Proc. Natl. Acad. Sci. USA
Vol. 92, pp. 4061–4065, April 1995
Biochemistry

Bipartite function of a small RNA hairpin in transcription antitermination in bacteriophage λ

(transcription termination/antiterminator/RNA polymerase/RNA–protein interaction)

SAMIT CHATTOPADHYAY, JAIME GARCIA-MENA, JOSEPH DEVITO, KRYSTYNA WOLSKA, AND ASIS DAS

Department of Microbiology, University of Connecticut Health Center, Farmington, CT 06030

Communicated by Mary J. Osborn, University of Connecticut Health Center, Farmington, CT, January 20, 1995

ABSTRACT **Transcription of downstream genes in the early operons of phage λ requires a promoter-proximal element known as *nut*. This site acts in cis in the form of RNA to assemble a transcription antitermination complex which is composed of λ N protein and at least four host factors. The *nut*-site RNA contains a small stem–loop structure called boxB. Here, we show that boxB RNA binds to N protein with high affinity and specificity. While N binding is confined to the 5′ subdomain of the stem–loop, specific N recognition relies on both an intact stem–loop structure and two critical nucleotides in the pentamer loop. Substitutions of these nucleotides affect both N binding and antitermination. Remarkably, substitutions of other loop nucleotides also diminish antitermination *in vivo*, yet they have no detectable effect on N binding *in vitro*. These 3′ loop mutants fail to support antitermination in a minimal system with RNA polymerase (RNAP), N, and the host factor NusA. Furthermore, the ability of NusA to stimulate the formation of the RNAP–boxB–N complex is diminished with these mutants. Hence, we suggest that boxB RNA performs two critical functions in antitermination. First, boxB binds to N and secures it near RNAP to enhance their interaction, presumably by increasing the local concentration of N. Second, boxB cooperates with NusA, most likely to bring N and RNAP in close contact and transform RNAP to the termination-resistant state.**

The positive control of genes that facilitate the bimodal development of λ and related phages in *Escherichia coli* depends on two distinct operon-specific antiterminators (1). The N antiterminator activates the early operons, whereas the Q antiterminator activates the late operon. Both proteins function by a common mechanism: they capture RNA polymerase (RNAP) during early phases of transcription and mask RNAP's response to the downstream terminators (2–8). However, each antiterminator recognizes the respective genetic signal and captures RNAP by distinct mechanisms. The signals for Q action span the late promoter and the early transcribed region. Q binds to a DNA sequence within the late promoter and acts upon RNAP paused at a defined site (9). Specific nucleotides in the nontemplate strand of this region interact with RNAP not only to induce pausing but also to endow upon RNAP the conformation that is essential for engagement by Q (10). In contrast, the *nut* site, required for N action, functions in the form of RNA (11–13). It can facilitate the productive interaction between N and RNAP at remote sites, suggesting that *nut* RNA may act similarly to DNA enhancers, binding N and delivering N to RNAP through RNA looping (11). Finally, while a single host factor (NusA) appears to be sufficient for Q activity, processive antitermination by N demands three additional factors: NusB, S10 ribosomal protein (NusE), and NusG (2, 14–16).

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

The *nut* site contains two important domains: boxA and boxB. boxA is a conserved sequence. Genetic studies suggest that it is recognized by NusB (12, 17). RNAs with some natural versions of boxA, but not all, bind to a complex of NusB and S10 *in vitro* (18). boxB is an interrupted palindrome with the potential to form a hairpin (stem–loop) structure. Its sequence varies among relatives of λ which encode distinct genome-specific N homologues (19). Chimeric *nut* sites that contain the same boxA but unique boxB elements permit antitermination only by the cognate N proteins (20). Hence, N might recognize boxB. The specificity of hybrid N proteins suggests that a putative boxB recognition domain maps in the amino terminus of each N protein (20). This domain comprises an essential, arginine-rich motif conserved in some RNA-binding proteins (20, 21). Although these and other biochemical evidence are consistent with N–boxB RNA interaction (7, 8, 11–13), none has demonstrated the binary interaction to date. By a combination of mutagenesis, gel shift assays, footprinting, and transcription studies, we show here that N binds to boxB RNA in the absence of RNAP and host factors and that N binding is one of two functions performed by boxB.

MATERIALS AND METHODS

The reporter plasmid pGS1, the parent of all boxB mutants, was derived from pDL174 (20). It contained wild-type boxA and boxB elements from λ*nutR* between the *lac* promoter and the *rrnB* T1 and T2 terminators followed by the *galK* gene. Stem mutants were created with synthetic oligonucleotides containing desired substitutions in each strand of the *Xho* I–*Eco*RI cassette. The loop mutants were generated through two oligonucleotides: 5′-CGGGAI$_5$TCCCGTAGCTTAA-3′ and 5′-TCGAGCCCTN$_5$AGGGCATCG 3′, where I represents deoxyinosine and N, equimolar mixture of A, T, G, and C. Upon cloning of the annealed oligonucleotides into pGS1, clones containing desired substitution mutants were identified by dideoxy sequencing of plasmids isolated by alkaline lysis (22, 23). *E. coli* N100 (*recA galK*) was used for cloning by standard procedures. Activity of boxB mutants was assessed by galactokinase assays (20).

RNAs were synthesized *in vitro* by transcription of linearized plasmids that contained a *Hin*dIII–boxA–boxB–*Eco*RI cassette or an *Xho*I–boxB–*Eco*RI cassette cloned downstream from the phage T3 or T7 promoter in standard reactions (Stratagene) with 50–500 μCi (1 μCi = 37 kBq) of [α-^{32}P]ATP (binding studies) or [^{3}H]UTP (competition studies). RNAs were purified by phenol/chloroform extraction and ethanol precipitation and quantitated as trichloroacetic acid-precipitable radioactivity. Over 95% of transcripts were judged to be full-length. Binding reactions used highly purified preparations of the soluble forms of N, NusA, and RNAP (16). Typical reaction mixtures (20 μl) contained fixed amounts of ^{32}P-labeled RNA (5–10 nM), varying amounts of N protein (5–200 nM), 40 mM Tris glutamate (pH 8.0), 10 mM magne-

Abbreviation: RNAP, RNA polymerase.

sium glutamate, 100 mM potassium glutamate, 25 mM NaCl, 5% (vol/vol) glycerol, 0.5% (vol/vol) Nonidet P-40, and *E. coli* tRNA at 50 μg/ml (except in competition experiments). After incubation at 30°C for 5 min, 10-μl samples were resolved in nondenaturing 5% polyacrylamide gels (24). Bands in dried gels were quantitated with a Betascope 603 blot analyzer (Betagen) and also autoradiographed.

RESULTS

N Protein Forms a Binary Complex with boxB RNA. Initial evidence that N forms a binary complex with boxB RNA was obtained by bandshift assays. The *nutR* RNA substrates contained either boxB alone or both boxA and boxB elements. In the absence of N, the native forms of each RNA displayed two prominent bands: a fast-mobility monomer band and a slow-mobility dimer band, which arose from RNA·RNA pairing due to self-complementarity of the vector-derived segments. N binding results in three complexes (Fig. 1*A*): one (designated C1) originated from the monomeric form of RNA and two (C2 and C3) from the dimer, reflecting the occupancy of one or both N binding sites. That the shifted bands represented RNA–protein complexes was evident from Western blotting. The complex formed with the monomeric boxB RNA migrated just above the protein-free RNA dimer. Note that heat denaturation converted the substrate RNA to the monomeric form (M), which resulted in a single shift upon N binding; that the vector-derived 5′ segment, rather than boxB, was responsible for pairing was shown by heterodimer (D) formation between the boxA–boxB RNA and a truncated counterpart that lacked the 3′ arm of boxB (data not shown). N bound to boxB RNA with high affinity and sequence specificity. Several natural and synthetic RNAs without boxB failed to compete for N binding. While the affinity of N for boxB RNA was similar ($K_d \approx 10$–20 nM) whether boxA was present or not, N did not bind to a truncated form of the boxA–boxB RNA devoid of the 3′ half of boxB (data not shown).

N Binds to an Asymmetric Subdomain of the boxB Stem–Loop. By RNase footprinting, we next assessed the secondary structure of boxB and localized the N-binding surface. Consistent with a stem–loop structure, cleavage of the RNA by RNase T1 occurred after numerous guanines, including the one in the presumptive loop (GAAAA), but not after those guanines present in the stem (Fig. 2*A*, lane b). Similarly, cleavage by another single-strand specific nuclease, RNase T2, occurred after all but the last nucleotide of the loop (lane f), and a double-strand-specific nuclease, V1, cleaved most phosphodiester bonds within the 5′ and 3′ arms of the boxB stem (lane j). In each case, N reduced strand scission specifically within boxB. However, the entire boxB sequence was not protected by N from RNase attack. While the stem 5′ arm and the adjacent loop sequence were protected, the 3′ arm was clearly available for cleavage by V1, even with excess N (lanes k and l). Hence, N binding is confined to an asymmetric domain of boxB that encompasses one face of the helix and the adjacent loop (Fig. 2*B*).

Sequence Determinants Critical for N Recognition. The boxB stem is made of a stem with 5 bp and a pentanucleotide loop. In agreement with previous work (26–28), mutations in both the stem and the loop affected antitermination *in vivo* (Table 1). A single base substitution in the 5′ arm of the stem (GCGCU, with the mutant base underlined) decreased antitermination by a factor of ≈2 (line 2). It also caused a modest decrease in N binding (data not shown). The substitution of three additional bases (CGGGU), expected to abolish the stem structure, also abolished antitermination (line 3). The mutant RNA did not form a complex even with micromolar amounts of N (data not shown). This binding deficiency might result from the disruption of stem structure or the loss of base-specific contacts or both. Therefore, we engineered boxB

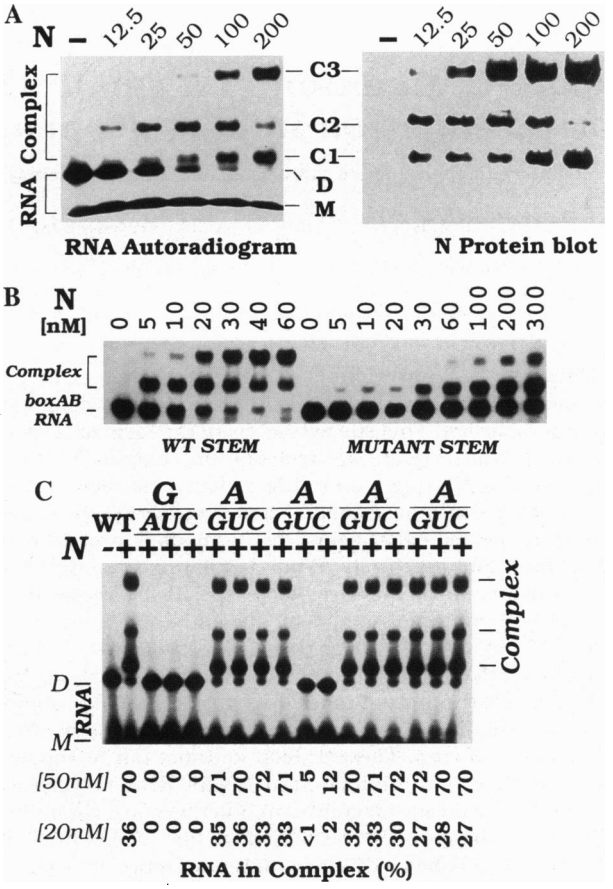

FIG. 1. N–boxB interaction. (*A*) Demonstration of complex formation by bandshift assay. Binding reactions programmed with a 53-nt wild-type boxB RNA (25 nM) and varying amounts of N are displayed (*Left*) along with a Western blot of a duplicate gel with N antibody (*Right*). For blotting, gel contents were electrophoretically transferred onto a Millipore poly(vinylidene difluoride) membrane, and the filter was incubated with purified N antibody, as described (7), followed by a secondary antibody conjugated to alkaline phosphatase and a chemoluminescent substrate from Tropix (Bedford, MA). Although the absolute mass of N in complexes could not be deduced, because N alone did not enter nondenaturing gel due to its high basicity, densitometry revealed that complex C3 contained twice as much N per RNA molecule when compared with complex C2. Additional saturation binding experiments with a fixed amount of N and varying amounts of monomerized RNA suggested 1:1 binding stoichiometry. (*B*) Binding activity of the compensated stem mutant (see text). Reaction mixtures contained 81-nt boxA–boxB RNA (10 nM) with wild-type (WT) or mutant boxB. (*C*) Activity of loop mutants. Reaction mixtures contained 53-nt boxB RNAs with various loop substitutions (10 nM each) and 50 nM N; percent total complexes formed with 50 nM N and 20 nM N are shown at the bottom.

mutants with compensatory base-pair substitutions in the stem. One such mutant (CGGGUACCCG), which theoretically maintained the palindromic structure but diverged from the wild-type stem sequence in all but the closing base pair, showed a substantive defect in antitermination (line 4). Likewise, compared with wild-type RNA, about 10-fold more N was required to convert 50% of the mutant RNA into nucleoprotein complexes (Fig. 1*B*). Thus, optimal N binding requires not only a stable stem structure but also a specific stem sequence.

The analysis of base substitutions in the loop identified two most crucial determinants of N recognition (Fig. 1*C*). One is the guanine residue (1G). Its replacement by A, U, or C abolished specific bandshifts as well as antitermination. Competition experiments determined that substitution of A for G reduced N-binding affinity by a factor of ≈200. The other

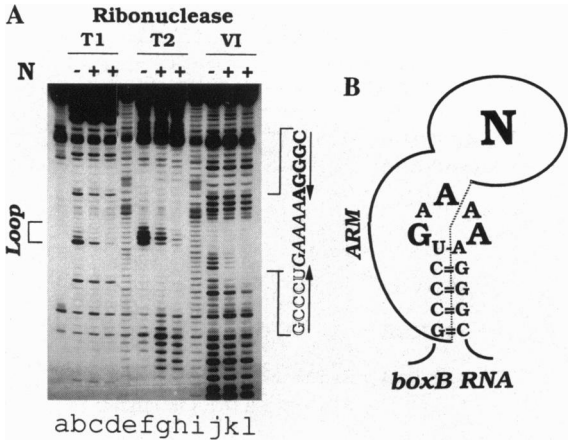

FIG. 2. N-binding surface in boxB. (*A*) RNase footprints. Protection studies were done by standard procedure (25) with 10 nM 93-nt boxA–boxB RNA (5′ end labeled by standard transcription with [γ-^{32}P]GTP) and 200 nM tRNA in the absence or presence of 50 nM N (lanes c, g, and k) or 200 nM N (lanes d, h, and l) and RNase T1 (2.5 units), T2 (5 units), or V1 (0.7 unit), as indicated. RNA was incubated with N at 30°C for 5 min in binding reaction mixtures. After RNase treatment for 10 min at 0°C, RNAs were extracted and precipitated. Resuspended samples were resolved by electrophoresis in 10% polyacrylamide gel with 7 M urea. Marker RNAs and an RNA ladder (produced by alkaline hydrolysis of the substrate RNA) were used to deduce the cleavage sites. (*B*) Model for N–boxB complex. ARM designates the arginine-rich motif in N; the most critical loop nucleotides are shown in large type.

critical residue is the adenine at position 3. Although its replacement by guanine did not affect N-binding or antitermination, either uracil or cytosine substitutions caused a defect in each activity (Table 1, lines 12–14; Fig. 1*C*). Titration experiments showed that these mutants possessed detectable N-binding activity; competition studies determined that these substitutions reduced the binding affinity by a factor of 20–30.

Table 1. *In vivo* activity of stem–loop mutants

	BoxB sequence*	% activity
(1)	GCCCUGAAAAAGGGC	100
(2)	..G.....	59
(3)	CGGG.....	<1
(4)	CGGG.....CCCG	16
(5)	....A.....U....	7
(6)	.....A.....	<1
(7)	.....U.....	<1
(8)	.....C.....	<1
(9)	......G.....	66
(10)	......U.....	32
(11)	......C.....	2
(12)	.......G.....	89
(13)	.......U.....	1
(14)	.......C.....	<1
(15)	........G.....	57
(16)	........U.....	20
(17)	........C.....	8
(18)	.........G.....	7
(19)	.........U.....	<1
(20)	.........C.....	<1

Antitermination activity was monitored in *E. coli* strain N99 (F$^-$*sup*^{0} *strA relA galK*) containing individual *nut*-tester plasmids and the compatible N-supplier plasmid pDL280, a derivative of pACYC177 with the λ *N* gene fused to the *lac* promoter and controlled by the plasmid-borne *lacI* gene (20). Relative antitermination efficiency was measured from rates of galactokinase synthesis. Levels of galactokinase reflect antitermination efficiency rather than plasmid copy number, as determined by quantitative plasmid extraction and estimation of plasmid content. One hundred percent activity represents 3.2 units of galactokinase synthesized per minute per milliliter of pGS1 culture in the presence of N. Less than 1% activity was detected in control cultures without N.

*Line 1 shows the wild-type sequence. Dots in the mutant sequences indicate identity with the wild type.

Duality in boxB Function. That mutations which affect N binding also affect antitermination establishes that N–boxB interaction is a prerequisite for antitermination. The converse is not true. Not all antitermination-defective boxB mutants showed a defect in N binding. For instance, the substitution of adenine residues at positions 2, 4, and 5 in boxB loop did not affect N binding, even when N was limiting (Fig. 1*C*). Yet, some of these mutants allowed very little or no detectable antitermination. The substitutions 2C, 4C, and 5G reduced antitermination by a factor of 10–50 (Table 1, lines 11, 17, and 18), and the 5U/C substitutions abolished antitermination (lines 19 and 20). Likewise, somewhat disproportionate to the 2- to 3-fold N-binding defect (data not shown), the A·U stem showed antitermination reduced by a factor of 15 (line 5). Competition assays confirmed that the defect of none of these boxB mutants in antitermination was attributable to a proportionate decrease in N-binding affinity (unpublished results). Therefore, the mere ability of boxB in securing N may not be not sufficient for a productive N–RNAP interaction.

Cooperativity Between boxB and NusA for Productive N–RNAP Interaction. One simple model that can account for the apparent discrepancy just described is that a cellular protein binds to the particular mutant RNAs and inhibits antitermination *in vivo* by precluding N binding. A more attractive model is that the particular mutations affect a second attribute of boxB: the allosteric modification of N or the interaction of boxB with another component of the antitermination complex—i.e., RNAP or a host factor (7). To distinguish between these models, we cloned the boxB mutants in a transcription vector and examined their antitermination capacity *in vitro* with purified components (Fig. 3). In agreement with previous work (14), N and NusA promoted significant transcription through a test terminator, about 15-fold stimulation of readthrough, when the terminator was preceded by wild-type boxB. However, the templates with each of two boxB mutants that are proficient in N binding did not display appreciable N antitermination, just as a template without the *nut* site. It is then unlikely that the antitermination defect of respective boxB mutants *in vivo* is due to inhibition of N binding by a cellular inhibitor. Rather, the failure to cause antitermination is most likely due to an intrinsic defect of the mutant boxB–N complexes in productive interaction with NusA and/or RNAP.

We therefore examined the binding of wild-type and mutant RNAs with N, NusA, and RNAP by bandshift assays. These reactions did not reveal a NusA–RNA complex, even in the presence of N (Fig. 4*A*, lanes c and e), somewhat contrary to reports that NusA binds to N (29) and also RNA (30). However, in agreement with evidence for RNAP–RNA interactions (31, 32), a bandshift representing an RNAP–RNA complex was indeed visible in the absence of N and NusA (lane d). RNAP bound to boxB RNA in the presence of excess tRNA, and the complex contained the core enzyme, as revealed by Western blotting. NusA did not affect RNAP–RNA binding; neither the quantity of the RNAP–RNA complex nor its mobility differed significantly with NusA. Notably, a distinct complex of RNAP, N, and RNA formed in the absence of NusA (lane f), and NusA stimulated the accumulation of this complex 12 to 15-fold, without a marked change in its mobility (lane h). The presence of N in this complex was confirmed by Western blotting; although some NusA did comigrate with the complex, suggesting a loose association, an accurate determination of NusA content was obscured due to anomalous mobility (smearing) of NusA in nondenaturing gels. These results are consistent with our recent transcription studies

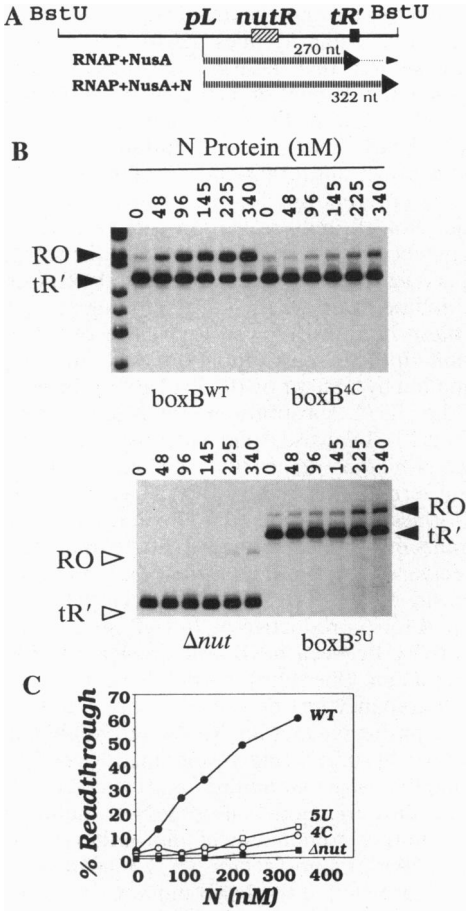

FIG. 3. Effects of 3′-loop mutations on antitermination *in vitro*. Runoff transcription assays were performed as described (16), with highly purified *E. coli* RNAP (17 nM) and NusA (90 nM) and indicated amounts of N. Wild-type and mutant boxB oligonucleotides were cloned as *Eco*NI–*Bst*XI cassettes producing pL-*nutR* hybrids. (*A*) Template and transcript map. (*B*) Gels displaying transcription products [terminated (tR′) and runoff (RO)] from templates with wild-type *nutR* (pJD12; boxB^{WT}) and boxB mutants [pWW31 (Δnut), pCS55 (boxB^{4C}), and pAJ49 (boxB^{5U})]. (*C*) Transcription readthrough as a function of N concentration.

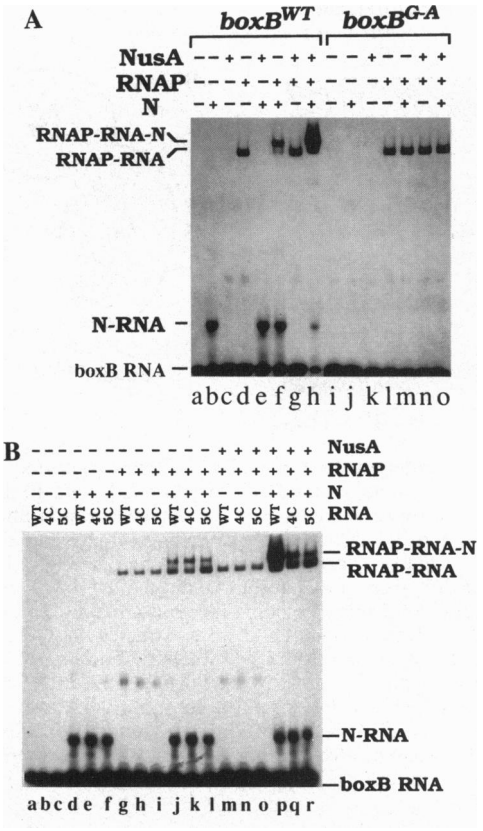

FIG. 4. RNAP–RNA–N complex. (*A*) Cooperative effects of boxB and NusA. (*B*) Defect of boxB loop mutants in cooperativity with NusA. Binding reaction mixtures contained boxB RNA (5 nM), tRNA (200 nM), RNAP (20 nM), NusA (100 nM), and N (50 nM in *A* and 20 nM in *B*). RNA was converted to the monomeric form by heat denaturation. Note that the RNAP preparation contained about an equal mixture of core and holoenzyme. The identity of bands marked N–RNA, RNAP–RNA, and RNAP–RNA–N complexes was determined by blotting with specific antibodies against N, NusA, and RNAP core and σ (K.W., S.C., and A.D., unpublished work). WT, wild type.

demonstrating the basal, NusA-independent antitermination by N (16). Together, they indicate that NusA and boxB RNA cooperate and facilitate a direct, productive interaction between N and RNAP.

Two further pieces of evidence lend support to our proposal that boxB acts as an intermediary. First, as expected, the boxB loop mutant that contains the G → A substitution and does not bind to N failed to produce any RNAP–RNA–N complex, despite the presence of NusA (Fig. 4A, lane o); the mutant RNA did bind to RNAP similarly to wild type (lane 1). Second, each of two 3′ subdomain boxB mutants formed binary complexes with N and RNAP, individually (Fig. 4*B*, lanes d–i) but did not produce an appreciable amount of the RNAP–RNA–N complex in the presence of NusA (lanes p–r); compared with the 12-fold stimulation of the complex with wild-type boxB, the 4C and 5C mutants were stimulated about 2-fold. Clearly, the mutants are defective in cooperating with NusA to activate (or stabilize) N–RNAP interaction. We conclude that while the 5′ subdomain of boxB secures N near RNAP, its 3′ subdomain cooperates with NusA actively to bring N and RNAP in close contact.

DISCUSSION

The evidence that boxB RNA binds to N and that this interaction is required for antitermination is consistent with the hypothesis that boxB RNA acts as an enhancer (11). boxB might merely serve to secure N in the vicinity of its ultimate target, the RNAP elongation complex, similar to the conventional function of a DNA enhancer (33). The biological specificity demands that N action is confined to the λ genome. By tethering N to RNAP on the λ genome, boxB would bring the desired partners together and facilitate their engagement through RNA looping (Fig. 5). According to this model, N must find boxB soon after its emergence in the nascent RNA and bind to it with a high affinity before RNAP reaches the terminator. Our results conform to these requirements: (*i*) the N–boxB complex forms in the presence of a vast excess of tRNA; (*ii*) the affinity is high enough to allow the occupancy of boxB when N concentration is as low as five protomers per cell; (*iii*) the N–boxB complex is formed rapidly, with equilibrium reached in at most a few seconds (unpublished results); and (*iv*) the complex is stable enough to resist RNase attack for minutes.

Is tethering N near RNAP the sole function of boxB? A significant finding reported here suggests that it is not. Some boxB mutants fail to support antitermination *in vivo*, yet they do not affect N binding *in vitro* (Fig. 1 and Table 1). That these mutants are defective in N-mediated antitermination *in vitro* with NusA and RNAP (Fig. 3) rules out the possibility that their antitermination defect in the cell is manifested by an aberrant interaction with an inhibitor that precludes N binding. It follows that the particular boxB mutants must secure N in the vicinity of RNAP just as wild-type boxB, but they fail to

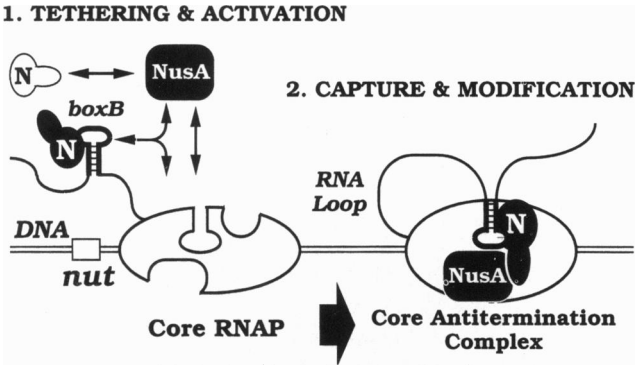

FIG. 5. Model for the core antitermination complex. Details are described in the text. The model suggests that the binding of N to boxB induces an allosteric change in N (or stabilizes a particular conformation of N) necessary for a productive N–NusA–RNAP interaction. The model suggests further that the 3′ subdomain of boxB contacts the NusA–RNAP complex directly.

convert RNAP to the termination-resistant form due to a block in another crucial function of boxB (Fig. 5). Conceivably, N–boxB interaction not only secures N but also activates N to the form essential for capturing RNAP. boxB might activate a direct N–RNAP interaction through an allosteric change in N. Alternatively, boxB could enhance the binding of N to NusA which binds to RNAP. A more appealing model, not mutually exclusive from the others, is that boxB contacts both N and the NusA–RNAP complex. Although N does form a binary complex with NusA which in turn binds to RNAP independently of a nucleic acid signal (29, 34), boxB is still necessary to facilitate the assembly of a functional antitermination complex (11, 14). Indeed, we have shown here that boxB and NusA cooperate to stimulate the formation of a RNAP–boxB–N complex in the absence of transcription (Fig. 4). Both classes of antitermination-defective boxB mutants, one which binds to N and one which does not, are defective in this cooperative interaction.

Further consistent with the bipartite function of boxB is our evidence that N binding is confined to an asymmetric, 5′ subdomain of boxB that is constituted by one helical face and the adjacent loop (Fig. 2). Clearly, the other helical face, if not parts of the loop, is left vacant for interaction with another component. There are hints that the growing segment of the nascent RNA chain interacts with RNAP to modulate elongation and that upstream sequences may similarly play a role in switching the elongation-termination conformations of RNAP (5, 32, 35–37). It is tempting to postulate that boxB contacts the NusA–RNAP complex directly. Perhaps, N binding changes the structure of boxB to facilitate this second interaction. The postulated contact, be it with RNAP or with NusA, would influence antitermination several ways. First, through this additional contact, boxB should strengthen N–RNAP interaction, stabilizing the termination-resistant state of RNAP. Second, the tethering of boxB to RNAP might hinder termination indirectly through a stable association of the nascent RNA in the transcription complex. Third, as envisioned previously (7), the contact might facilitate antitermination directly by masking RNAP's interaction with pause and termination hairpins.

We are indebted to David Lazinski for gifts of plasmids and help in oligonucleotide mutagenesis; to Balaram Ghosh and William Whalen for gifts of N, NusA, and respective antibodies; to Richard Burgess for generously providing RNAP antibodies; and to Yan Wang for help in deriving binding constants. These experiments were supported by the Public Health Service Grant GM28946.

1. Friedman, D. I. & Gottesman, M. (1983) in *Lambda II*, eds. Hendrix, R. W., Roberts, J. W., Stahl, F. W. & Weisberg, R. A. (Cold Spring Harbor Lab. Press, Plainview, NY), pp. 21–51.
2. Das, A. (1992) *J. Bacteriol.* **174,** 6711–6716.
3. Roberts, J. (1992) in *Transcription Regulation*, eds. McKnight, S. & Yamamoto, K. (Cold Spring Harbor Lab. Press, Plainview, NY), pp. 389–406.
4. Greenblatt, J., Nodwell, J. R. & Mason, S. W. (1993) *Nature (London)* **364,** 401–406.
5. Das, A. (1993) *Annu. Rev. Biochem.* **62,** 893–930.
6. Grayhack, E. J., Yang, X., Lau, L. F. & Roberts, J. W. (1985) *Cell* **42,** 259–269.
7. Barik, S., Ghosh, B., Whalen, W., Lazinski, D. & Das, A. (1987) *Cell* **50,** 885–899.
8. Horwitz, R. J., Li, J. & Greenblatt, J. (1987) *Cell* **51,** 631–641.
9. Yarnell, W. & Roberts, J. (1992) *Cell* **69,** 1181–1189.
10. Ring, B. Z. & Roberts, J. W. (1994) *Cell* **78,** 317–324.
11. Whalen, W. & Das, A. (1990) *New Biol.* **2,** 975–991.
12. Friedman, D. I., Olson, E. R., Johnson, L. L., Alessi, D. & Craven, M. G. (1990) *Genes Dev.* **4,** 2210–2222.
13. Nodwell, J. R. & Greenblatt, J. (1991) *Genes Dev.* **5,** 2141–2151.
14. Whalen, W., Ghosh, B. & Das, A. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 2494–2498.
15. Mason, S. W., Li, J. & Greenblatt, J. (1992) *J. Biol. Chem.* **267,** 19418–19426.
16. DeVito, J. & Das, A. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 8660–8664.
17. Patterson, T. A., Zhang, Z., Baker, T., Johnson, L. L., Friedman, D. I. & Court, D. L. (1993) *J. Mol. Biol.* **236,** 217–228.
18. Nodwell, J. R. & Greenblatt, J. (1993) *Cell* **72,** 261–268.
19. Franklin, N. C. (1985) *J. Mol. Biol.* **181,** 75–84.
20. Lazinski, D., Grzadzielska, E. & Das, A. (1989) *Cell* **57,** 207–218.
21. Franklin, N. C. (1993) *J. Mol. Biol.* **231,** 343–360.
22. Birnboim, H. C. & Doly, J. (1979) *Nucleic Acids Res.* **7,** 1513–1523.
23. Sanger, F., Nicklen, S. & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 5463–5467.
24. Carey, J. (1991) *Methods Enzymol.* **208,** 103–117.
25. Knapp, G. (1989) *Methods Enzymol.* **180,** 192–212.
26. Szybalski, W., Brown, A. L., Hasan, N., Podhajska, A. J. & Somasekhar, G. (1987) in *RNA Polymerase and the Regulation of Transcription*, eds. Reznikoff, W. S., Burgess, R. R., Dahlberg, J. E., Gross, C. A., Record, M. T., Jr., & Wickens, M. P. (Elsevier, New York), pp. 381–390.
27. Doelling, J. H. & Franklin, N. C. (1989) *Nucleic Acids Res.* **17,** 5565–5577.
28. Baron, J. & Weisberg, R. A. (1992) *J. Bacteriol.* **174,** 1983–1989.
29. Greenblatt, J. & Li, J. (1981) *J. Mol. Biol.* **147,** 11–23.
30. Tsugawa, A., Kurihara, T., Zuber, M., Court, D. L. & Nakamura, Y. (1985) *EMBO J.* **4,** 2337–2342.
31. Busby, S., Spassky, A. & Buc, H. (1981) *Eur. J. Biochem.* **118,** 443–451.
32. Chamberlin, M. J. (1994) *Harvey Lect.* **88,** 1–22. (Wiley, New York).
33. Ptashne, M. (1992) *A Genetic Switch: Phage λ and Higher Organisms* (Blackwell Scientific, Cambridge, MA), 2nd Ed.
34. Greenblatt, J. & Li, J. (1981) *Cell* **24,** 421–428.
35. Landick, R. & Yanofsky, C. (1987) *J. Mol. Biol.* **196,** 363–377.
36. Telesnitsky, A. P. W. & Chamberlin, M. J. (1989) *J. Mol. Biol.* **205,** 315–330.
37. Clerget, M., Jin, D. J. & Weisberg, R. A. (1995) *J. Mol. Biol.*, in press.