Involvement of boxA Nucleotides in the Formation of a Stable Ribonucleoprotein Complex Containing the Bacteriophage λ N Protein*

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The association of the transcriptional antitermination protein N of bacteriophage λ with Escherichia coli RNA polymerase depends on nut site RNA (boxA + boxB) in the nascent transcript and the host protein, NusA. This ribonucleoprotein complex can transcribe through Rho-dependent and intrinsic termination sites located up to several hundred base pairs downstream of nut. For antitermination to occur farther downstream, this core antitermination complex must be stabilized by the host proteins NusB, NusG, and ribosomal protein S10. Here, we show that the assembly of NusB, NusG, and S10 onto the core complex involves nucleotides 2–7 of λ boxA (CGCUCUUACACA) and is a fully cooperative process that depends on the presence of all three proteins. This assembly of NusB, NusG, and S10 also requires the carboxyl-terminal region (amino acids 73–107) of N, which interacts directly with RNA polymerase. NusB and S10 assemble in the absence of NusG when λ boxA is altered at nucleotides 8 and 9 to create a consensus version of boxA (CGCUCUUACA). These experiments suggest that multiple protein-protein and protein-RNA interactions are required to convert a core antitermination complex into a complete complex.

The bacteriophage λ N protein allows Escherichia coli RNA polymerase to transcribe through termination signals in the early operons of the phage (1). In vivo, N requires a set of host proteins to mediate transcriptional antitermination. Mutations in the nua, nub, and nusE (which encodes ribosomal protein S10), and nusG genes have been isolated that affect N function (2, 3). The importance of these proteins in preventing termination at Rho-dependent and intrinsic termination sites has also been demonstrated in vitro (4–7).

A cis-acting element, the nut site,1 is situated between the promoter and first terminator of the early operons and is necessary for N-mediated antitermination (8, 9). It consists of two elements, boxA and boxB (8, 10, 11). boxB is a 15-bp sequence of hyphenated dyad symmetry downstream of the 12-bp boxA element. Transcription of the nut site initiates the formation of a stable ribonucleoprotein complex consisting of N, the Nus factors, nut site RNA, and RNA polymerase (12–14). Many weak protein-protein and protein-RNA interactions are required for this complex to form: N binds boxB and NusA (15–18); NusA, NusG, and S10 bind RNA polymerase (14, 19, 20); nucleotides in boxA and boxB have been implicated in binding NusA (16); and NusB and S10 form a heterodimer that binds a consensus boxA sequence (CGCUCUUACA) and the functionally related boxA elements of the seven ribosomal RNA (rrn) operons (UGGCUCUUACA), but does not bind λ boxA (CGGCUUACACA) (21, 22).

Whereas the complete antitermination complex can transcribe through multiple termination signals kilobases downstream of the nut site, a core complex consisting of a subset of these factors can only antiterminate over short distances (6, 23, 24). Thus, the core complex, which consists of N, NusA, and the nut site, contains all of the components required for antitermination, and the remaining components (NusB, NusG, and S10) are processivity factors that stabilize the core complex.

Experiments that demonstrated that a boxA point mutation was more deleterious to antitermination in vivo than a boxA deletion raised the possibility that there exists an inhibitor of antitermination that binds boxA (25). The existence of such an inhibitor would imply that, in addition to stabilizing the core complex, NusB and S10 would prevent the inhibitor from accessing boxA. In support of this is the observation that the nubS5 mutation has little effect on antitermination when boxA is deleted (25). The nusE71 mutation, however, still impairs antitermination when boxA is deleted (25). Considering that NusB is dispensable for antitermination in certain conditions (7), the primary role of NusB may be counterinhibition, whereas S10 may be critical for stabilizing the complex as well as for counterinhibition.

N, RNA polymerase, and all four Nus factors can be assembled in vitro onto nut site RNA in the absence of a DNA template, and this process can be analyzed by sequential gel mobility shift assays utilizing 32P-labeled nut site RNA (16). The present study was initiated to further understand the roles of NusB, NusG, S10, and boxA in stabilizing the assembly of a core ribonucleoprotein complex containing N, NusA, and RNA polymerase. We have determined that the assembly of any one of NusB, NusG and S10 onto the core complex in vitro depends on the co-assembly of the other two proteins and boxA, and is stronger with consensus boxA. Furthermore, consensus boxA allows for the assembly of NusB and S10 in the absence of NusG. The systematic mutagenesis of boxA has identified nucleotides 2, 3, 4, 5, 6, and 7 (CGCUCUUACACA) as being specifically involved in the assembly of the complete complex. Using a series of N deletion mutants, we have determined that its carboxyl-terminal region is important for the formation of the core complex as well as for the assembly of NusB-S10.

1 The abbreviations used are: nut site, N utilization site; bp, base pair(s).
that was equilibrated with 0.15M potassium acetate P11 buffer (0.05 M
slurry and incubated at room temperature for 1 h. After centrifugation
of phosphate-buffered saline. Thrombin (400 units) was added to the
12,000 rpm in a Sorvall SS34 rotor. Glutathione-Sepharose beads (4 ml;
was loaded on a P11 column that was equilibrated with P11 buffer
acetate, P11 buffer containing 2M urea and 0.15M potassium acetate. The column was
was incubated on ice for 2.5 h and then centrifuged at 12,000 rpm for 20
with 0.5 mM isopropyl-1-thio-
was grown to an
linear gradient to 1.5 M potassium acetate in P11 buffer.
Preparation of Mutant N Proteins—pNUT mutants were constructed as
described previously (16).
Construction of Glutathione S-Transferase-N Mutants—PCR primers were
designed to amplify N or fragments of N from the plasmid pT7N
(generous gift of M. Gottesman). Forward and reverse primers con-
tained BamHI and EcoRI restriction sites, respectively, for subsequent
cloning into the vector pGEX-2T.
Purification of Mutant N Proteins—The e. coli strain TOP2 (Strat-
agene) (6L) containing glutathione S-transferase-N fusion plasmids
was grown in LB medium to an A600 of 0.5 and induced with 0.5 mM
isopropyl-1-thio-β-ν-galactopyranoside. Cells were harvested by centrif-
ugation at 4500 rpm, resuspended, and sonicated in 50 ml of 1 M NaCl
Buffer A (20 mM Tris-HCl pH 7.8, 0.2 mM EDTA, 1 mM dithiothreitol, 1
mM phenylmethylsulfonyl fluoride), and then centrifuged for 20 min
at 12,000 rpm in a Sorvall SS34 rotor. Glutathione-Sepharose beads (4 ml;
Pharmacia) were added to the supernatant, and this suspension was
rotated at 4 °C for 1 h. The beads were washed and resuspended in 4 ml
of phosphate-buffered saline. Thrombin (400 units) was added to the
slurry and incubated at room temperature for 1 h. After centrifugation
at 3000 rpm, the supernatant was collected and loaded on a P11 column
that was equilibrated with 0.15 M potassium phosphate buffer P11 buffer (0.05 M
Tris acetate, pH 8.0, 0.01 M potassium phosphate, pH 7.0, 7 mM
mercaptoethanol, and 1 mM EDTA). Mutant N proteins were eluted with a
linear gradient to 1.5 M potassium phosphate in P11 buffer.

The Production of N—The E. coli strain TOP2 (Strat-
agene) (6L) containing pT7N was grown to an
A600 of 0.5 in LB medium and induced with 0.5 mM
isopropyl-1-thio-β-ν-galactopyranoside. Cells were har-
cvested by centrifugation, resuspended, and sonicated in 50 ml of 1 M
Cl Buffer A and then centrifuged for 20 min at 12,000 rpm. The
supernatant was discarded, and the pellet was resuspended in 15 ml
of buffer containing 6 M urea, 0.1 M Tris-HCl, pH 7.8, 0.5 M NaCl, 1 mM
EDTA, 1 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride. This
was incubated on ice for 2.5 h and then centrifuged at 12,000 rpm for
20 min. The supernatant was added to 50 ml of 6 M urea P11 buffer and
was loaded on a P11 column that was equilibrated with P11 buffer
containing 6 M urea and 0.15 M potassium phosphate, P11 buffer containing 6 M urea and 0.15 M potassium acetate. The column was
washed sequentially with P11 buffer containing 4 x urea and 0.15 M
potassium acetate, P11 buffer containing 2 x urea and 0.15 M potassium acetate, and P11 buffer containing 0.15 M potassium acetate. N was
eluted with a linear gradient to 1.5 M potassium phosphate in P11 buffer.
 Fractions containing N were pooled and dialyzed against buffer con-
taining 0.05 M potassium phosphate pH 7.0, 1 mM EDTA, and 7 mM
β-mercaptoethanol and then loaded on a hydroxyapatite column equil-
ibrated with the same buffer. N was eluted with a linear gradient to
buffer containing 0.45 M potassium phosphate, pH 7.0, 1 mM EDTA, and
7 mM β-mercaptoethanol. The yield of N with this procedure was 5
per cent of culture.

In Vitro Transcription and RNA Purification—32P-Labeled RNA for
gel mobility shift experiments was produced in 10 μl reactions contain-
ing 1 × BRL T7 RNA polymerase buffer, 5 μM dithiothreitol, 20 μCi of
(α-32P)UTP (NEN Life Science Products), 500 μM UTP, 500 μM ATP, 500
μM CTP, and 500 μM GTP, 1 unit/μl RNAguard, 50 μg/ml Sall-cut
plasmid DNA, and 1.5 units/μl T7 RNA polymerase. The reactions were
incubated at 37 °C for 4 h. A total of 5 μl of 95% deionized formamide,
0.05% bromophenol blue, 0.05% xylene cyanol was added to the reac-
tions, which were then incubated in a boiling water bath for 2 min and
loaded on a 15% (37.5:1 acrylamide:bisacylamide) polyacrylamide, 7 M
urea gel and electrophoresed until the bromphenol blue had migrated
two-thirds of the way down the gel. The labeled RNA was located by
autoradiography; the gel slice containing the RNA was cut out and
soaked in diethylpyrocarbonate-treated water overnight. The yield of
RNA was determined by liquid scintillation counting.

Gel Mobility Shift Experiments—Gel mobility shift assays were per-
duced as described previously (16), except that the reactions contained
approximately 25 nM of radiolabeled nut site RNA. The reactions con-
tained 25 nM RNA polymerase core component, 100 nM NusA, 500 nM N,
100 nM NusB, 100 nM NusG, and 100 nM S10 or as otherwise indicated.

RESULTS

The Assembly of NusB, NusG, and S10 onto the Core Complex—Purification of N from the insoluble fraction derived from a high level
overproducer of N led us to the realization that the N protein used in our previous gel mobility shift studies (16) contained a small amount of ribosomal protein S10 (data not
shown). Our previous experiments had suggested that NusG could be assembled onto the core complex in the absence of
NusB or S10. Therefore, we re-examined the assembly of NusB,
NusG, and S10 onto the core complex in gel mobility shift experiments using the more highly purified N protein as well
as a more highly purified NusG protein preparation (Fig. 1, lanes 1–9). Incubation of N, NusA, and RNA polymerase with
RNA containing the nut site resulted in two weak slow migrating
bands (lane 2). The lower of these bands forms in the presence of only RNA polymerase and NusA and is likely a
complex of NusA, RNA polymerase, and the nut site RNA (16). The upper weak band depends on the presence of N, NusA, and
RNA polymerase, and its formation is impaired by boxB muta-
tions that prevent the assembly of N-nut RNA or NusA-nut RNA complexes (16). Thus, this band likely represents the
unstable core ribonucleoprotein complex. Addition of any one of
NusB, NusG or S10 alone did not supershift the core complex.
Furthermore, the addition of NusB-NusG, NusB-S10, or NusG-S10 did not supershift the core complex (lanes 6–8). Therefore, the complexes that we previously observed when subsets of NusB, NusG, and S10 were used to supershift the core complex could be attributed to small amounts of cross contamination in our purified protein preparations. A strong supershifted band was observed when NusB, NusG, and S10 were added together (lane 9). Thus, the assembly of NusB, NusG, and S10 onto the core antitermination complex depends on the presence of all three proteins.

The substitution of consensus boxA for l boxA (lanes 10–18) still did not allow NusB, NusG, S10, NusB-NusG, or NusG-S10 to supershift the core complex (lanes 12–15). However, the addition of NusB and S10 together supershifted the complex (lane 16). This result is consistent with our previous observation that a heterodimer of NusB and S10 can bind consensus boxA RNA in the absence of other proteins (22). Further stabilization of this complex occurred when NusG was added to the reaction (compare lanes 16 and 18). As well, the addition of NusB-NusG-S10 to the core complex shifted more probe when the probe contained consensus boxA (compare lane 9 with lane 18), presumably because of the strengthened interaction between NusB-S10 and boxA.

Nucleotides 2–7 of boxA Are Specifically Involved in the Assembly of the Complete Complex—Point mutations were made at each position of boxA to further understand its role in the assembly of the complete antitermination complex (see Fig. 2 (A–D) and Table 1). Mutating position 1, 8, or 10 had little effect on the assembly of any of the complexes (e.g. Fig. 2A, compare lanes 2–5 to lanes 7–10). Mutating position 2, 3, 4, 5, or 6 of boxA allowed the formation of the core complex but almost completely prevented the formation of the complete complex (e.g. Fig. 2A, compare lanes 3–5 to lanes 13–15). Since the critical importance of nucleotides 2–6 for the assembly of NusB-S10 onto rrr boxA had been demonstrated previously (22), it was not unexpected that mutation of these nucleotides

| Probe  | boxA sequence | Core complex | Complete complex | Complete/core |
|--------|---------------|--------------|------------------|--------------|
| NUTWT  | CGCUCUUAACA | ++           | ++               | >10          |
| NUTC1G | CGCUCUUAACA | ++           | ++               | >10          |
| NUTC2U | CGCUCUUAACA | ++           | ++               | 0.5          |
| NUTC3G | CGCUCUUAACA | ++           | ++               | 0.8          |
| NUTC4G | CGCUCUUAACA | ++           | ++               | 0.1          |
| NUTC5G | CGCUCUUAACA | ++           | ++               | 0.6          |
| NUTC6G | CGCUCUUAACA | ++           | +                | 0.1          |
| NUTC7G | CGCUCUUAACA | ++           | +                | 3            |
| NUTC8C | CGCUCUUAACA | ++           | +                | >10          |
| NUTC9G | CGCUCUUAACA | ++           | +                | >10          |
| NUTC10C| CGCUCUUAACA | ++           | +                | >10          |
| NUTC11G| CGCUCUUAACA | ++           | +                | >10          |
| NUTC12G| CGCUCUUAACA | ++           | +                | 4            |
| NUTC13G| CGCUCUUAACA | ++           | +                | >10          |
| NUTC14G| CGCUCUUAACA | ++           | +                | >10          |

NUTCON CGCUCUUAACA ++          +++          >10

NUTCON CGCUCUUAACA ++          +++          >10

RNA polymerase, NusA, NusB, NusG, S10, and N (as indicated) were electrophoresed on nondenaturing gels, dried, and exposed to film.
would also prevent the formation of the complete $\lambda$ complex. Mutating position 7 also had little effect on formation of the core complex but in this case partially impaired formation of the complete complex (Fig. 2B, compare lanes 3–5 to lanes 8–10). Similarly, mutating this nucleotide of $rrn$ boxA moderately impaired the formation of the $rrn$ boxA-NusB-S10 complex (22). Mutating position 11 slightly reduced the formation of the core and complete complexes (Fig. 2D, compare lanes 3–5 to lanes 8–10), whereas mutating nucleotide 12 affected the complete complex more than the core complex (Fig. 2D, lanes 13–15 and Table I). Therefore, nucleotides 11 and 12 may weakly interact with a component of the core complex, perhaps NusA. Mutating position 9 slightly weakened the formation of the RNA polymerase-NusA-nut site complex as well as the higher complexes (Fig. 2C, compare lanes 2–5 to lanes 7–10). Thus, only nucleotides 2–7 and perhaps 12 of $\lambda$ boxA are specifically involved in the assembly of the complete antitermination complex (see last column of Table I).

Deletion of the Carboxyl Terminus of N Impairs the Formation of the Core and Complete Complexes—N is a 107-amino acid protein with an RNA-binding domain (amino acids 1–22) (17, 28), a NusA-binding region (amino acids 34–47), and a carboxyl-terminal region (amino acids 73–107) that binds RNA polymerase (29). We have used carboxyl-terminal deletion mutants of N to determine which parts of the protein are important for the formation of the core and complete complexes (see Fig. 3A). N1–89 (the first 89 amino acids of N), which has a truncated RNA polymerase-binding region, supported the formation of much less complete complex and only trace amounts of core complex compared with full-length N (compare lanes 13 and 14 to lanes 15 and 16). N1–47, N1–58, and N1–73, which contain only the RNA-binding domain and NusA-binding region, supported the formation of similar trace amounts of core complex as N1–89, but did not support the formation of the complete complex (lanes 7–12). N1–22 and N1–39, which contain only the RNA-binding domain, did not support the formation of either the core or complete complexes (lanes 3–6). These results showed that the carboxyl-terminal region of N is important for the stability of the core complex and critical for the formation of the complete complex.

To further assess the importance of the carboxyl-terminal region of N for the assembly of NusB, NusG, and S10, we utilized a nut site with consensus boxA for the mobility shift assays (see Fig. 3B). Addition of NusB-S10 supershifted more core complex containing N1–89 and consensus boxA than core complex containing N1–73 and consensus boxA (compare lane 4 to lane 7). Similarly, a smaller amount of complete complex was detectable with N1–73 than with N1–89 (compare lane 5 to lane 8). Thus, the assembly of NusB-S10 is weakened when the carboxyl-terminal 16 amino acids of N1–89 are deleted, which likely explains the inability of N1–73 to support the complete complex with $\lambda$ boxA. The formation of some complete complex in Fig. 3B with N1–73 indicates that using consensus boxA for forming the complete complex can partly bypass a requirement for N to interact with RNA polymerase.

DISCUSSION

Our observation that the N protein fraction used in our previous studies contained a small amount of ribosomal protein S10 prompted us to re-examine the assembly of N, RNA polymerase and the Nus factors onto nut site RNA. This new N preparation is free of significant contamination by S10 (or any other Nus factor) since it behaves identically to gel-purified N in mobility shift assays (data not shown). Our preparations of NusA, NusB, NusG, and S10 are also unlikely to be cross-contaminated to any significant extent since the addition of all four is required to form the slowest migrating complex with a wild type nut site (Ref. 16 and Fig. 1).

Our new experiments show that the stable assembly of NusB, NusG, or S10 onto a core complex containing N, NusA, and RNA polymerase depends on the co-assembly of the other two proteins. These results agree with in vitro transcription experiments that demonstrated that the omission of NusB, NusG, or S10 is equally deleterious to antitermination several kilobases downstream of the nut site (6). Furthermore, omission of any one of these factors prevented the protection from ribonuclease of the nut site RNA in active transcription complexes (30). In contrast, other in vitro experiments indicated that omission of NusB did not severely impair antitermination at terminators situated less than a kilobase downstream of the nut site (7). These results suggest that an antitermination complex that lacks only NusB may be stable enough to support antitermination at a terminator that is less than a kilobase downstream from the nut site, but too unstable to antiterminate over longer distances or to survive in certain in vitro conditions.
Several groups have examined the effects of mutations in boxA on antitermination by N in vivo (25, 31–34), although no systematic mutagenesis of boxA has been performed. Mutations in nucleotides 1–3, 5, and 6 have been shown to impair antitermination (25, 31, 33, 34). However, deletion of boxA had little effect on antitermination within several hundred bp of the nut site (25). The more deleterious effects of point mutations than a simple deletion on antitermination have been explained by the possible existence of an inhibitor of antitermination that binds boxA RNA (25). The existence of such an inhibitor makes it difficult to determine the role of boxA in stabilizing the assembly of the N-modified elongation complex from in vivo results. Deletion of boxA does impair the assembly of N-modified elongation complexes isolated in vitro (14, 30), and deletion of the boxA and interbox regions impairs the formation of antitermination complexes in gel mobility shift assays (16).

We have now determined which boxA nucleotides are important for the in vitro assembly of N-containing ribonucleoprotein complexes by singly mutating each nucleotide of boxA away from the wild type sequence. Mutation of nucleotides 2–7 of boxA did not affect formation of a core complex containing N, NusA, and RNA polymerase and, instead, specifically impaired the formation of the complete complex. Mutating positions 1 and 8–12 of boxA either had no effect or slightly impaired the formation of both the core and complete antitermination complexes (see Table I). Some of these nucleotides in the 3′ region of boxA have been implicated in the binding of NusA to the N-nut site complex,2 suggesting that NusA may interact with this region of the RNA. NusA contains three putative RNA binding domains, one S1 domain and two KH domains (35, 36), and has been implicated in interacting with nucleotides in both boxA and boxB (16). The observation that the nusA1 mutation, which localizes to the S1 domain, still impairs antitermination when boxA is deleted led to the suggestion that NusA might not interact with boxA (32). It is possible, however, that NusA can bind mutant forms of boxA (or unrelated sequences in this region), but with lower affinity. Alternatively, the S1 domain of NusA may recognize nucleotides in boxB or the interbox region, which is also important for antitermination (32, 33), while one of the KH domains interacts with boxA.

The boxA mutations that affect the assembly of the complete complex, but not the core complex (i.e., mutations in nucleotides 2, 3, 4, 5, 6, and 7), correlate very well with rnr boxA mutations that prevent the binding of NusB and S10 to rnr boxA in the absence of NusA, RNA polymerase, or other proteins (22). Mutation of nucleotides 2–6, 8, and 9 prevented the detection of NusB-S10-rrn boxA complexes, mutation of nucleotides 7 and 12 impaired the formation of these complexes, while mutation of nucleotides 1 and 11 had no effect on the assembly of NusB-S10 onto rnr boxA. Taken together, these results are consistent with the idea that NusB-S10 interacts with nucleotides 2–7 of λ boxA and with nucleotides 2–9 of rnr and consensus boxA. Thus, λ boxA is effectively a mutant form of rnr boxA in which nucleotides 8 and 9 are altered so as to prevent the binding of NusB and S10 unless N, NusA, RNA polymerase, and NusG are also present.

NusB-S10 can assemble onto an N-containing complex in the absence of NusG if nucleotides 8 and 9 of boxA are mutated to consensus nucleotides. What then is the role of NusG in stabilizing the assembly with a wild type nut site? Our experiments provide no indication that NusG interacts specifically with any nucleotides in the nut site, but NusG does interact with RNA polymerase (20). Although NusG does not bind strongly enough to NusB or S10 to supershift a NusB-S10 complex on rnr boxA RNA (22), it may nevertheless interact weakly with NusB or S10 and thereby stabilize the interaction of S10 with RNA polymerase (14). This would also explain the observation that NusG can stably assemble onto the core complex only in the presence of NusB-S10.

The assembly of the complete antitermination complex depends on the carboxyl-terminal region of N. Compared with full-length N (107 amino acids), N1–89 supports the formation of reduced amounts of the complete complex and almost undeetectable amounts of the core complex. We have shown elsewhere (29) that amino acids 73–107 of N interact with the core component of RNA polymerase and are critical for antitermination even in the absence of NusB, NusG, and S10. Moreover, deletion of amino acids 90–107 substantially weakens binding to RNA polymerase (29). Presumably, N1–89 makes fewer contacts with RNA polymerase than does the full-length protein, resulting in the formation of less stable complexes. Eliminating the RNA polymerase-binding site by making a larger deletion in N to amino acid 73 eliminated all formation of the complete complex. This result implies either that interaction of N with RNA polymerase is necessary for forming the complete complex or that N73–107 has an additional interaction with NusB, NusG, or S10.

When the nut site contains consensus boxA, N1–73 does assemble NusB-S10 and NusG onto the core complex, but still less efficiently than is the case with N1–89. Again, this may only mean that N1–73, which completely lacks the carboxyl-terminal RNA polymerase-binding domain of N, does not stabilize the core complex as effectively as N1–89, which can still weakly bind RNA polymerase. Nevertheless, even though the core complexes formed by N1–73 and N1–89 are barely detectable, it does seem that they are formed in similar amounts, suggesting that deleting the carboxyl-terminal 16 amino acids of N1–89 may specifically impair the assembly of NusB-S10. It is, therefore, also possible that N facilitates the assembly of this complex by interacting directly with either NusB or S10. Independent evidence for an interaction between N and NusB comes from the observation that mutations in NusB that suppress the nusAI mutation are specific for either the λ N protein or the related N protein of phage 21 (37). It is also possible that an interaction between the carboxyl-terminal region of N and RNA polymerase alters the conformation of RNA polymerase in such a way as to increase the affinity between RNA polymerase and S10.

These experiments suggest that the conversion of a core antitermination complex containing N, NusA, and RNA polymerase to a complete antitermination complex additionally containing NusB, NusG, and S10 depends on nucleotides 2–7 of λ boxA and perhaps on the carboxyl-terminal 34 amino acids of N. The assembly of NusB, NusG, and S10 onto the core complex seems fully cooperative since subcomplexes lacking one or two of these proteins are evidently too unstable to detect by gel mobility shift assays. Our results suggest that numerous protein-protein and protein-RNA interactions are required for the cooperative assembly of NusB, NusG, and S10 onto the core complex.

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