally, it seems plausible that any loop of the form $G(N_x)RA$ or $GNR(N_x)A$ (x = 1, 2, 3...) might be capable of forming a GNRA fold. In that case, GNRA folds may be very common in ribosomal RNAs and elsewhere.

DOMAINS OF N

Nuclear magnetic resonance (NMR) and circular dichroic (CD) experiments have shown that the 107-amino-acid λ N protein is disordered (Van Gilst et al. 1997; Legault et al. 1998). This lack of secondary and tertiary structure and the large number of basic residues in N (22% arginine plus lysine) probably explain why N has a half-life of only 2 minutes in vivo (Konrad 1968; Rabovsky and Konrad 1970; Schwartz 1970; Greenblatt 1973). We and other investigators have used various fragments of N in protein binding, gel mobility shift, and antitermination assays and found that N has at least three functionally important regions (see Fig. 1): The amino-terminal ARM (amino acids 1–19) interacts with *nut* site RNA and generates the operon specificity in this control system (Lazinski et al. 1989; Tan and Frankel 1995; Su et al. 1997a; Mogridge et al. 1998b); a central region of N (amino acids 34–47) interacts directly with NusA (Mogridge et al. 1998b); and a carboxy-terminal region (amino acids 73–107) interacts with RNA polymerase (Mogridge et al. 1998b). There may also be an interaction with RNA polymerase of the amino-terminal half of N (amino acids 1–47). In analogy with the nomenclature used for proteins that activate transcriptional initiation, the middle and carboxy-terminal regions of N behave as activation domains for antitermination in transcription assays (Fig. 6b) (Mogridge et al. 1998b). NMR experiments have shown that only the amino-terminal ARM of N, and not its downstream antitermination regions, becomes folded when N interacts with *boxB* RNA (Van Gilst and von Hippel 1997; Van Gilst et al. 1997; Mogridge et al. 1998b). Therefore, N is a multidomain disordered protein whose domains must fold independently when they encounter their targets.

INTERACTION OF THE N-*NUT* COMPLEX WITH NUSA

Transcriptional antitermination requires that the N-*nut* site complex communicate via RNA looping with elongating *E. coli* RNA polymerase, as illustrated in Figure 2. A key aspect of this link to RNA polymerase is provided by NusA, an *E. coli* elongation factor that can interact di-

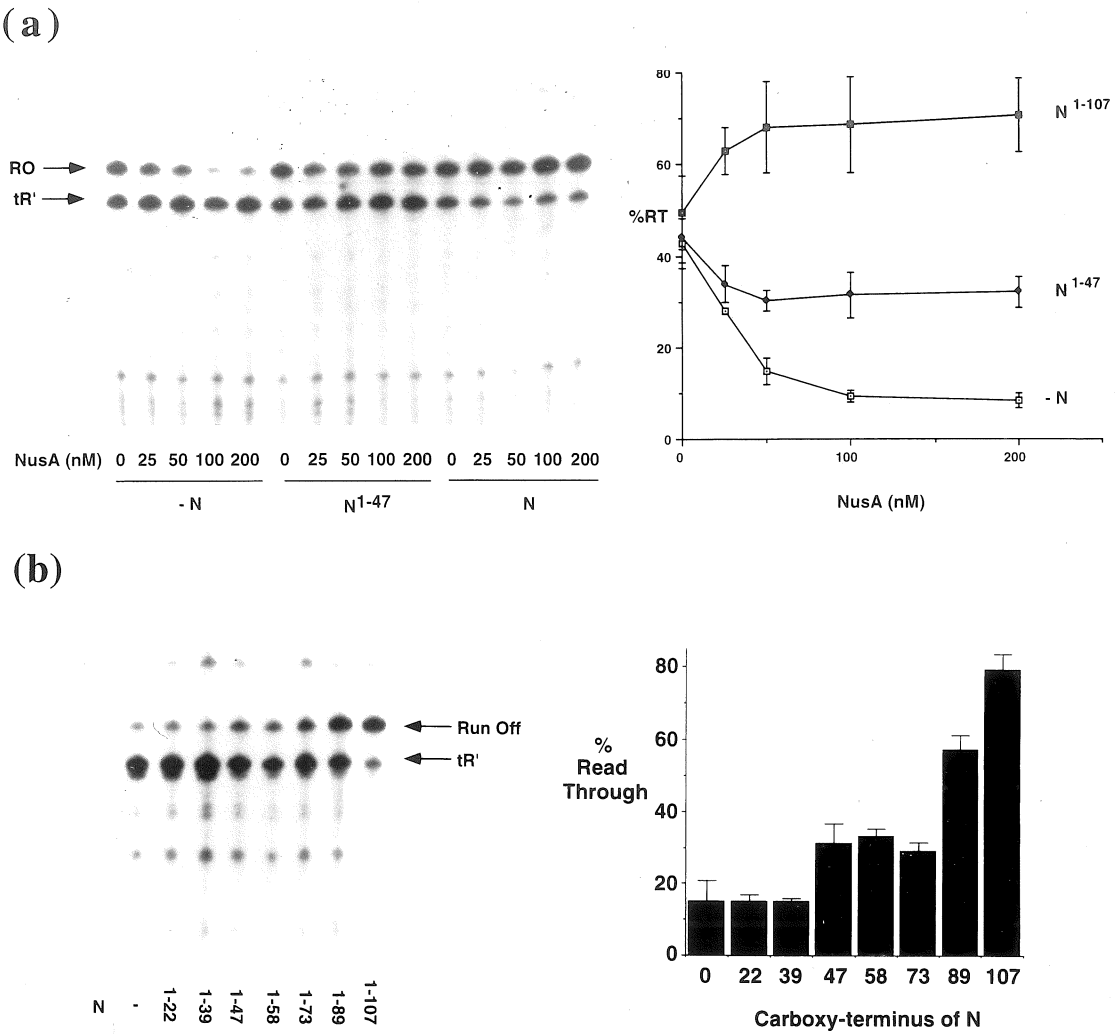

Figure 6. Effects on transcription of the antitermination domains of the λ N protein. (*a*) N^{1-47} supports an intermediate level of antitermination. (*b*) The carboxy-terminal region of N is also important for antitermination. Transcription reactions containing 25 nM RNA polymerase, 100 nM NusA unless otherwise indicated, and 100 nM full-length N or various deletions mutants of N, as indicated, were analyzed on a denaturing polyacrylamide gel and autoradiographed. The DNA fragment used as a template contained p_L, *nutL*, and t'_R. The positions of terminated (t'_R) and runoff (RO) transcripts are indicated. The experiments were quantitated and expressed as percent terminator read-through (RT). (Reprinted, with permission, from Mogridge et al. 1998b [copyright Cell Press].)

rectly with both RNA polymerase (Greenblatt and Li 1981b) and a central region of N (Greenblatt and Li 1981a). If these direct protein-protein interactions were strong enough, there would probably be no *nut* site specificity for antitermination mediated by N, and indeed, *nut* site specificity is lost when N is overproduced (Schauer et al. 1987; Franklin and Doelling 1989; Lazinski et al. 1989). That a *nut* site is normally required for antitermination implies that the N-NusA and NusA-RNA polymerase interactions are too weak to drive the assembly process at physiological concentrations of N (about 10^{-7} M; Greenblatt 1973; Greenblatt et al. 1980). Since we have estimated that the affinity of N for NusA is about 10^6 M^{-1} (Formosa et al. 1992 and unpubl.) and have found that the affinity of N for a NusA-RNA polymerase complex is about 5–10 times lower than its affinity for free NusA (J. Li and J. Greenblatt, unpubl.), *nut*-site-independent interaction of N with RNA polymerase should occur only 1–2% of the time in vivo and have little biological effect. Strengthening of the N-NusA interaction by RNA looping (Fig. 2a) and by direct interactions between NusA and the *nut* site RNA would then make antitermination *nut*-site-specific.

The interaction of NusA with the N-*nut* site complex can be assessed by gel mobility shift assays utilizing ^{32}P-labeled *nut*-site-containing RNA, as shown in Figure 3b (Mogridge et al. 1995). NusA does not bind the RNA detectably on its own but does cause a supershift in the presence of N. Most importantly, NusA does not bind to the N-*nut* site complex when nucleotide 2 or 4 of the *boxB* loop is changed from G or A to C (Fig. 3b) (Mogridge et al. 1995). These loop nucleotides have little effect on the binding of N but are required for antitermination by N (Fig. 3a) (Doelling and Franklin 1989; Chattopadhyay et

al. 1995; Mogridge et al. 1995). When nucleotide 4, which extrudes from the GNRA fold of the *boxB* loop (Fig. 5b), is deleted, there is very little effect on the binding of N, but NusA no longer binds to the N-*nut* site complex (Legault et al. 1998). Therefore, nucleotide 4 of the loop has a key role in enabling the antitermination factor N to communicate with the *E. coli* transcription apparatus via NusA. In analogy with the ways in which protein modules like the SH2, SH3, PTB, PDZ, and WW domains organize signaling complexes (Pawson and Scott 1997), *boxB* serves as an adaptor module that helps couple N to NusA.

The *nut* site RNA upstream of *boxB* also has a strong effect on the binding of NusA to the N-*nut* site complex. As shown in Figure 3c (Mogridge et al. 1995), the reversal of the upstream *boxA* and *interbox* sequences prevents the binding of NusA. Further analysis with single and multiple point mutations in the *boxA* and *interbox* regions (T.-F. Mah and J. Greenblatt, unpubl.) has shown that the identities of nucleotides in the 3′ half of *boxA* are most important for the binding of NusA.

The data in Figure 3, b and c, imply that NusA might have multiple direct interactions with the *nut* site RNA. Sequence comparisons have revealed that NusA has an S1 domain (Fig. 7c), which is a presumptive RNA-binding domain whose prototypes are found in bacterial ribosomal protein S1. The S1 domain is a member of the family of OB folds (Bycroft et al. 1997), which bind oligonucleotides and oligosaccharides. As well, NusA has two KH domains, which are also thought to bind RNA (Fig. 7c) (Musco et al. 1996, 1997). The *nusA1* mutation prevents antitermination by N in vivo and in vitro (Friedman and Baron 1974; Das and Wolska 1984; Goda and Greenblatt 1985; Horwitz et al. 1987) without affecting the binding of N to NusA (Greenblatt and Li 1981a). Instead, the *nusA1* mutation prevents the binding of NusA to the N-*nut* site complex (Fig. 7a) (Mogridge et al. 1995), presumably because the NusA1 mutant protein has a defect in RNA binding. Consistent with this idea, the *nusA1* mutation is located in the S1 domain of NusA (Craven and Friedman 1991; Ito et al. 1991). This and other observations (Y.N. Yu et al., in prep.) indicate that the S1 domain of NusA has an important role in binding the *nut* site RNA. Since we have also found that ribosomal protein S1 can itself bind quite selectively to RNAs that contain the *rrn* or λ *boxA* sequence (Mogridge and Greenblatt 1998), one possibility is that the S1 domain of NusA interacts with the *boxA* region of the λ *nut* site RNA. The particular region of NusA that recognizes the loop of *boxB* has not yet been identified.

In any case, the interaction of NusA with an N-*nut* site complex is likely to be at least tripartite (Fig. 8). First, there is a direct interaction of NusA with amino acids 34–47 of N (Mogridge et al. 1998b). Second, there may be an interaction of NusA with extruded nucleotide 4 of the *boxB* loop and/or the minor groove face of the *boxB* A-form stem, which is not occupied by N (Mogridge et al. 1995; Legault et al. 1998). Third, there may be a direct interaction of NusA with *boxA*. It would not be surprising if these interactions had a substantial effect on the conformation of NusA and altered its interaction with RNA polymerase.

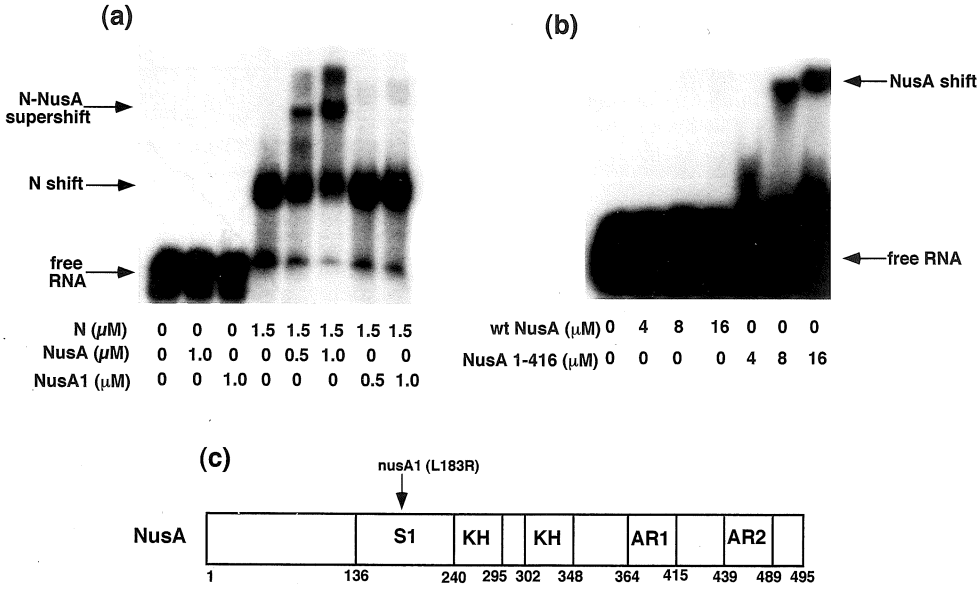

Figure 7. Interaction of NusA with *nut* site RNA. (*a*) Gel mobility shift assays showing the effect of the *nusA1* mutation in the S1 domain of NusA on the binding of NusA to the N-*nut* site complex. Reactions contained ^{32}P-labeled *nut* site RNA and the indicated concentration of N and NusA (Mogridge et al. 1995). (*b*) Gel mobility shift assays showing the effect of deleting the carboxy-terminal 79 amino acids of NusA on the binding of NusA to ^{32}P-labeled *nut* site RNA. (*c*) Diagram showing the S1 and KH domains of NusA and the position of the *nusA1* mutation. AR1 and AR2 are acidic, degenerate, repeated sequences in NusA.

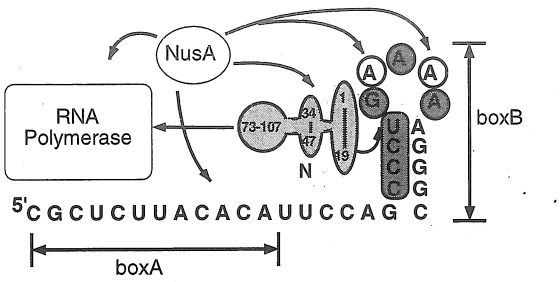

Figure 8. Diagram emphasizing the role of NusA in stabilizing an N-modified transcription complex. NusA interacts with *boxA*, the *boxB* loop, a central region of N, and RNA polymerase. It may facilitate the interaction of the carboxyl terminus of N with RNA polymerase.

MECHANISM OF ANTITERMINATION

The presence of S1 and KH domains in NusA and the inability of NusA to bind RNA on its own imply that there might be occlusion of one or more of the RNA-binding domains in NusA. As shown in Figure 7b, the deletion of 79 amino acids from the carboxyl terminus of NusA does allow NusA to bind *nut*-site-containing RNA weakly in a gel mobility shift assay. This implies that the carboxy-terminal region of NusA has a role in inhibiting the binding of NusA to RNA. Perhaps the interaction of full-length NusA with RNA is only possible in the presence of N or in the context of a transcription complex.

NusA has been shown to bind to the α-subunit of *E. coli* RNA polymerase (Liu et al. 1996). As well, cross-linking experiments using NusA derivatized with a photoactivatable cross-linking reagent have shown that NusA is close to the large subunits, β and β′, of RNA polymerase (J. Li and J. Greenblatt, unpubl.). In analogy to the way in which interaction of σ^{70} with RNA polymerase relieves the inhibitory effect of the amino terminus of σ^{70} on promoter-specific DNA binding (Dombroski et al. 1992, 1993), it is possible that the interaction of NusA with RNA polymerase relieves the inhibitory effect of the carboxyl terminus of NusA and allows NusA to bind RNA. Nuclease protection experiments and protein-RNA cross-linking experiments (Landick and Yanofsky 1987; Liu and Hanna 1995) have shown that NusA interacts with or is close to RNA nucleotides upstream of the 3′ end of the nascent transcript in a transcription complex. Interaction of NusA with the nascent transcript may be important for NusA to enhance pausing and termination by RNA polymerase.

Amino acids 34–47 of N suffice to bind NusA (see Figs. 1b and 8) (Mogridge et al. 1998b). The presence of N, as well as NusA, in a transcription complex may therefore redirect one or more of the RNA-binding domains of NusA away from the 3′ region of the transcript and onto the *nut* site RNA. This phenomenon, if it occurs, is likely to contribute to the ability of N to inhibit pausing and termination by RNA polymerase, both of which are stimulated by NusA (for review, see Richardson and Greenblatt 1996) and likely to involve an interaction of NusA with the nascent transcript. Amino acids 1–47 of N can bind *boxB* and NusA (see Fig. 1b) and suffice to partly suppress termination of transcription in the presence of NusA (Fig. 6a) (Mogridge et al. 1998b). In contrast, amino acids 1–39 of N can bind *boxB* but not NusA and have no effect on termination at an intrinsic terminator (Fig. 6b). Perhaps amino acids 39–47 of N influence antitermination because they bind NusA and serve to position an RNA-binding domain of NusA near the *nut* site RNA, rather than near the 3′ end of the transcript. Subsequent occupation of the S1 and KH domains of NusA by *nut* site RNA would then prevent the binding to NusA of newly synthesized RNA and therefore prevent NusA from contributing to the termination of transcription.

N has little effect on transcription in the absence of NusA in standard reactions containing 10^{-7} M N protein (Whalen et al. 1988; Mason et al. 1992a). At higher concentrations of N and under conditions of low ionic strength, N can suppress termination in the absence of NusA (Rees et al. 1996), and this effect depends on amino acids 73–107 of N (Mogridge et al. 1998b). Amino acids 73–107 of N, which bind to RNA polymerase, also have a major effect on the efficiency of antitermination in the presence of NusA when the concentration of N is 10^{-7} M (Fig. 6) (Mogridge et al. 1998b). In these circumstances, it seems likely that the interaction of RNA polymerase-bound NusA molecules with N and the *nut* site RNA would position amino acids 73–107 of N near a critical surface of RNA polymerase (Fig. 8). In this view, the interaction of the N-*nut* site complex with NusA would suppress termination both by interfering with the ability of NusA to enhance termination and by positioning amino acids 73–107 of N near RNA polymerase (see Figs. 1b and 8).

How amino acids 73–107 of N suppress termination is not yet clear. One possibility, which we favor, is that N might prevent the reverse translocation by RNA polymerase which can occur at pause sites and terminators (Nudler et al. 1997). Reverse translocation necessarily involves the extrusion from the RNA-DNA hybrid in the transcription bubble of the 3′ end of the nascent transcript. In principle, N could inhibit reverse translocation simply by occupying the space that would otherwise be occupied by the extruded 3′ end of the transcript.

ACKNOWLEDGMENTS

This research was supported by grants from the Natural Sciences and Engineering Research Council of Canada (L.E.K.) and the Medical Research Council of Canada (J.G. and L.E.K.). P.L. is a Terry Fox research fellow of the National Cancer Institute of Canada supported with funds provided by the Terry Fox Run. J.G. is a Distinguished Scientist of the Medical Research Council of Canada. J.G. and L.E.K. are International Research Scholars of the Howard Hughes Medical Institute.

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