Assembly of transcription elongation complexes containing the N protein of phage λ and the *Escherichia coli* elongation factors NusA, NusB, NusG, and S10

Stephen W. Mason and Jack Greenblatt¹

Department of Medical and Molecular Genetics, and Banting and Best Department of Medical Research, University of Toronto, Toronto, Canada

The transcription antitermination protein, N, of bacteriophage λ ; the *Escherichia coli* elongation factors NusA, NusB, ribosomal protein S10, and NusG; and a DNA template containing a λ nut (N-utilization) site are necessary and sufficient for the highly cooperative formation in vitro of stable transcription complexes containing all five elongation factors. Mutations in the *nut* site, NusA, or the β -subunit of RNA polymerase (RNAP) that impair antitermination in vivo also abolish the assembly of a stable complex containing the antitermination factors in vitro. The effects of RNAP mutations on assembly imply that the antitermination factors assemble on the surface of RNAP. We have shown previously that NusA binds directly to transcribing RNAP ($K_a \sim 10^7 \text{ M}^{-1}$); K_a = association constant and we show here that S10 also binds directly and specifically to RNAP with an apparent K_a of 10^6 M^{-1} . These observations led to a model for the ordered assembly of the N-modified transcription complex.

[Key Words: Antitermination; N protein; Nus factors; ribosomal protein S10; complex assembly]

Received April 19, 1991; revised version accepted June 6, 1991.

The N protein of bacteriophage λ positively regulates delayed-early gene expression by preventing termination of transcription at termination sites located downstream of the λ early genes (for review, see Friedman 1988). This modification of RNA polymerase (RNAP) to a termination-resistant form by N, called antitermination, requires a phage-encoded site called *nut* (N-utilization; Friedman et al. 1973; Adhya et al. 1974; Franklin 1974; Salstrom and Szybalski 1978; Olson et al. 1982). A *nut* site is located between the promoter and the first terminator of each of the two early operons of the phage (Friedman et al. 1973; Salstrom and Szybalski 1978). Antitermination by the N protein in vivo also involves the host *Escherichia coli* proteins NusA (Friedman and Baron 1974) and NusB (Keppel et al. 1974; Friedman et al. 1976), and ribosomal protein S10 (Friedman et al. 1981). These factors are also important for antitermination by N in vitro in reactions containing crude *E. coli* extracts (Das and Wolska 1984; Goda and Greenblatt 1985; Horwitz et al. 1987). The N protein can antiterminate transcription to some extent in vitro in reactions that contain only NusA (Whalen et al. 1988; Whalen and Das

1990); however, efficient, long-distance antitermination in vitro also requires NusB, S10, and a recently discovered additional host elongation factor, NusG (J. Li, S. Mason, and J. Greenblatt, unpubl.), encoded by the essential *E. coli nusG* gene (Downing et al. 1990). N-modified transcription elongation complexes isolated from reactions containing N and *E. coli* extracts have been shown to contain N (Barik et al. 1987; Horwitz et al. 1987), NusA, NusB (Horwitz et al. 1987; Horwitz 1988), and NusG (Horwitz 1988). Therefore, N and at least some of the host factors are carried along by the RNA polymerase as it transcribes DNA located distal to a *nut* site.

We have now analyzed the protein composition of transcription complexes isolated from a reconstructed antitermination system that contains only purified proteins, namely RNAP, N, NusA, NusB, S10, and NusG. We show that N, NusA, NusB, NusG, and S10 are sufficient for the formation of a stable N-modified transcription complex; that N-modified complexes contain all five elongation factors; that formation of the complex is highly cooperative, because all of the antitermination factors must be present for a stable N-modified complex to be isolated; that S10 binds directly and specifically to RNAP; and that mutations in the β -subunit of RNAP

¹Corresponding author.

that affect antitermination in vivo prevent the formation of stable N-modified transcription complexes in vitro.

Results

Protein composition of N-modified transcription complexes

To analyze the protein composition of N-modified transcription complexes, we have carried out transcription reactions using purified RNAP holoenzyme, NusA, NusB, ribosomal protein S10, λ N protein, and the recently identified *E. coli* antitermination cofactor NusG (J. Li and J. Greenblatt, unpubl.). Figure 1 shows an analysis, by SDS-polyacrylamide gel electrophoresis (Laemmli 1970) and Coomassie staining, of the preparations of the purified proteins used in these experiments. For the in vitro transcription reactions, several different DNA templates were employed. The plasmid pLS1 (Lau and Roberts 1985) contains a λ *pR-nutR-tR1* insert, and pLS9 and pLS11 (Lau and Roberts 1985) are derivatives of this same plasmid containing deletions within the *nut* site. Transcription complexes formed in these reactions were separated from free proteins by chromatography on a high-performance liquid chromatography (HPLC) gel filtration column as described previously by Horwitz et al. (1987). The protein components present in the isolated complexes were analyzed by electrophoresis through SDS-urea-polyacrylamide gels (Swank and Munkres 1971) followed by silver staining (Morrissey 1981). This particular gel system can resolve NusB, N, S10, and the ω -subunit of RNAP (Burgess et al. 1986), all of which have molecular masses between 10 and 15 kD.

Figure 1. Proteins used in transcription experiments. Purified proteins (3 μ g of each), as indicated, were subjected to electrophoresis on a 13.5% SDS-polyacrylamide gel and stained with Coomassie Brilliant blue R. (M) 4 μ g each of phosphorylase B (97 kD), BSA (66 kD), ovalbumin (43 kD), carbonic anhydrase (31 kD), soybean trypsin inhibitor (21.5 kD), and lysozyme (14.4 kD).

The N-modified transcription complexes, isolated and analyzed as described above, contained N, NusB (Fig. 2A, lane 1), NusA (data not shown), and NusG, as described previously (Horwitz et al. 1987; Horwitz 1988). In addition, they contained S10 in amounts that were similar to the amounts of NusB and N present in these complexes (Fig. 2A, lane 1). Therefore, all of the antitermination factors are present in N-modified transcription complexes, and the set of five purified elongation factors that we used is sufficient for the formation of an elongation complex that is stably modified by N. The association of elongation factors with the elongation complex was specific: Not all proteins present in a reaction assemble into elongation complexes because BSA, which was present in all our reactions, did not copurify with isolated elongation complexes (data not shown). Although Rho factor can bind selectively to the *rutA* RNA sequence, which overlaps the *boxA* component of *nutR* (Faus and Richardson 1990), Rho had no effect on the presence of the antitermination factors in the isolated transcription complexes (data not shown). Unlike the stoichiometrically modified elongation complexes isolated previously from reactions containing *E. coli* S100 extracts (Horwitz et al. 1987), only ~50% of the RNAP isolated from reactions containing purified proteins was N-modified. It is likely, therefore, that under these conditions about half of the RNAP isolated in these complexes was transcribing plasmid DNA other than the λ *pR-nutR-tR1* region. The difference in promoter utilization between the two systems is probably due to DNA-binding proteins that are present in the crude S100 extracts, but not in our purified protein system, and affect the relative efficiency of different promoters on the plasmid DNA.

To test the biological relevance of this in vitro system, transcription complexes were isolated from reactions programmed with plasmids containing an altered *nut* site (Lau and Roberts 1985). Elongation complexes isolated from reactions programmed with either the *boxA*⁻ template, pLS9 (Fig. 2A, lane 2), or the *nutR*⁻ (*boxA*⁻ *boxB*⁻) template, pLS11 (Fig. 2A, lane 3), did not contain NusB or N and had greatly reduced amounts of NusG and S10. Therefore, although NusA binds directly to the core component of RNAP (Greenblatt and Li 1981b), a wild-type *nutR* site is important for the incorporation of N, NusB, NusG, and S10 into the transcription elongation complex.

Because the *nusA1* mutation has a strong effect on antitermination by N in vivo (Friedman and Baron 1974) and in vitro (Das and Wolska 1984; Goda and Greenblatt 1985), we expected that the *nusA1* mutation might affect the assembly of N-modified transcription complexes in vitro. As shown in Figure 2B, when the purified NusA1 protein replaced wild-type NusA in the transcription reaction, the resulting transcription complexes contained greatly reduced amounts of N, NusB, NusG, and S10 (cf. lanes 1 and 4, Fig. 2B). The *E. coli* strain *groN785* contains three mutations: one in *rpoB*, which encodes the β -subunit of RNAP (Georgopoulos 1971), one in *nusA* (Greenblatt et al. 1981), and one in *rpsJ*, which encodes S10 (C. Georgopoulos, pers. comm.). The *nusA*^{*groN785*}

Figure 2. The effects of mutations in the *nut* site, NusA, and RNAP on the assembly of N-modified transcription complexes. (A) Transcription complexes isolated from reactions containing RNAP, NusA, NusB, S10, NusG, and N were programmed with a plasmid DNA template containing either a wild-type *nut* site (pLS1; lane 1) or one of two mutant *nut* sites with deletions as indicated [lanes 2 and 3; pLS9 (*boxA*[−]) and pLS11 (*boxA*[−]*boxB*[−]), respectively]. Isolated transcription complexes were processed and subjected to electrophoresis on a 10% (wt/vol) SDS-urea-polyacrylamide gel and the proteins were visualized by silver-staining. (Alpha and omega) RNAP α - and ω -subunits, respectively. The electrophoretic position of each protein is indicated at right. (M) 50 ng each of ovalbumin (43 kD), α -chymotrypsinogen (25 kD), β -lactoglobulin (18 kD), and lysozyme (14 kD). (B) Transcription elongation complexes were isolated from reactions containing pLS1 plasmid DNA, NusB, NusG, S10, N, and either wild-type or mutant versions of RNAP or NusA, as indicated at bottom. The position of each protein is indicated at left.

mutation has a relatively weak effect on antitermination by N in vivo (C. Georgopoulos, pers. comm.) and in vitro (J. Li and J. Greenblatt, unpubl.). When the purified NusA^{groN785} protein replaced wild-type NusA in the reaction, the isolated transcription complexes contained small amounts of N, NusB, NusG, and S10, but only approximately one-fifth of that in transcription complexes isolated from reactions containing wild-type NusA (Fig. 2B, cf. lanes 1 and 5).

Mutations in RNAP block the assembly of N-modified transcription complexes

Mutations in the gene encoding the β -subunit of RNAP (*rpoB*) have also been shown to impair antitermination in vivo (Georgopoulos 1971; Ghysen and Pironio 1972; Jin et al. 1988). To test whether these antitermination-defective RNAPs had any effect on the assembly of N-modified transcription complexes, the purified mutant proteins RNAP^{ron} (Ghysen and Pironio 1972) and RNAP^{groN785} (Georgopoulos 1971) were substituted in transcription reactions for wild-type RNAP, and transcription elongation complexes were isolated and analyzed. When either of the RNAP mutant proteins was present in the transcription reactions, the resulting transcription elongation complexes contained NusA (data not shown), but did not contain N or NusB, and had reduced levels of NusG and S10 (Fig. 2B, lanes 2 and 3). Therefore, mutations in RNAP that impair antitermination in vivo also prevent the formation of stable N-modified transcription complexes in vitro.

Assembly of an N-modified transcription complex is highly cooperative

To determine whether each antitermination factor was required for the assembly of an N-modified elongation complex, transcription complexes were isolated from reactions containing all but one of the factors (Fig. 3). As might be expected, no elongation factor was recovered when RNAP was omitted from the reaction (Fig. 3B, lane 3); therefore, none of the factors binds directly to DNA. When any single elongation factor was omitted from the reaction, the remaining elongation factors were obtained in low yield (NusG and S10) or were not detected (NusB and N; Fig. 3A, lanes 2–6). NusA was present in all elongation complexes except when NusA was omitted from the reaction (data not shown). Although the presence of a small amount of NusG did not seem to depend on any other factor, NusG was not present in initiation complexes isolated from reactions lacking nucleoside triphosphates (Fig. 3A, lane 7). The absence of N, NusG, and NusB in Figure 3A, lane 7, suggested that these proteins only associate with transcribing RNAP. We conclude from this experiment that N, NusA, NusB, NusG, and S10 are all important for the *nut* site-dependent stable association of N, NusB, NusG, and S10 with the transcribing RNAP. Therefore, the assembly of these N-modified elongation complexes is highly cooperative.

Ribosomal protein S10 binds directly to RNAP

The role of S10 in the assembly of N-modified elongation complexes was investigated in more detail by varying

Figure 3. The effect of each antitermination factor on the assembly of the N-modified transcription complex. Transcription complexes were isolated from reactions programmed with pLS1 plasmid DNA template. Each reaction contained either all of the proteins (+ NTP) listed (lane 1 of each panel) or a subset of these with individual components singly omitted, as indicated. Isolated transcription complexes were subjected to electrophoresis and visualized as in Fig. 1. (A) Transcription reactions contained 1 μ g/ml of S10 and 1.0 μ g/ml of N. A small amount of NusG was present in lane 5 even though transcription complexes were isolated from reactions that did not contain added NusG, probably because NusG was present as a minor contaminant in the RNAP. However, the amount of NusG present in this reaction was insufficient to allow the incorporation of N, NusB, and S10. (B) Transcription reactions in lanes 1–9 contained 4 μ g/ml of S10 and 2.0 μ g/ml of N, and those in lanes 10 and 11 contained 20 μ g/ml of S10. Superscript notes below lanes 10 and 11 indicate that RNAP mutants were used in place of wild-type RNAP [(a) RNAP^{ron}; (b) RNAP^{groN785}]. Complexes in lanes 10 and 11 were analyzed on a separate gel from lanes 1–9.

the concentration of S10 in a series of transcription reactions (Fig. 4). A small amount of S10 (~1 μ g/ml) was sufficient for the assembly of NusB, S10, NusG, and N into the elongation complex (lanes 1–3). However, the presence in the reaction of much higher amounts of S10 led to much higher yields of S10 in the isolated elongation complexes, although the yields of the other elongation factors were unaffected (lanes 5–7). This suggested that S10 might be able to associate with RNAP independently of the other elongation factors. Therefore, we repeated the experiment shown in Figure 3A except that each reaction contained a fourfold higher concentration of S10 (4 μ g/ml; Fig. 3B). Ribosomal protein S10 was present in isolated elongation complexes when N (lane 2), NusA (lane 5), NusG (lane 6), or NusB (lane 7) was omitted from the reaction, but not when S10 (lane 8) or the RNAP (lane 3) was omitted. S10 was even present in initiation complexes isolated from reactions from which nucleoside triphosphates had been omitted (Fig. 3B, lane 4). Finally, S10 was present in initiation complexes isolated from reactions containing only RNAP, DNA, and S10 and from which all of the other elongation factors were omitted (Fig. 3B, lane 9). These results suggest that

Figure 4. The effect of S10 concentration on the incorporation of the antitermination factors into the transcription complex. Transcription elongation complexes were isolated from transcription reactions programmed with pLS1 plasmid DNA and containing a constant amount of RNAP, NusA, NusB, NusG, and N (2.0 μ g/ml) and various concentrations of S10, as indicated. Complexes were analyzed as in Fig. 1.

S10 binds to the RNAP and can be carried along with the RNAP during elongation. Only when the concentration of S10 was low (as in Fig. 3A) would the stable association of S10 with an elongation complex depend on N, NusA, NusB, and NusG.

To test whether S10 was binding specifically to RNAP, transcription initiation complexes were isolated from reactions containing only RNAP, DNA, and various concentrations of S10 (Fig. 5). The amount of S10 recovered in association with the RNAP–DNA complex saturated at a molar ratio of $\sim 1 : 1$. Even at the highest concentrations of S10 in the reactions, the presence of S10 in the isolated fraction depended on the RNAP (data not shown). Half-maximal binding of S10 occurred at an S10 concentration of $\sim 7.5 \mu\text{g/ml}$, corresponding to an apparent dissociation constant of $\sim 7 \times 10^{-7} \text{ M}$. Therefore, S10 binds specifically and with a relatively low affinity to DNA-bound RNAP in the absence of any other factors.

The isolation of S10–RNAP complexes in the experiments shown in Figures 2–5 depended on the RNAP being bound to the DNA. To determine whether S10 can bind to RNAP that is free in solution we examined the chromatographic properties of purified RNAP and S10 (both together and separately) on a Bio-Gel A1.5m gel filtration column (Fig. 6). Fractions were analyzed by electrophoresis on SDS–polyacrylamide gels (Laemmli 1970). The proteins were transferred to a nitrocellulose filter that was probed with affinity-purified anti-S10. When RNAP and S10 were mixed prior to gel filtration, some S10 coeluted with the RNAP (Fig. 6A, lanes 3 and 4). S10 was not present in lanes 3 and 4 when either RNAP (Fig. 6B) or S10 (Fig. 6C) was chromatographed alone. These results indicate that S10 binds to both DNA-bound and free RNAP.

Figure 5. The effect of S10 concentration on the binding of S10 to RNAP in initiation complexes. Transcription initiation complexes were isolated from reactions containing RNAP, pLS1 plasmid DNA, various concentrations of S10, and no NTPs. The molar ratio of S10 to RNAP in the isolated initiation complex was assessed by densitometric scans of silver-stained SDS–urea–polyacrylamide gels. The data were standardized by scanning various known amounts of S10 and RNAP.

Figure 6. Binding of S10 to free RNAP. RNAP and S10 (A), RNAP alone (B), and S10 alone (C) were chromatographed on a 0.5-ml BioGel A-1.5m column. Fractions were collected and subjected to SDS–PAGE. Western blots probed with affinity-purified anti-S10 are shown. The lane numbers correspond to fractions collected for each column run. The bold arrows at top correspond to the chromatographic position of RNAP and S10, as indicated. The chromatographic position of RNAP was determined by electrophoresis of the fractions on a separate gel followed by Coomassie staining (not shown). The arrows at right show the electrophoretic position of S10 in each gel. (S) 55 ng of purified S10 on each filter. A minor 50-kD protein contaminant present in the RNAP preparation chromatographed in lanes 4–6 of A and B and cross-reacted with the anti-S10.

Because both S10 and NusA (Greenblatt and Li 1981b) bind RNAP and are essential for the assembly of stable N-modified transcription complexes (Fig. 3A, lanes 3 and 6; Fig. 3B, lanes 5 and 8), and because the *ron* and *groN785* mutations in RNAP block the assembly of the N-modified transcription complex (Fig. 2B, lanes 2 and 3), we tested whether these RNAP mutations would interfere directly with the binding of S10 or NusA to the RNAP in the absence of other factors. Transcription initiation complexes isolated from reactions containing only DNA, one of the two mutant RNAPs, and 20 $\mu\text{g/ml}$ of S10 still contained S10 (Fig. 3B, lanes 10 and 11). In an independent experiment these mutations in RNAP did not affect the binding of NusA to RNAP either (data not shown). Therefore, the *ron* and *groN785* mutations must interfere with the modification of RNAP by N in some

way other than by simply blocking the binding of NusA or S10 to RNAP.

Discussion

N, NusA, NusB, NusG, and S10 are necessary and sufficient for the assembly of a stable N-modified transcription elongation complex

We have shown that transcription elongation complexes isolated from reactions containing the λ N protein and the *E. coli* antitermination factors NusA, NusB, NusG, and S10 contain all of these proteins. Mutations in the *nut* site, the β -subunit of RNAP, and in NusA that impair antitermination in vivo also prevented the assembly of an N-modified transcription complex in vitro (Fig. 2A,B), suggesting that the complexes that we have formed in vitro mirror the in vivo situation properly. We have shown previously that the *nusA1* mutation does not affect the binding of N to NusA (Greenblatt and Li 1981a) or the association of NusA with RNAP (Greenblatt et al. 1981; Horwitz et al. 1987). However, the effect of the *nusA1* modification in NusA is manifested as a defect in the stable assembly of N-modified transcription complexes (Fig. 2B). It would appear, therefore, that the *nusA1* mutation directly or indirectly affects a protein-protein or protein-RNA interaction that is important for the assembly of the N-modified complex.

We have also shown that the omission of any single factor from the transcription reaction resulted in destabilized transcription complexes that lacked N and NusB and had reduced amounts of S10 and NusG (Fig. 3A). The assembly of transcription complexes containing λ N protein must be highly cooperative, as each factor is required for the stable association of the other factors with the complex. These observations suggest that N-modified transcription complexes are stabilized by multiple intermolecular interactions.

In work described here we have not directly analyzed the effects of the host factors NusA, NusB, S10, and NusG on transcription in the presence of N. However, in work that will be published elsewhere, we have found that long transcripts (>2000 bp) are produced in much greater yield in transcription reactions containing N, NusA, NusB, NusG, and S10 than in reactions containing only N and NusA (J. Li, S. Mason, and J. Greenblatt, unpubl.). Thus, we think that the assembly of stable transcription complexes containing all of these factors is important for processive, long-distance antitermination.

The role of ribosomal protein S10 in transcription

Ribosomal protein S10 is an intrinsic part of the antitermination reaction, because mutations in S10 prevent antitermination in vivo (Friedman et al. 1981) and in vitro (Das et al. 1985; Horwitz et al. 1987). The role of S10 in antitermination by N may be to couple NusB to RNAP, as NusB binds to S10 (S. Mason, J. Li, and J. Greenblatt, submitted), and, as we have shown here, S10 binds to RNAP.

The rRNA (*rrn*) operons of *E. coli* contain elements that closely resemble the *boxA* sequences in λ *nut* sites (Li et al. 1984; Morgan 1986). When elongation complexes synthesizing rRNA in vitro are isolated from reactions containing *E. coli* extracts, these complexes contain both NusB and NusG (Horwitz 1988). Because NusB binds to S10, it would not be surprising if elongation complexes synthesizing rRNA also contained S10. Therefore, this raises the interesting possibility that S10 may regulate the rate of rRNA synthesis in *E. coli*.

The order of assembly of an N-modified elongation complex

Horwitz et al. (1987) outlined an order for the assembly of an N-modified transcription complex. The experiments presented here enable us to add further to the details of that model (see Fig. 7). Ribosomal protein S10 was able to bind to free RNAP as well as to promoter-bound RNAP that was, presumably, still associated with the initiation subunit σ^{70} (Figs. 3B and 5). This is in contrast to NusA, which can only bind to the core component of RNAP lacking σ^{70} (Greenblatt and Li 1981b). Therefore, S10 can enter the transcription complex by binding weakly to the RNAP in initiation complexes or early elongation complexes (Fig. 7A,B). When the RNAP begins to elongate RNA chains, the σ^{70} is released (Travers and Burgess 1969; Hansen and McClure 1980) and NusA can then bind directly to the RNAP (Fig. 7B; Greenblatt and Li 1981b; Horwitz et al. 1987).

The presence of a small amount of NusG in each of the isolated transcription elongation complexes shown in Figure 3 suggested that NusG can also bind to RNAP independently of other factors or a *nut* site. NusG was absent in isolated transcription initiation complexes (Fig. 3A, lane 7), suggesting that NusG binds only to the core component of RNAP. Recently, RNAP affinity chromatography was used to show that NusG can bind to the core component of RNAP (S. McCracken, unpubl.). As was the case for S10, the binding of NusG to the RNAP in an elongation complex was weak unless it was stabilized by the presence of N and the other elongation factors, as well as a *nut* site (Figs. 2A and 3).

The transcription of the *nut* site allowed N and NusB to bind to the transcription complex, which stabilized and/or enhanced the binding to the elongation complex of S10 and NusG (Fig. 7C,D). Affinity chromatography with S10 and NusB as the immobilized ligands has shown that NusB binds to S10 (S. Mason, J. Li, and J. Greenblatt, submitted). We propose that this interaction also occurs in N-modified transcription complexes. Therefore, just as the N protein binds to the RNAP-bound NusA (Greenblatt and Li 1981a; Horwitz et al. 1987), NusB can bind to the RNAP-bound S10 (Fig. 7D).

Mutations in the β -subunit of RNAP that abolish antitermination in vivo prevent the assembly of elongation complexes containing the λ N protein in vitro (Fig. 2B). Three antitermination factors, NusA (Greenblatt and Li 1981b), S10 (Figs. 5 and 6), and NusG (S. McCracken, unpubl.), bind directly to the RNAP. Therefore, the sur-

Figure 7. Model for the ordered assembly of an N-modified transcription complex. (A) Transcription initiation complexes contain σ^{70} and may have S10 weakly associated. (B) Early in RNA chain elongation σ^{70} is released and NusA associates with the RNAP. NusG and S10 may also be present, although weakly bound to the RNAP. (C) After transcription of the *nut* site, the antitermination factors all assemble onto the surface of RNAP. (D) The stable N-modified transcription complex with known interactions (NusA–RNAP; N–NusA; S10–RNAP; S10–NusB; NusG–RNAP), as well as hypothesized interactions (unknown factors with the *nut* site RNA). This model shows only one molecule of NusA even though we believe that N-modified transcription complexes contain two molecules of NusA (Horwitz et al. 1987), because we do not know where to place the second NusA molecule in our model.

face of the *E. coli* RNAP could function as a platform on which the antitermination complex assembles. Consistent with this idea, Barik et al. (1987) have shown that T7 RNAP cannot substitute for *E. coli* RNAP in the assembly of an N-modified transcription complex. The formation of a multiprotein complex on the surface of RNAP would align the elongation factors so that two or more of them can simultaneously bind to the *nut* site RNA (J. Nodwell and J. Greenblatt, submitted). The final N-modified elongation complex would be stabilized by many protein–protein and protein–RNA interactions.

Materials and methods

Materials

RNAP holoenzyme saturated with σ^{70} was purified by the method of Burgess and Jendrisak (1975) from JG148 (Greenblatt 1972), *ron* (Ghyzen and Pironio 1972), and *groN785* (Georgopoulos 1971) *E. coli* strains. N, NusA, and NusB were purified as described previously (Greenblatt et al. 1980, 1981; Swindle et al. 1988). The purification of NusG will be described elsewhere (J. Li and J. Greenblatt, in prep.) Purified S10 was a generous gift from V. Nowotny. The plasmids pLS1, pLS9, and pLS11 were described by Lau and Roberts (1985). Plasmid DNA was purified by alkaline lysis, followed by CsCl gradient ultracentrifugation (Maniatis et al. 1982).

Rabbit antiserum raised against S10 (gift from Dr. I. Kahan) was affinity purified on a column containing an immobilized glutathione-S-transferase–S10 fusion protein as described elsewhere (S. Mason, J. Li, and J. Greenblatt, submitted).

Transcription reactions and isolation of transcription complexes

Transcription reactions (1 ml) containing 27 $\mu\text{g}/\text{ml}$ of RNAP holoenzyme, 65 $\mu\text{g}/\text{ml}$ of plasmid DNA, 36 $\mu\text{g}/\text{ml}$ of NusA, 5 $\mu\text{g}/\text{ml}$ of NusB, 1 $\mu\text{g}/\text{ml}$ of S10 (unless otherwise indicated in the figure legends), 7 $\mu\text{g}/\text{ml}$ of NusG, and 2.0 $\mu\text{g}/\text{ml}$ of N (in all figures except Fig. 3A, where 1.0 $\mu\text{g}/\text{ml}$ was used) were preincubated in buffer containing 10 mM HEPES (pH 7.3), 20 mM potassium acetate, 20 mM $(\text{NH}_4)_2\text{SO}_4$, 12 mM MgSO_4 , and 25 $\mu\text{g}/\text{ml}$ of bovine serum albumin. Elongation complexes were formed in reactions containing 0.57 mM ATP, and 0.14 mM GTP and UTP that were incubated for 15 min at 37°C. Processive chain elongation was started by the addition of 0.14 mM CTP to the reaction, and incubation at 37°C was continued for 2 min. Initiation complexes were formed in reactions containing no nucleoside triphosphates. Transcription complexes were isolated by HPLC gel filtration as described previously (Horwitz et al. 1987). The isolated transcription complexes were analyzed by electrophoresis on 10% SDS–urea–polyacrylamide gels (Swank and Munkres 1971) with a 10 : 1 ratio of acrylamide/bis, and the proteins were visualized by staining with silver (Morrisey 1981).

Minigel filtration

RNAP (43 μg) and/or S10 (550 ng) in a total volume of 20 μl was incubated for 5 min at 37°C, followed by chromatography on a 0.5-ml column containing Bio-Gel A-1.5m (Bio-Rad) in 10 mM HEPES (pH 7), 20 mM MgSO_4 , and 75 mM NH_4 acetate. Fractions of 60 μl were collected, lyophilized, and resuspended in 15 μl of Laemmli sample buffer. Samples were analyzed by electrophoresis on 13.5% SDS–polyacrylamide gels, transferred to nitro-

cellulose, and probed with 0.5 μ g/ml of affinity-purified anti-S10 as described in the Bio-Rad Immuno-Blot assay kit.

Acknowledgments

We thank all the members of the laboratory for helpful discussion and advice. In particular, we thank Dr. S. McCracken for suggesting the use of the SDS-urea-polyacrylamide gel system and the minigel filtration technique and J. Li for advice and for purifying most of the proteins used in this work. We also thank Dr. V. Nowotny for the gift of purified S10, Dr. L. Kahan for the gift of anti-S10 antiserum, and Drs. J. Segall and C.J. Ingles for critical review of this manuscript. This work was supported by the Medical Research Council of Canada.

The publication costs of this article were defrayed in part by payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 USC section 1734 solely to indicate this fact.

References

- Adhya, S., M. Gottesman, and B. deCrombrughe. 1974. Release of polarity in *Escherichia coli* by gene *N* of phage λ : Termination and antitermination of transcription. *Proc. Natl. Acad. Sci.* **71**: 2534–2538.
- Barik, S., B. Ghosh, W. Whalen, D. Lazinski, and A. Das. 1987. An antitermination protein engages the elongating transcription apparatus at a promoter-proximal recognition site. *Cell* **50**: 885–899.
- Burgess, R.R. and J.J. Jendrisak. 1975. A procedure for the rapid, large-scale purification of *E. coli* DNA-dependent RNA polymerase involving polymin P precipitation and DNA-cellulose chromatography. *Biochemistry* **14**: 4634–4638.
- Burgess, R.R., B. Erickson, D. Gentry, M. Gribskov, D. Hager, S. Lesley, M. Strickland, and N. Thompson. 1986. Bacterial RNA polymerase subunits and genes. In *RNA polymerases and the regulation of transcription. A Steenbock symposium* (ed. W.S. Reznikoff, R.R. Burgess, J.E. Dahlberg, C.A. Gross, M.T. Record, and M.P. Wickens) pp. 3–15. Elsevier, New York.
- Das, A. and K. Wolska. 1984. Transcription antitermination in vitro by lambda *N* gene product: Requirement for phage *nut* site and the products of host *nusA*, *nusB*, and *nusE* genes. *Cell* **38**: 165–173.
- Das, A., B. Ghosh, S. Barik, and K. Wolska. 1985. Evidence that ribosomal protein S10 itself is a cellular component necessary for transcription antitermination by phage λ N protein. *Proc. Natl. Acad. Sci.* **82**: 4070–4074.
- Downing, W.L., S.L. Sullivan, M.E. Gottesman, and P.P. Dennis. 1990. Sequence and transcriptional pattern of the essential *Escherichia coli* *secE-nusG* operon. *J. Bacteriol.* **172**: 1621–1627.
- Faus, I. and J.P. Richardson. 1990. Structural and functional properties of the segments of λ *cro* mRNA that interact with transcription termination factor Rho. *J. Mol. Biol.* **212**: 53–66.
- Franklin, N.C. 1974. Altered reading of genetic signals fused to the N operon of bacteriophage λ : Genetic evidence for modification of polymerase by the protein product of the N gene. *J. Mol. Biol.* **185**: 701–712.
- Friedman, D.I. 1988. Regulation of phage gene expression by termination and antitermination of transcription. In *The bacteriophages* (ed. R. Calendar), Vol. 2. pp. 263–319. Plenum Press, New York.
- Friedman, D.I. and L.S. Baron. 1974. Genetic characterization of a bacterial locus involved in the activity of the N function of phage λ . *Virology* **58**: 141–148.
- Friedman, D.I., G.S. Wilgus, and R.J. Mural. 1973. Gene N regulator function of phage lambda *imm21*: Evidence that a site of N action differs from a site of N recognition. *J. Mol. Biol.* **81**: 505–516.
- Friedman, D.I., M.F. Baumann, and L.S. Baron. 1976. Cooperative effects of bacterial mutations affecting lambda N gene expression. I. Isolation and characterization of a *nusB* mutant. *Virology* **73**: 119–127.
- Friedman, D.I., A.T. Schauer, M.R. Baumann, L.S. Baron, and S.L. Adhya. 1981. Evidence that ribosomal protein S10 participates in control of transcription termination. *Proc. Natl. Acad. Sci.* **78**: 1115–1118.
- Georgopoulos, C.P. 1971. Bacterial mutants in which the gene N function of bacteriophage lambda is blocked have an altered RNA polymerase. *Proc. Natl. Acad. Sci.* **68**: 2977–2981.
- Ghyssen, A. and M. Pironio. 1972. Relationship between the N function of bacteriophage λ and host RNA polymerase. *J. Mol. Biol.* **65**: 259–272.
- Goda, Y. and J. Greenblatt. 1985. Efficient modification of *E. coli* RNA polymerase *in vitro* by the N gene transcription antitermination protein of bacteriophage lambda. *Nucleic Acids Res.* **13**: 2569–2582.
- Greenblatt, J. 1972. Positive control of endolysin synthesis *in vitro* by the gene N protein of phage λ . *Proc. Natl. Acad. Sci.* **69**: 3606–3610.
- Greenblatt, J. and J. Li. 1981a. The *nusA* gene protein of *E. coli*: Its identification and a demonstration that it interacts with the gene N transcription antitermination protein of bacteriophage lambda. *J. Mol. Biol.* **147**: 11–23.
- . 1981b. Interaction of the sigma factor and the *nusA* gene protein of *E. coli* with RNA polymerase in the initiation-termination cycle of transcription. *Cell* **24**: 421–428.
- Greenblatt, J., P. Malnoe, and J. Li. 1980. Purification of the gene N transcription antitermination protein of bacteriophage λ . *J. Biol. Chem.* **255**: 1465–1470.
- Greenblatt, J., M. McLimont, and S. Hanly. 1981. Termination of transcription by *nusA* gene protein of *E. coli*. *Nature* **292**: 215–220.
- Hansen, U.M. and W.R. McClure. 1980. Role of the σ subunit of *Escherichia coli* RNA polymerase in initiation. II. Release of σ from ternary complexes. *J. Biol. Chem.* **255**: 9564–9570.
- Horwitz, R.J. 1988. "Genetic and structural analysis of bacterial transcription complexes." Ph.D thesis, University of Toronto, Toronto, Canada.
- Horwitz, R.J., J. Li, and J. Greenblatt. 1987. An elongation control particle containing the N gene transcriptional antitermination protein of bacteriophage lambda. *Cell* **51**: 631–641.
- Jin, D.J., M. Cashel, D.I. Friedman, Y. Nakamura, W.A. Walter, and C.A. Gross. 1988. Effects of rifampicin resistant *rpoB* mutations on antitermination and interaction with *nusA* in *Escherichia coli*. *J. Mol. Biol.* **204**: 247–261.
- Keppel, F., C.P. Georgopoulos, and H. Eisen. 1974. Host interference with expression of the lambda N gene product. *Biochimie* **56**: 1503–1509.
- Laemmli, U.K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227**: 680–685.
- Lau, L.F. and J.W. Roberts. 1985. p-dependent transcription termination at t_{R1} requires upstream sequences. *J. Biol. Chem.* **260**: 574–584.
- Li, S.C., C.L. Squires, and C. Squires. 1984. Antitermination of *E. coli* rRNA transcription is caused by a control region segment containing lambda *nut*-like sequences. *Cell* **38**: 851–860.

Mason and Greenblatt

- Maniatis, T., E.F. Fritsch, and J. Sambrook. 1982. *Molecular cloning. A laboratory manual*. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.
- Morgan, E.A. 1986. Antitermination mechanisms in rRNA operons of *E. coli*. *J. Bacteriol.* **168**: 1–5.
- Morrissey, W.H. 1981. Silver stain for proteins in polyacrylamide gels: A modified procedure with enhanced uniform sensitivity. *Anal. Biochem.* **117**: 307–310.
- Olson, E.R., E.L. Flamm, and D.I. Friedman. 1982. Analysis of *nutR*: A region of phage lambda required for antitermination of transcription. *Cell* **31**: 61–70.
- Salstrom, J.S. and W. Szybalski. 1978. Coliphage λ *nutL*[−]: A unique class of mutants defective in the site of gene *N* product utilization for antitermination of leftward transcription. *J. Mol. Biol.* **124**: 195–221.
- Swank, R.T. and K.D. Munkres. 1971. Molecular weight analysis of oligopeptides by electrophoresis in polyacrylamide gel with sodium dodecyl sulphate. *Anal. Biochem.* **39**: 462–477.
- Swindle, J., M. Zylicz, C. Georgopoulos, and J. Greenblatt. 1988. Purification and properties of the NusB protein of *Escherichia coli*. *J. Biol. Chem.* **263**: 10229–10235.
- Travers, A.A. and R.R. Burgess. 1969. Cyclic re-use of the RNA polymerase sigma factor. *Nature* **222**: 537–540.
- Whalen, W.A. and A. Das. 1990. Action of an RNA site at a distance: Role of the *nut* genetic signal in transcription antitermination by phage- λ *N* gene product. *New Biologist* **2**: 975–991.
- Whalen, W., B. Ghosh, and A. Das. 1988. NusA protein is necessary and sufficient *in vitro* for phage λ *N* gene product to suppress a Rho-independent terminator placed downstream of *nutL*. *Proc. Natl. Acad. Sci.* **85**: 2494–2498.

Assembly of transcription elongation complexes containing the N protein of phage lambda and the Escherichia coli elongation factors NusA, NusB, NusG, and S10.

S W Mason and J Greenblatt

Genes Dev. 1991, **5**:

Access the most recent version at doi:[10.1101/gad.5.8.1504](https://doi.org/10.1101/gad.5.8.1504)

References

This article cites 36 articles, 12 of which can be accessed free at:
<http://genesdev.cshlp.org/content/5/8/1504.full.html#ref-list-1>

License

Email Alerting Service

Receive free email alerts when new articles cite this article - sign up in the box at the top right corner of the article or [click here](#).