Role of *Escherichia coli* RNA polymerase alpha subunit in modulation of pausing, termination and anti-termination by the transcription elongation factor NusA

Kebin Liu¹, Yuying Zhang¹,
Konstantin Severinov², Asis Das³ and
Michelle M. Hanna^{1,4,5}

¹Department of Botany and Microbiology and ⁴Department of Chemistry and Biochemistry, The University of Oklahoma, Norman, OK 73019, ²The Rockefeller University, New York, NY 10021 and

³Department of Microbiology, University of Connecticut Health Center, Farmington, CT 06030, USA

⁵Corresponding author

The alpha subunit (α) of RNA polymerase (RNAP) is critical for assembly of polymerase and positive control of transcription initiation in *Escherichia coli*. Here, we report that α also plays a role in transcription elongation, and this involves a direct interaction between α and NusA factor. During *in vitro* transcription without NusA, α interacts with the nascent RNA, as revealed by photocrosslinking. When NusA is present, RNA crosslinks to NusA rather than to α . We show that this NusA–RNA interaction is diminished during transcription with an RNAP mutant that lacks the C-terminus of α beyond amino acid 235, including the so-called α CTD. The absence of α CTD also affects NusA's ability to enhance transcription pausing, termination at intrinsic terminators and anti-termination by the phage lambda Q anti-terminator, but not anti-termination by the lambda N anti-terminator. NusA functions are not recovered even when transcription with mutant RNAP is done with excess NusA, a condition which does restore NusA–RNA crosslinking. By affinity chromatography, we show that NusA interacts directly with α , and also with β and β' , but not with mutant α . Hence, α –NusA interaction is vital for the control of transcript elongation and termination.

Keywords: elongation factor/*Escherichia coli*/RNA polymerase/transcription

Introduction

Our understanding of the mechanism and regulation of transcription requires dissection of the specific roles of the individual components of the multi-subunit enzyme RNA polymerase (RNAP). The single form of RNAP from *Escherichia coli* consists of a core enzyme ($\beta\beta'\alpha_2$), capable of RNA synthesis and factor-independent termination, and a holoenzyme ($\beta\beta'\alpha_2\sigma$), which is capable of specific initiation of transcription. The core enzyme contains one copy each of two large subunits beta prime (β' , $M_r = 155.2$ kDa) and beta (β , $M_r = 150.6$ kDa) and two copies of the smaller subunit alpha (α , $M_r = 36.5$ kDa) (Burgess, 1969). The α subunit is involved in both the assembly of the core enzyme and in the regulation of transcription initiation at some promoters. Ishihama and co-workers have generated a set

of C-terminal deletion mutations of *rpoA*, the gene for the α subunit, that result in production of truncated forms of α . When these are reconstituted into core enzyme, the mutant enzymes are affected in their ability to activate transcription *in vitro* from several positively controlled promoters. Core assembly therefore requires the N-terminal domain (NTD) of α (Igarashi *et al.*, 1991), while it is the C-terminal domain (CTD) which is the target of several regulatory molecules (reviewed in Igarashi and Ishihama, 1991; Ishihama, 1992, 1993; Russo and Silhavy, 1992). A direct interaction between the CTD of α and protein activators or even the promoter DNA somehow activates transcription from some otherwise poor promoters (Zou *et al.*, 1992; Ross *et al.*, 1993; Blatter *et al.*, 1994; Chen *et al.*, 1994). The α C-terminal domain (α CTD) and α N-terminal domain (α NTD) are separated by a flexible linker of 13–36 amino acids. It has been proposed that this linker allows the α CTD to be positioned at different distances from the remainder of RNAP at different promoters (Blatter *et al.*, 1994).

The RNAP holoenzyme contains one of several sigma (σ) factors which confers promoter specificity upon the core enzyme (reviewed in Gross *et al.*, 1992). Shortly after promoter recognition and initiation of transcription, the sigma factor is released from core, and the elongation factor NusA ($M_r = 54.6$ kDa) can then bind to the transcription complex (Burgess and Travers, 1969; Greenblatt and Li, 1981a; Gill *et al.*, 1991). NusA interacts with both the core enzyme and the nascent RNA (Liu and Hanna, 1995a). As a component of the elongation complex, NusA slows the elongation rate and enhances pausing (Greenblatt *et al.*, 1981; Kassavetis and Chamberlin, 1981; Kingston and Chamberlin, 1981; Farnham *et al.*, 1982; Yager and von Hippel, 1987). This may be due to direct competition for NTPs (Schmidt and Chamberlin, 1984), or alternatively, through a NusA-directed change in the interactions between the RNA and the core subunits β and β' (Zhang and Hanna, 1994). Pausing occurs even in the absence of other factors (Dahlberg and Blattner, 1973; Maizels, 1973). However, NusA enhances pausing at some sites, and often these are found immediately downstream from RNA stem-loop structures or hairpins (Chan and Landick, 1993). Pausing can be independent of hairpin structures however, and can also be affected by untranscribed DNA sequences both upstream and downstream of a pause site (Lee *et al.*, 1990; Ring and Roberts, 1994). NusA also increases termination efficiency at intrinsic terminators (Schmidt and Chamberlin, 1987). These terminators include RNA hairpin structures, and NusA interaction with the base of the stem of RNA hairpins has been suggested by both RNase protection (Landick and Yanofsky, 1987; Dissinger and Hanna, 1991) and RNA crosslinking studies (Dissinger and Hanna, 1991; Liu and Hanna, 1995a). In addition to the role of NusA in transcription of *E. coli* genes, it is one of four host factors

(including NusB, NusE and NusG) which are involved in transcriptional anti-termination by the bacteriophage λ N protein (see DeVito and Das, 1994). Anti-termination by a second λ protein, Q, is also enhanced by NusA (Grayhack *et al.*, 1985).

By photochemical crosslinking in active transcription elongation complexes, we have recently demonstrated that the α subunit of RNAP contacts the nascent RNA, and we have mapped this contact to the C-terminal region of α beyond amino acid 208 (Liu and Hanna, 1995b). We have incorporated a photocrosslinking UMP analog at internal positions in the nascent RNA. As the analogs are moved away from the 3' end of the transcript, the RNA crosslinks to different polymerase subunits, including α . When NusA is present in the reaction, the RNA contacts with α , but not β or β' , are virtually eliminated, and RNA crosslinks to NusA are observed instead. We now describe experiments examining the relationship between NusA and the C-terminal region of α , in transcription elongation and termination.

Results

RNAPs

Throughout this study, we have examined four different forms of RNAP holoenzyme. Two were isolated from cells in native form using standard procedures (Burgess and Jendrisak, 1975; Gonzalez *et al.*, 1977). The first was the wild-type enzyme (WT RNAP), and the second contained a point mutation in α CTD (α D305E RNAP). This point mutation, an aspartic to glutamic acid change at amino acid 305, restores N-mediated anti-termination *in vivo* in *nusA* and *nusE* mutant strains (D.Friedman, personal communication). Two other forms of polymerase were prepared by reconstitution of individual subunits. Both contained a hexa-histidine tag at the N-terminal end of α (Tang *et al.*, 1995). One was otherwise essentially wild-type (WT^{His} RNAP), while the other ($\alpha 235^{\text{His}}$ RNAP) contained an α subunit which was truncated after amino acid 235 and lacked the entire CTD of α . We refer to this missing region as α CTD, even though a small region of the linker is also deleted. The elongation, termination, and anti-termination properties of these enzymes, in the absence and presence of NusA were examined. In addition, photocrosslinking was used to probe for RNA interactions with the altered α subunits of these polymerases.

Deletion of α CTD affects interaction between NusA and nascent RNA in elongation complexes

Transcription complexes containing a 49 nucleotide (nt) long RNA molecule, which contained the photocrosslinking nucleotide analog 5-APAS-UMP in place of several UMPs between +6 and +36, were prepared from the λ P_R promoter on template 1 (see Materials and methods). Upon irradiation, the RNA was crosslinked to the β and/or β' and α subunits of WT RNAP (Liu and Hanna, 1995b; Figure 1A, lane 5). In identical experiments, the α subunits of WT^{His} RNAP and α D305E RNAP were also crosslinked to the nascent RNA (Figure 1A, lanes 6 and 8, respectively). In contrast, the α subunit of $\alpha 235^{\text{His}}$ RNAP, which lacks α beyond amino acid 235, was not crosslinked to the RNA (Figure 1A, lane 7).

The addition of NusA at 100 nM to WT^{His} RNAP

Fig. 1. RNA crosslinking to α and NusA with wild-type and α -modified RNAPs. **(A)** Transcription complexes were formed with all four RNAPs in which the polymerase was paused after synthesis of a 49mer RNA on P_R by a Gln111 transcription roadblock. The RNA was radioactively labeled and contained the photocrosslinking nucleotide analog 5-APAS-UMP at the 12 UMP residues between +6 and +36. After irradiation of transcription complexes RNAP subunits crosslinked to the RNA were identified by separation of subunits by SDS-PAGE, transfer of protein-RNA complexes to nitrocellulose, and autoradiography of the nitrocellulose membrane (WT RNAP, lanes 1 and 5; WT^{His} RNAP, lanes 2 and 6; $\alpha 235^{\text{His}}$ RNAP, lanes 3 and 7; α D305E RNAP, lanes 4 and 8). The position of the alpha subunit truncated at amino acid 235, lacking the CTD, is indicated as $\alpha 235^{\text{His}}$. **(B)** Transcription complexes containing the radioactive, analog-tagged RNA were formed with WT^{His} RNAP and $\alpha 235^{\text{His}}$ RNAP. NusA was added at the concentrations indicated before irradiation of the transcription complexes. After irradiation, labeled proteins were identified as described in (A).

elongation complexes completely eliminated the α -RNA crosslinks, with concomitant RNA crosslinking to NusA (Figure 1B, lane 3). By contrast, crosslinking of NusA to RNA was not observed with $\alpha 235^{\text{His}}$ RNAP when NusA was present at 100 nM in the reaction (Figure 1B, lane 8). Crosslinks to NusA could be fully restored in the reaction with mutant RNAP by increasing NusA concentration 5-fold (Figure 1B, lane 10), and a low level of crosslinking could be detected at 300 nM NusA (data not shown). NusA crosslinking to RNA in complexes with α D305E RNAP occurred, with concomitant loss of the α -RNA crosslink, at the same NusA concentration as with the wild-type enzyme (data not shown). Therefore, an intact α CTD enhances NusA-RNA interactions at a limiting concentration of NusA.

α CTD is required for enhancement of pausing by NusA

To determine if α CTD contributes to NusA-RNAP interaction functionally, we examined NusA's influence on pausing of transcription from two promoters. The first was the bacteriophage T7 A1 promoter on template

pAR1707 (template 2). There is a strong NusA-enhanced pause site (designated P1) 80 nt downstream from the start site (Dissinger and Hanna, 1991). The second is the λ P_R' promoter, at which the λ Q protein modifies RNAP to an anti-termination conformation. This modification is enhanced by NusA. In both cases, synchronously started transcription complexes were formed by initiation of transcription in the absence of one nucleoside triphosphate so that polymerase was poised at the position at which the missing nucleotide was first encoded. Complexes generated at the A1 promoter contained an RNA 20 nt long, and those generated at the P_R' promoter (template 3) contained an RNA 15 nt long. All four nucleoside triphosphates were then added, and transcription kinetics were examined by removing aliquots at different times and analyzing the RNA by denaturing PAGE.

During transcription from the A1 promoter, WT RNAP paused at two regions, designated P1 and P2 (Figure 2A). After 2 min, however, over 50% of the polymerases had reached the end of the template in the absence of NusA. When present at 200 nM, NusA enhanced pausing at both P1 and P2, so that after 2 min, over 80% of the polymerases were still paused at P2 (Figure 2A, compare lanes 5, with and without NusA). The decrease in elongation rate caused by NusA could also be assessed by comparing the 5 min time points (Figure 2A, lanes 7): in the absence of NusA virtually all polymerase had reached the end of the template, while nearly half of the polymerases were still paused at P1 when NusA was present. RNAP containing the hexa-His tag at the NTD of α (WT^{His} RNAP) also showed increased pausing in the presence of 200 nM NusA (Figure 2A, compare lanes 5 and 7, with and without NusA). Surprisingly, however, polymerase lacking α CTD did not show a decreased elongation rate and enhanced pausing, even in the presence of 500 nM NusA. The pause half-life at P1 was ~50 s for both WT^{His} and α 235^{His} RNAPs in the absence of NusA. In the presence of NusA, the half-life of this pause was increased ~3-fold, to 130 s with WT^{His} RNAP, but it remained virtually unchanged at 50 s when α CTD was missing (Figure 2B). The lack of response to NusA by α 235^{His} RNAP was also observed during transcription from the λ P_R' promoter (Figure 2C). Although only one NusA-sensitive pause site (P1, ~40 nt long) was observed on this template under the conditions used, an overall slower elongation rate in the presence of NusA can be seen by comparing the amount of time required to reach the terminator t_R' (Figure 2C, compare lanes 4).

α CTD is required for optimizing intrinsic terminators by NusA

We next tested how α CTD contributes to the ability of NusA to enhance termination at Rho-independent termina-

tors. Three constructs were tested with all four forms of RNAP. Results for two of these templates are shown in Figure 3. WT RNAP initiating transcription from the λ P_R' promoter on template 1 terminated with ~47% efficiency (53% readthrough) at the λ t₀ terminator placed 465 nt downstream (Figure 3A, lane 1 and Figure 3B). The addition of NusA increased termination efficiency ~2-fold (Figure 3A, lane 2) so that readthrough was reduced to 27%. This enhancement of terminator efficiency was also observed with both WT^{His} RNAP and α D305E RNAP (Figure 3A, lanes 3 versus 4 and lanes 7 versus 8). In contrast, polymerase containing only α NTD did not respond to NusA (Figure 3A, lanes 5 versus 6). Both polymerases which contained the hexa-His tag on the NTD of α did show a slightly higher termination efficiency than wild-type polymerase in the absence of NusA (Figure 3B). Similar results were obtained for NusA's influence on termination at the T7 t_e terminator by monitoring transcription from the T7 A1 promoter (Figure 3C and D).

The defect of RNAP containing only α NTD in responding to NusA for enhancement of pausing could not be suppressed by increasing the NusA concentration. Similarly, NusA-enhanced termination efficiency could not be restored with the polymerase containing only α NTD by increasing the NusA concentration (Figure 4). For the His-tagged polymerases (WT^{His} RNAP and α 235^{His} RNAP), the efficiency of termination was ~62% without NusA (38% readthrough, Figure 4A, lanes 1 and 7; Figure 4B). The addition of NusA to 0.2 μ M increased termination efficiency to 83% with WT^{His} RNAP (Figure 4A, lane 3), and no further increase in termination was observed as the NusA concentration was increased to 1 μ M (Figure 4A, lanes 4–6). By comparison, when NusA was added to polymerase containing only α NTD, termination efficiency remained at ~62–65%, even when the NusA concentration was raised to 1 μ M (Figure 4A, lanes 8–12 and Figure 4B). Compared with the λ t₀ and T7 t_e terminators, λ t_R' was >90% efficient, even in the absence of NusA. The WT^{His} RNAP and α 235^{His} RNAP showed 97–99% termination at t_R' (Figure 5B and F). Termination efficiency was not increased by NusA with α 235^{His} RNAP on these templates from either promoters P_R' or P_L.

Deletion of α CTD abolishes NusA-enhanced anti-termination by the λ Q protein but not the λ N protein

We next examined whether the influence of NusA on factor-dependent anti-termination also requires α CTD. To monitor λ Q-mediated anti-termination, we utilized template 3 that contains the λ P_R' promoter, 6S RNA gene and t_R' terminator. The λ Q protein modifies RNAP at this promoter to a termination-resistant form, and NusA is

Fig. 2. Effect of changes in α on NusA-enhanced pausing. (A) An autoradiogram of the RNA gel showing the transcripts made during the transcription time course from the T7 A1 promoter on template 2 is shown. Ternary transcription complexes containing RNA 20 nt long were formed with WT RNAP, WT^{His} RNAP and α 235^{His} RNAP. The 20mer RNA was chased with and without NusA for 0 min (lanes 1), 10 s (lanes 2), 30 s (lanes 3), 1 min (lanes 4), 2 min (lanes 5), 3 min (lanes 6) or 5 min (lanes 7). Pause RNAs are identified as RNA species which persist with time, but eventually chase to full-length RNA. A NusA-enhanced pause site is one in which the RNA persists longer in the presence of NusA than in the absence. P1 and P2 indicate the positions of the major pause site RNAs from this template, and RO indicates the position of the run-off transcript. The NusA concentration used for WT RNAP and WT^{His} RNAP was 200 nM, and the NusA concentration for α 235^{His} RNAP was 500 nM. (B) The half-life of the P1 pause on template 2 in the absence or presence (■) of NusA was determined by densitometric analysis of autoradiograms like those shown in (A). The amount of RNA at P1 at 10 s was taken as 100%, and the data from three separate experiments were averaged. (C) Pausing from the P_R' promoter with WT RNAP and α 235^{His} RNAP. Transcription complexes containing a 15mer RNA were formed and the RNA was then chased for 20 s (lanes 1), 40 s (lanes 2), 1 min (lanes 3), 2 min (lanes 4), 3 min (lanes 5) or 5 min (lanes 6), with and without NusA. The major pause RNA from this template is designated P1, and the position of the full-length terminated transcript is designated t_R'.

known to increase anti-termination efficiency (Grayhack *et al.*, 1985). With the wild-type polymerase, anti-termination at t_R' was ~20% in the presence of Q; upon addition of NusA, anti-termination increased to ~50% (Figure 5A,

lanes 3 versus 4 and Figure 5B). The anti-termination efficiency and the effect of NusA were essentially the same with α D305E mutant polymerase (Figure 5A, lanes 15 and 16, and Figure 5B). Anti-termination was somewhat

Fig. 3. Effect of changes in α on NusA-enhanced intrinsic termination. (A) Transcription was from the $P_{R'}$ promoter of template 1 with all four RNAPs in the presence or absence of NusA. Positions of the transcripts generated by termination at the Rho-independent terminator t_o (465 nt) or from readthrough of t_o to produce the run-off RNA (RO, 619 nt) are indicated. (B) The data from three separate experiments, including that shown in (A), were averaged to determine the percentage readthrough of the t_o terminator, with and without NusA. (C) Termination efficiency of all four polymerases at the T7 t_e terminator on template 4, in the presence and absence of NusA. The terminated (160 nt) and readthrough (502 nt) transcripts are indicated. (D) The data of three separate experiments, including that shown in (C), were averaged to determine the percentage readthrough of the t_e terminator.

lower with the His-tagged wild-type polymerase, but NusA still increased anti-termination efficiency ~2.5-fold (Figure 5A, lanes 7 versus 8, and Figure 5B). By comparison, polymerase containing only αNTD showed only negligible enhancement of anti-termination (1.2-fold) with NusA (Figure 5A, lanes 11 versus 12, and Figure 5B). Increasing the NusA concentration as high as 2.5 μM still did not restore the level of NusA-enhanced anti-termination to that obtained with the wild-type polymerase (Figure 5C and D). It is noteworthy that although the effect of NusA on Q anti-termination was diminished when αCTD was deleted, the basal level of anti-termination by Q alone was unaffected.

In striking contrast, the deletion of αCTD had no effect on NusA-mediated enhancement of N-mediated anti-termination. We monitored N activity on template 5 that contains the λp_L promoter, the N-recognition site (*nutL*) and the cloned $\lambda t_{R'}$ terminator downstream. The λ N protein interacts with RNAP to produce a basal level of anti-termination in the absence of other factors (DeVito and Das, 1994). In agreement with this, N produced ~27% anti-termination at $t_{R'}$ with WT RNAP (Figure 5E, lanes 1 versus 3, and Figure 5F). NusA increased the anti-termination efficiency ~2.4-fold, to 63% (Figure 5E, lanes 3 versus 4). An enhancement of N-mediated anti-termination efficiency was observed with all four polymerases, even with NusA present at only 30 nM. We conclude that αCTD is important for most, but not all, functions of NusA in the modulation of transcription elongation and termination.

Fig. 4. Effect of NusA concentration on the termination efficiency at t_o . (A) Transcription was initiated from the $P_{R'}$ promoter of template 1 with WT^{His} RNAP and $\alpha 235^{\text{His}}$ RNAP. NusA was present at the indicated concentrations. The positions of the transcripts resulting from termination at t_o or from readthrough of t_o to generate the run-off RNA (RO) are indicated. (B) The amount of readthrough of the t_o terminator was determined as described. The histogram represents the average of three individual experiments.

Fig. 5. Effect of changes in α on Q and N-dependent transcriptional anti-termination. (A) Q anti-termination assay: transcription was from the $P_{R'}$ promoter of template 3, which contains the λ 6S gene, including the *gut* site, and the ρ -independent $t_{R'}$ terminator. Positions of the terminated (194 nt) and readthrough/anti-terminated (390 nt) transcripts are shown. (B) The percentage of readthrough (or anti-termination) was determined by scanning of the autoradiograms, as described. The average of three separate experiments is shown. (C) Effect of NusA concentration on Q anti-termination: the effect of increasing the concentration of NusA on Q anti-termination with α 235^{His} RNAP was tested. Transcription reactions contained 0.8 μ M Q (where present) with various concentrations of NusA, as indicated. (D) The data from three separate experiments like that shown in (C) were averaged to determine the extent of anti-termination at different NusA concentrations. (E) N anti-termination assay: transcription was from the P_L promoter of template 5, which contains the λ *nutL* site and the λ $t_{R'}$ terminator. When added, NusA was present at 30 nM and N was at 100 nM. The terminated (245 nt) and readthrough/anti-terminated (295 nt) transcripts are indicated. (F) The data of the three separate experiments like that shown in (E) were averaged to determine the percentage of readthrough or anti-termination of the $t_{R'}$ terminator.

α CTD is required to stabilize interaction of NusA with RNAP

To determine if the loss of NusA function with α 235^{His} RNAP occurred because NusA could not bind to polymerase, we analyzed NusA binding to the core and the holoenzyme (sigma saturated) forms of both WT^{His} and α 235^{His} RNAPs (Figure 6). Polymerase was mixed with NusA and then the reaction mixture was incubated with Ni-NTA beads, resulting in the binding of polymerase to the resin through the hexa-His tag on α . After washing the resin to remove proteins not interacting with poly-

merase, the polymerase and associated proteins were eluted with imidazole. The results for polymerase with or without α CTD were identical at 100 mM NaCl. In agreement with previous work (Greenblatt and Li, 1981a; Gill *et al.*, 1991), NusA bound to the core enzyme (Figure 6B and C, lanes 5) but not to the holoenzyme (Figure 6A, lanes 5). However, when the NaCl concentration was reduced 2-fold, NusA could only weakly interact with core polymerase lacking α CTD (Figure 6D). α CTD is therefore required for a stable interaction of NusA with the enzyme.

Fig. 6. Interactions between immobilized RNAP and NusA. (A–C) Interaction of core or holo RNAPs (WT^{His} or α235^{His}) with NusA. Holo (A) or core (B and C) enzymes were mixed with NusA, and an aliquot was removed for analysis (lanes 1). The remainder of the reaction was added to Ni-NTA resin. The resin was spun down and the unbound proteins were removed (lanes 2). The resin was then washed twice with buffer containing 10 mM imidazole to remove non-specifically bound proteins (lanes 3 and 4). Specifically bound proteins were then eluted with buffer containing 100 mM imidazole (lanes 5). The stained protein gels are shown and the positions of the polymerase subunits and NusA are indicated on the left. The position of the truncated α subunit of α235^{His} RNAP is indicated as α235^{His}. The concentration of NusA was 2 μM in (A) and (B) and 100 nM in (C). (D) Interaction of core RNAP with NusA (2 μM) in low-salt buffer. Interaction was assessed by two methods. In the first, WT^{His} core RNAP or α235^{His} core RNAP were immobilized to the Ni²⁺-NTA resin and NusA was then added to the resin (lanes 1 and 3). In the second method, core RNAP was mixed with NusA and the mixture was then mixed with the resin (lanes 2 and 4). In both methods, after incubation of NusA and RNAP at 37°C for 10 min, the resin was spun down, washed three times with buffer containing 10 mM imidazole, and specifically bound proteins were then eluted with 100 mM imidazole. NusA eluted only with WT^{His} RNAP (lanes 1 and 2).

NusA makes direct contact with β, β' and α, but not αNTD

To determine if the contribution of αCTD to the stability of the NusA–polymerase interaction involves a direct interaction between NusA and α, we carried out binding studies utilizing an immobilized glutathione (GST)–NusA protein. Fusion of GST to NusA does not interfere with its function in enhancement of pausing, termination, or Q-mediated anti-termination (Zhang and Hanna, 1995). Both purified WT^{His} core RNAP and WT core RNAP (data not shown) bound to GST–NusA, while α235^{His} core RNAP lacking αCTD did not (Figure 7A, lanes 3 versus 6). Neither RNAP bound to the immobilized GST alone (Figure 7A, lanes 2 and 5). Similarly, purified αWT^{His} subunit bound to NusA (Figure 6B, lane 2), while purified α235^{His} subunit did not (Figure 6B, lane 4). When αWT^{His} was overexpressed and the cell lysate was passed over the GST–NusA and GST control columns, the αWT^{His} subunit was specifically retained only on the GST–NusA column, even in the presence of other cellular proteins (Figure 7C, lane 2 versus 4). In contrast, the α235^{His} subunit, containing only αNTD, was not retained in the GST–NusA column (Figure 7C, lane 8). When the β and β' subunits were overexpressed and isolated from cells as inclusion bodies and then solubilized, each subunit bound specifically to the GST–NusA column (Figure 7D, lanes 2 and 4), but not to the GST column (lanes 1 and 3). Although the affinity of NusA appears to be greater for β' than β, the β inclusion bodies contain more β proteolytic

products which also bound to NusA. We conclude that NusA binds directly to β, β' and α, but not to αNTD.

Discussion

The principal findings reported in this paper illustrate the role of the RNAP α subunit in transcription elongation and termination in *E. coli*. The α subunit has long been known to play a pivotal role in the assembly of the core enzyme (Igarashi *et al.*, 1991; Kimura *et al.*, 1994). Recent work has shown that αCTD is the target of a large group of activator proteins, and that it also interacts with an A/T-rich upstream element of certain promoters to enhance transcription initiation (Igarashi and Ishihama, 1991; Ishihama, 1992, 1993; Russo and Silhavy, 1992; reviewed in Busby and Ebright, 1994). We show here that α also participates in the productive interaction of RNAP with a key elongation modulatory factor (NusA), and that this also involves a direct interaction with α. These results reveal a new function for the α subunit of *E. coli* RNAP: α takes part in the control of transcription pausing, termination and anti-termination and hence, α participates in all aspects of the transcription cycle.

That α might play a role in elongation was indicated from a recent study examining the interaction of nascent RNA with various components of the ternary transcription complex by photochemical crosslinking (Liu and Hanna, 1995b). Besides demonstrating an interaction between RNA and the β and β' subunits, these studies have

Fig. 7. Interactions between immobilized NusA and RNAP subunits and core enzymes. **(A)** GST (lanes 2 and 5) or GST-NusA (lanes 3 and 6) were immobilized on glutathione agarose beads and then mixed with the WT^{His} core RNAP (lanes 2 and 3) or α235^{His} core RNAP (lanes 5 and 6) for 10 min at 37°C. The resin was then washed three times with buffer and then bound proteins were eluted from the resin with SDS loading buffer and heating at 100°C for 3 min. The last wash was analyzed and found to contain no protein (not shown). Released proteins were analyzed by SDS-PAGE, followed by staining of the gel. Lanes 1 and 4 contain WT^{His} core RNAP and α235^{His} core RNAP, respectively. The positions of the GST and GST-NusA, which are released from the glutathione beads in the presence of SDS are indicated to the left of the gels. **(B)** GST (lanes 1 and 3) and GST-NusA (lanes 2 and 4) were immobilized and incubated with purified αWT^{His} (lanes 1 and 2) and α235^{His} (lanes 3 and 4). After washing, the bound proteins were analyzed as described in (A). **(C)** GST (lanes 1, 2, 5, 6) or GST-NusA (lanes 3, 4, 7 and 8) were immobilized on glutathione agarose, mixed with crude cell extracts containing overexpressed αWT^{His} (lanes 1–4) and α235^{His} (lanes 5–8) subunits. After incubation, an aliquot of unbound protein was removed for analysis (lanes 1, 3, 5, 7). The resin was then washed three times and proteins bound to the resin were released and analyzed, as in (A). Only α containing CTD bound to the GST-NusA column (lanes 4 versus 8). **(D)** Inclusion bodies containing β and β' were incubated with immobilized GST (lanes 1 and 3) or immobilized GST-NusA (lanes 2 and 4). After incubation the resin was spun down, washed three times, and bound proteins were then eluted from the resin and analyzed as above.

revealed an interaction of α with RNA in reactions without NusA. We have mapped the RNA interaction to the C-terminal region of α beyond amino acid 208. The α-RNA interaction is abolished by NusA while it binds itself to the nascent RNA. A remarkable finding reported here is that the NusA-RNA interaction in the transcription complex is affected when the α subunit contains only αNTD. This finding has led us to examine whether the C-terminal region of α also plays a role in NusA function.

We have monitored four different functions of NusA with RNAP containing only αNTD. NusA failed to exert its effect on the mutant polymerase in three of these processes: pausing, termination at intrinsic terminators and anti-termination by λ Q anti-terminator. This defect was apparent even with a high concentration of NusA that restored NusA-RNA crosslinking in the transcription complex. Hence, while αCTD does influence NusA-RNA interaction, it also plays an additional role in modulating some NusA functions. Surprisingly, the mutant polymerase without αCTD responded normally to NusA for anti-termination mediated by the λ N protein, consistent with our findings that the absence of αCTD does not abolish RNAP-NusA interaction altogether.

A role of αCTD in elongation control has been suggested independently from genetic studies by D. Friedman and

coworkers (personal communication). These authors have shown that *in vivo*, a suppressor mutation (*sneA16*) that can compensate for the anti-termination defect of a *nusE* mutant or a *nusA* mutant maps in αCTD. The fact that the deletion of αCTD also suppresses the *nusA/nusE* mutations has led to the proposal that αCTD might be the target of an inhibitor of anti-termination by λ N. Our finding that αCTD is not essential for N-mediated anti-termination *in vitro* is consistent with this hypothesis. In addition, the possibility that αCTD itself is an inhibitor is ruled out since its deletion does not increase the efficiency of anti-termination.

Our evidence that αCTD helps NusA to enhance anti-termination by Q but not by N suggests that there is a difference in the manner in which NusA influences the interaction of RNAP with each anti-terminator. Although each anti-terminator is thought to act by increasing the elongation rate and accelerating RNAP through pause and termination sites, the two anti-terminators differ in the mechanism by which they capture RNAP (Roberts, 1988; reviewed in Das, 1993; Greenblatt *et al.*, 1993). Q is a DNA-binding protein; it interacts with RNAP paused at a specific site near the promoter (Yarnell and Roberts, 1992), and although NusA stabilizes the interaction of Q with the paused complex, there is no influence of NusA

on the length of this pause (Grayhack *et al.*, 1985). In contrast, N is an RNA-binding protein (Chattopadhyay *et al.*, 1995); it can capture RNAP at non-specific sites hundreds of base pairs away from the promoter (Whalen and Das, 1990). Both genetic and biochemical evidence suggest N–NusA interaction (Friedman *et al.*, 1981; Greenblatt and Li, 1981b). This binding must play a role in the recruitment of N protein to the RNAP elongation complex since the requirement for NusA in N-anti-termination is at least partly overcome by supplying N in excess (DeVito and Das, 1994). For Q anti-termination, however, the requirement of NusA for efficient anti-termination could not be overcome by a large supplement of Q. Hence, we imagine that the enhancement of Q-mediated anti-termination by NusA depends upon a proper interaction between NusA with RNAP which is somehow influenced by an intact α CTD.

While α CTD enables NusA to enhance pausing, it does not appear to influence the intrinsic pausing capacity of RNAP. At present, we do not have a clear understanding of the molecular details of pausing, though it is clear that pausing is induced by multiple mechanisms and that interactions of RNAP with both the nascent RNA and the DNA template contribute. The movement of RNAP along DNA is affected by several different activities of the polymerase. Current models suggest that at least three separable regions of the polymerase that are flexibly connected to each other can move independently along the DNA template (Nudler *et al.*, 1994, 1995; Wang *et al.*, 1995; Zaychikov *et al.*, 1995). If the front-end domain of polymerase, the RNA product binding site, and the upstream domain of polymerase each move independently, then factors decreasing the movement of any of these domains would lead to transcriptional pausing.

Our binding data, together with genetic data (Jin *et al.*, 1988; Ito *et al.*, 1991; Sparkowski and Das, 1992), suggest that the primary interaction of NusA is with β and β' . This interaction appears to be non-ionic, as it is weakened by decreasing the salt concentration. This interaction may involve an NTD of NusA, as this interaction is not observed even at a higher salt concentration when the N-terminus of NusA is blocked by immobilization and α CTD is absent (compare Figures 6C and 7A). We have now presented evidence that NusA requires an additional contact with α , requiring α CTD, for a stable, functional interaction with the transcription complex. Although α CTD is not absolutely required for the binding of NusA with the transcription complex, it is required for NusA to enhance pausing and intrinsic termination.

We propose two simple models to explain how a loss of α CTD affects NusA function. First, the loss of NusA function with polymerase lacking α CTD may be caused by the conformational change in polymerase induced by NusA (Zhang and Hanna, 1994). This change is mediated by a direct interaction between NusA and α , and α CTD must be present for the polymerase to adopt the appropriate conformation for NusA function. NusA then competes directly for binding to nascent RNA, probably by occupying the RNA-binding site on α (Figure 8B). A second model which emerges from these results is shown in Figure 8C. During initiation, α CTD has been shown to interact with the DNA template centered around –40 to –50 at some promoters (Ross *et al.*, 1993). Perhaps, during

Fig. 8. Models for the effects of deletion of α CTD on transcription. Deletion of a portion of the interdomain linker and the entire CTD of the α subunit of RNAP has at least three effects on the structure and function of the *E. coli* transcription complex. First, the nascent RNA no longer crosslinks to the α subunit. Second, interaction of the elongation factor NusA with the transcription complex is weakened. Third, even at high concentrations of NusA, where RNA crosslinking indicates it is interacting with the transcription complex, NusA can no longer enhance transcriptional pausing with polymerase containing only α NTD. With WT RNAP, in the absence of NusA, the nascent RNA contacts α (A). When NusA is added, α is no longer crosslinked to the RNA, and NusA is crosslinked to the RNA. When NusA is added, it binds to β , β' and α , but not to α NTD. Through this interaction, it may lift the RNA so that it no longer contacts α , and it prevents RNA– α crosslinking by directly occupying an RNA binding site on α (B). This could cause the α CTD to lower down onto the DNA template, thus imposing 'drag' on the polymerase (C). Such an α –DNA interaction could cause RNAP to pause, until NusA dissociates and α –DNA contacts are broken. α CTD235 contains the CTD of α and a region of the linker starting at amino acid 235.

elongation in the absence of NusA, α CTD does not contact the DNA (Figure 8A). This is supported by DNase footprinting of elongation complexes with WT^{His} RNAP and α 235^{His} RNAP (unpublished results). NusA interaction with the transcription complex could cause α CTD to now contact the DNA (Figure 8C). Interaction between α and the DNA could prevent polymerase from escaping the pause if movement of the back-end domain of polymerase is affected by α interactions with the template. This model is consistent with our observations that, even though NusA could bind to polymerase containing only α NTD at high concentrations, it could not enhance transcriptional pausing. Experiments to test this model are underway.

The binding studies also provide information on the nature of the interaction between NusA and the core subunits. When the core polymerase is immobilized through the NTD of α via the hexa-His tag (Figure 6), NusA can bind to core at high salt concentrations (100 mM), even if the α CTD is missing. If the salt concentration is lowered, however, NusA binds only to core which contains an intact α CTD. This suggests that the interaction between NusA and core at high salt is independent of the α CTD, and is therefore due to NusA interactions with β and β' . At the lower salt levels, however, the $\beta\beta'$ interactions are not sufficient to hold NusA in the complex, and the contribution of the NusA– α CTD interaction becomes important. This suggests that the interaction between NusA and $\beta\beta'$ is a non-ionic interaction. When the NusA is immobilized via GST fused to its N-terminus, NusA binds only to core enzyme which contains an intact α CTD, even at high salt (Figure 7A). This suggests that the interaction between GST–NusA and $\beta\beta'$ does not contribute the same binding energy as the

interaction between native NusA and immobilized RNAP. Since the immobilization of NusA could sterically interfere with the N-terminus of NusA, the interaction between NusA and $\beta\beta'$ may involve an NTD of NusA.

In conclusion, NusA interacts with several components of the transcription complex: β , β' , α and the nascent RNA. Although α CTD is not essential for binding of NusA to RNAP, it stabilizes the interaction between NusA and the transcription complex. The functional interaction of NusA with the transcription complex, resulting in the modulation of elongation, termination and anti-termination, requires a direct interaction between NusA and α . Alpha is, therefore, a key regulatory target in RNAP, which functions through direct contacts with other molecules that regulate all stages of transcription.

Materials and methods

Buffers and nucleotides

Buffers and solutions used were as follows: Buffer A, 100 mM Tris-Cl (pH 7.5), 50 mM NaCl, 5 mM MgCl₂, 10 μ g/ml acetylated BSA; buffer B, 20 mM Tris-(OAc)₂ (pH 8.0), 5% (v/v) glycerol, 10 mM Mg(OAc)₂, 0.04 mM EDTA, 50 mM potassium glutamate; buffer C, 20 mM Tris-Cl (pH 8.0), 0.1 mM EDTA, 10 mM KCl, 2% (v/v) glycerol, 20 μ g/ml BSA; buffer D, 20 mM Tris-Cl (pH 8.0), 0.1 mM EDTA, 50 mM KCl, 2% (v/v) glycerol, 20 μ g/ml acetylated BSA; buffer E, 20 mM Tris-glutamate (pH 8.0), 100 mM K-glutamate, 5 mM MgCl₂, 5% (v/v) glycerol, 1 mM DTT; buffer F, 100 mM Tris-Cl (pH 7.9), 100 mM NaCl, 10 mM MgCl₂, 1 mM EDTA, 5% (v/v) glycerol; buffer G, 20 mM K₂PO₄ (pH 7.3), 150 mM KCl, 1% (v/v) Triton X-100 and 1 mM β -mercaptoethanol.

Solutions: solution A, 7 M urea, 1 mM EDTA, 0.02% (w/v) bromophenol blue, 0.02% (w/v) xylene cyanol; solution B, solution A + 60 mM DTT; solution C, 100 mM Tris-Cl (pH 8.0), 200 mM DTT, 4% (w/v) SDS, 0.02% (w/v) bromophenol blue, 20% (v/v) glycerol. Ultrapure nucleoside triphosphates were purchased from Pharmacia LKB Biotechnology Inc. (Piscataway, NJ). 5-[4-Azidophenacyl]thio] uridine-5'-triphosphate (5-APAS-UTP) was synthesized as described (Hanna, 1989; Hanna *et al.*, 1989; He and Hanna, 1995). Oligonucleotides were synthesized in our laboratory on an ABI Model 392 DNA-RNA synthesizer and purified by HPLC on a C18 column.

RNAPs and other proteins

WT RNAP was isolated from *E. coli* MRE 600 cells (Grain Processing Corp.) as described (Burgess and Jendrisak, 1975; Gonzalez *et al.*, 1977). WT^{His} RNAP and polymerase lacking part of the interdomain linker and the entire α CTD (α 235^{His} RNAP) were prepared by reconstitution, as described (Tang *et al.*, 1995). Holoenzyme was saturated with sigma during reconstitution. The RNAP with a single amino acid change in the α subunit (α D305E RNAP) was isolated from *E. coli* K4069, which was a gift from Dr David Friedman (The University of Michigan Medical School). The polymerase was purified with the following modifications to the published procedure. The polymin P pellet was washed five times with TGED (10 mM Tris-Cl, pH 7.9, 5% glycerol, 0.1 mM EDTA, 0.1 mM DTT) + 0.5 M NaCl. The pellet was resuspended in TGED + 1.0 M NaCl. After a 30 min centrifugation at 6000 g, the supernatant was precipitated at 75% saturated ammonium sulfate. The pellet was dissolved in TGED and applied to a 5 ml heparin HiTrap column (Pharmacia Biotech) equilibrated with TGED + 0.3 M NaCl. The column was washed with TGED + 0.3 M NaCl. The polymerase was eluted with TGED + 0.6 M NaCl and dialyzed against TGED + 0.1 M NaCl. The polymerase was further purified by anion exchange chromatography on a 5 ml Mono Q column (Bio-Rad). The polymerase was eluted with a 40 min gradient from TGED + 0.3 M NaCl to TGED + 0.5 M NaCl. NusA was expressed as a GST fusion protein (Zhang and Hanna, 1995) and purified by the method of Guan and Dixon (1991). Lambda Q protein was purified as described (Yang and Roberts, 1989). Lambda N protein was prepared as described by Ghosh and Das (1984). GST-NusA fusion protein and GST protein were prepared as described (Zhang and Hanna, 1995). The α WT^{His} subunit, α 235^{His} subunit, and the crude cell extracts containing overexpressed α WT^{His} and α 235^{His} subunits were purified as described (Tang *et al.*,

1995). β and β' inclusion bodies were prepared from strains which overexpressed β and β' and were renatured by dialysis against renaturation buffer G, as described (Tang *et al.*, 1995).

DNA templates

Five DNA templates were used in these studies. Template 1 contains the λ P_{R'} promoter, *qut* site, and the λ t₀ terminator, with an *Eco*RI site at position +63. The *Hind*III-*Sma*I fragment of pXY307 (Yang *et al.*, 1987) was subcloned into the corresponding sites of pBluescript II KS(-) phagemid (Stratagene) to generate phagemid pHA100-R63. The template for transcription was generated by PCR from pHA100-R63 using the T3 and T7 universal primers. Template 2 was a 302 bp PCR fragment amplified from pAR1707 (Briat *et al.*, 1987) which contains the bacteriophage T7 A1 promoter. Primer end points relative to the A1 transcription initiation site were -149 and +153. Template 3, which contains the λ P_{R'} promoter, 6S gene and t_R terminator was the 850 bp *Hind*III-*Eco*RI fragment of pXY310 (Yang *et al.*, 1987). Template 4 was the *Eco*RI-*Sall* restriction fragment of pAR1707 and contains the bacteriophage T7 A1 promoter and t_R terminator. Template 5 was made by linearizing pWW16 with *Hind*III (DeVito and Das, 1994). This template contains the λ P_L promoter and *nutL* site and the cloned λ t_R terminator.

RNA crosslinking to alpha and NusA

Transcription complexes containing RNA 49 nt long were prepared from the λ P_{R'} promoter of template 1 in buffer A with all four RNAPs, as described by Liu and Hanna (1995b). Paused transcription complexes were formed by binding the *Eco*RI mutant, Gln111, to the *Eco*RI site at +63. This mutant enzyme binds to the DNA, but it does not cleave the DNA, and RNAP does not displace the enzyme during transcription. The 3' end of the RNA present in the stalled transcription complex is located 14 nt upstream from the *Eco*RI cleavage site (Pavco and Steege, 1990). The DNA template (5 nM) was incubated with 20 nM Gln111 in buffer A at 37°C for 40 min. ATP, CTP (100 μ M each), 100 μ M [α -³²P]GTP (3 $\times$ 10⁵ d.p.m./pmol), UTP (5 μ M), 5-APAS-UTP (150 μ M) and RNAP (20 nM) were added, and the reactions were incubated at 37°C for another 10 min. Photocrosslinking and analysis of RNA and proteins was by electrophoresis and autoradiography, as described (Liu and Hanna, 1995a,b). For analysis of NusA-RNA interactions with the four polymerases, complexes were prepared as above. Reactions were divided into five aliquots, and different amounts of NusA were added to each. A control reaction contained no NusA, two reactions contained 500 nM NusA, one contained 100 nM NusA and the other contained 200 nM NusA. Reactions were incubated with NusA for 2 min at 37°C, and then irradiated and analyzed, as above. One of the reactions containing 500 nM NusA was kept in the dark as the minus irradiation control.

NusA-enhanced pausing with wild-type and α -modified polymerases

Transcription complexes containing polymerase paused after synthesis of a 20mer RNA (A₂₀) were prepared from the T7 A1 promoter of template 2 in buffer B with WT RNAP, WT^{His} RNAP and α 235^{His} RNAP. The DNA (10 nM) was incubated with 20 nM polymerase and 5 μ M ApU at 30°C for 5 min. ATP, CTP (1 μ M each), 1 μ M [α -³²P]GTP (10⁵ d.p.m./pmol) and heparin (25 μ g/ml) were added and the reaction was incubated at 30°C for 2 min. The A₂₀ RNA was then chased with ATP, CTP, GTP and UTP (10 μ M each) at 30°C, with or without NusA (200 nM for WT RNAP and WT^{His} RNAP and 500 nM for α 235^{His} RNAP). For the λ P_{R'} promoter, 5 nM DNA (template 3) was pre-incubated with 80 nM WT RNAP or α 235^{His} RNAP, 300 nM NusA (or buffer) and 100 μ M ApApC for 10 min at 37°C in buffer C. RNA synthesis was initiated by addition of 5 mM MgCl₂, 10 μ g/ml rifampicin, 20 μ M ATP, CTP, UTP and 5 μ M [α -³²P]GTP (2 $\times$ 10⁵ d.p.m./pmol). For both templates, aliquots were removed at various times and transcription was stopped by addition of an equal volume of solution B. The RNA was analyzed on a 30 $\times$ 0.75 cm 15% polyacrylamide-7 M urea gel (acrylamide/methylene bisacrylamide = 19/1).

NusA-enhanced intrinsic termination with WT and α -modified RNAPs

For all transcription reactions for analysis of termination or anti-termination efficiency, complexes were formed by pre-incubation of RNAP, DNA template, and 100 μ M initiator (ApApC for P_{R'} and ApU for A1 and P_L) for 10 min at 37°C in buffer A. Transcription was then initiated by addition of MgCl₂ (5 mM), rifampicin (10 μ g/ml), ATP, CTP and UTP (200 μ M each) and 50 μ M [α -³²P]GTP (2 $\times$ 10⁵ d.p.m./pmol). After 10 min at 37°C,

transcription was stopped by addition of an equal volume of solution B. Analysis of termination efficiency with each polymerase at the λ t_0 terminator utilized 5 nM template 1, 80 nM RNAP and 300 nM NusA (or storage buffer) in buffer D. Analysis of termination efficiency at the T7 t_0 terminator utilized 5 nM template 4, 80 nM RNAP and 300 nM NusA (or buffer). The NusA titration was done on template 1, at various concentrations of NusA, with 10 nM DNA and 20 nM WT^{His} RNAP or α 235^{His} RNAP in buffer A. RNA was analyzed on polyacrylamide-urea gels. The autoradiograms of the gels were scanned on an Apple OneScanner. The percentage of the RNA in the terminated and readthrough bands was determined using the Collage program (Fotodyne).

NusA-enhanced λ Q and N anti-termination with WT and α -modified RNAPs

To analyze the effect of changes in the α subunit on NusA-enhanced Q anti-termination, transcription was carried out with 5 nM template 3, 100 nM RNAP, 300 nM NusA (or buffer) and 0.8 μ M Q (or buffer). To determine if the function of NusA in Q-mediated anti-termination could be recovered at high NusA concentrations, transcription was carried out in buffer C with 5 nM template 3, 100 nM RNAP, 0.8 μ M Q (or buffer) and various concentrations of NusA. To analyze the effect of changes in the α subunit on NusA-enhanced N-mediated anti-termination, transcription was carried out in buffer E with 5 nM template 5, 30 nM RNAP, 30 nM NusA (or buffer) and 100 nM N (or buffer).

Binding of NusA to immobilized WT^{His} and α 235^{His} RNAPs

To determine if deletion of α CTD prevented interaction of NusA with the core enzyme, both WT^{His} and α 235^{His} RNAPs were incubated with NusA. RNAP core or holoenzymes (6 μ g) were incubated with 2.5 μ g (Figure 6A and B) or 0.125 μ g (Figure 6C) NusA in 20 μ l buffer F for 10 min at 37°C. Ni-NTA agarose beads (20 μ l, Qiagen) were washed with buffer F and left in ~25 μ l. The NusA-polymerase reactions were added to the beads and further incubated for 10 min at room temperature with occasional mixing of the beads. The beads were pelleted by brief centrifugation, and the supernatant fraction was withdrawn. The beads were then washed four times with 100 μ l of buffer F containing 10 mM imidazole (pH 7.9). The washes were carried out by adding the buffer, mixing the beads (~1 min) and then centrifuging briefly at room temperature. The washed beads were resuspended in 100 μ l buffer F with 100 mM imidazole. Samples (20 μ l) were analyzed by SDS Tris-glycine gel electrophoresis on 10% precast gels (Novex). In another experiment (Figure 6D), WT^{His} core RNAP or α 235^{His} core RNAP (6 μ g) were incubated with 2.5 μ g NusA in 20 μ l in 0.5 $\times$ buffer F for 10 min at 37°C and added to the resin as described above. Alternatively, the core enzymes were first incubated with the nickel resin in 0.5 $\times$ buffer F for 10 min at room temperature before NusA (2.5 μ g) was added. In both cases, after the core and NusA were incubated at 37°C for 10 min, the resin was washed three times with 0.5 $\times$ buffer F + 10 mM imidazole, and the bound proteins then eluted with 0.5 $\times$ buffer F + 100 mM imidazole and analyzed, as above.

Binding of WT^{His} core, α 235^{His} core and RNAP subunits to immobilized NusA

To determine if the α CTD contacts NusA directly and to determine if NusA also interacts with the β and/or β' subunits of RNAP, a GST-NusA fusion protein (Zhang and Hanna, 1995) was immobilized to glutathione agarose beads. WT^{His} core RNAP (6 μ g), α 235^{His} core RNAP (6 μ g), purified α WT^{His} subunit (1 μ g), purified α 235^{His} subunit (0.7 μ g), crude cell extract containing overexpressed α WT^{His} subunit, crude cell extract containing overexpressed α 235^{His} subunit, β inclusion bodies or β' inclusion bodies (Tang et al., 1995) were each incubated with 10 μ g of immobilized GST-NusA for 10 min at 37°C in buffer F. In some experiments, the proteins mentioned above were also incubated with immobilized GST as controls. The resin was centrifuged down, washed three times with buffer F as described above, and the washed beads were resuspended in solution C, heated at 100°C for 3 min and analyzed by 10% SDS Tris-glycine gel electrophoresis.

Acknowledgements

We thank Dr Juan Zhang for preparation of 5-APAS-UTP. This work was supported by NIH grant GM47493 to M.M.H. and in part by NIH grant GM28946 to A.D. K.S. is a Jane Coffin Childs Memorial Fund postdoctoral fellow.

References

- Blatter, E.E., Ross, W., Tang, H., Gourse, R.L. and Ebright, R.H. (1994) Domain organization of RNA polymerase α subunit: C-terminal 85 amino acids constitute a domain capable of dimerization and DNA binding. *Cell*, **78**, 889–896.
- Briat, J.-F., Bollag, G., Kearney, C.A., Molineux, I. and Chamberlin, M.J. (1987) Tau factor from *Escherichia coli* mediates accurate and efficient termination of transcription at the bacteriophage T3 termination site *in vitro*. *J. Mol. Biol.*, **198**, 43–49.
- Burgess, R.R. (1969) Separation and characterization of the subunits of ribonucleic acid polymerase. *J. Biol. Chem.*, **244**, 6168–6176.
- Burgess, R.R. and Jendrisak, J.J. (1975) A procedure for the rapid, large-scale purification of *Escherichia coli* DNA-dependent RNA polymerase involving polymin P precipitation and DNA-cellulose chromatography. *Biochemistry*, **14**, 4634–4638.
- Burgess, R.R. and Travers, A.A. (1969) Cyclic re-use of the RNA polymerase sigma factor. *Nature*, **222**, 537–540.
- Busby, S. and Ebright, R.H. (1994) Promoter structure, promoter recognition, and transcription activation in prokaryotes. *Cell*, **79**, 743–746.
- Chan, C.L. and Landick, R. (1993) Dissection of the *his* leader pause site by base substitution reveals a multipartite signal that includes a pause RNA hairpin. *J. Mol. Biol.*, **233**, 25–42.
- Chattopadhyay, S., Garcia-Mena, J., DeVito, J., Wolska, K. and Das, A. (1995) Bipartite function of a small RNA hairpin in transcription antitermination in bacteriophage lambda. *Proc. Natl Acad. Sci. USA*, **92**, 4061–4065.
- Chen, Y., Ebright, Y.W. and Ebright, R.H. (1994) Identification of the target of a transcription activator protein by protein-protein crosslinking. *Science*, **265**, 90–92.
- Dahlberg, J.E. and Blattner, F.R. (1973) In vitro transcription products of lambda DNA: nucleotide sequences and regulatory sites. In Fox, C.F. and Robinson, W.S. (eds), *Virus Research*. Academic Press, New York, pp. 533–543.
- Das, A. (1993) Control of transcription termination by RNA-binding proteins. *Annu. Rev. Biochem.*, **62**, 893–930.
- DeVito, J. and Das, A. (1994) Control of transcription processivity in phage lambda: Nus factors strengthen the termination-resistant state of RNA polymerase induced by N antiterminator. *Proc. Natl Acad. Sci. USA*, **91**, 8660–8664.
- Dissinger, S. and Hanna, M.M. (1991) RNA-protein interactions in a NusA-containing *E. coli* transcription complex paused at an RNA hairpin. *J. Mol. Biol.*, **219**, 11–25.
- Farnham, P.J., Greenblatt, J. and Platt, T. (1982) Effects of NusA protein on transcription termination in the tryptophan operon of *Escherichia coli*. *Cell*, **29**, 945–951.
- Friedman, D.I., Schauer, A.T., Baumann, M.R., Baron, L.S. and Adhya, S.L. (1981) Evidence that ribosomal protein S10 participates in control of transcription termination. *Proc. Natl Acad. Sci. USA*, **78**, 1115–1118.
- Ghosh, B. and Das, A. (1984) NusB: a protein factor necessary for transcription antitermination *in vitro* by phage N gene product. *Proc. Natl Acad. Sci. USA*, **81**, 6305–6309.
- Gill, S.C., Weitzel, S.E. and von Hippel, P.H. (1991) *Escherichia coli* Sigma⁷⁰ and NusA proteins I. Binding interactions with core RNA polymerase in solution and within the transcription complex. *J. Mol. Biol.*, **220**, 307–324.
- Gonzalez, N., Wiggs, J. and Chamberlin, M.J. (1977) A simple procedure for resolution of *Escherichia coli* RNA polymerase holoenzyme from core polymerase. *Arch. Biochem. Biophys.*, **182**, 404–408.
- Grayhack, E.J., Yang, X.J., Lau, L.F. and Roberts, J.W. (1985) Phage lambda gene Q antiterminator recognizes RNA polymerase near the promoter and accelerates it through a pause site. *Cell*, **42**, 259–269.
- Greenblatt, J. and Li, J. (1981a) 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. and Li, J. (1981b) The nusA gene protein of *Escherichia 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.
- Greenblatt, J., McLimont, M. and Hanly, S. (1981) Termination of transcription by nusA gene protein of *Escherichia coli*. *Nature*, **292**, 215–220.
- Greenblatt, J., Nodwell, J.R. and Mason, S.W. (1993) Transcriptional antitermination. *Nature*, **364**, 401–406.
- Gross, C.A., Lonetto, M.A. and Losick, R. (1992) Bacterial Sigma factors. In Yamamoto, K. and McKnight, S. (eds), *Transcription Regulation*.

- Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, pp. 129–176.
- Guan, K. and Dixon, J.E. (1991) Eukaryotic proteins expressed in *Escherichia coli*: an improved thrombin cleavage and purification procedure of fusion proteins with glutathione S-transferase. *Anal. Biochem.*, **192**, 262–267.
- Hanna, M.M. (1989) Photoaffinity cross-linking methods for studying RNA–protein interactions. *Methods Enzymol.*, **180**, 383–409.
- Hanna, M.M., Dissinger, S., Williams, B.D. and Colston, J.E. (1989) Synthesis and characterization of 5-[4-azidophenacyl]thio]uridine 5'-triphosphate, a cleavable photo-cross-linking nucleotide analogue. *Biochemistry*, **28**, 5814–5820.
- He, B. and Hanna, M.M. (1995) Preparation of probe-modified RNA with 5-mercapto-UTP for analysis of protein–RNA interactions. *Nucleic Acids Res.*, **23**, 1231–1238.
- Igarashi, K. and Ishihama, A. (1991) Bipartite functional map of the *E. coli* RNA polymerase alpha subunit: involvement of the C-terminal region in transcription activation by cAMP-CRP. *Cell*, **65**, 1015–1022.
- Igarashi, K., Fujita, N. and Ishihama, A. (1991) Identification of a subunit assembly domain in the alpha subunit of *Escherichia coli* RNA polymerase. *J. Mol. Biol.*, **218**, 1–6.
- Ishihama, A. (1992) Role of the RNA polymerase alpha subunit in transcription activation. *Mol. Microbiol.*, **6**, 3283–3288.
- Ishihama, A. (1993) Protein–protein communication within the transcription apparatus. *J. Bacteriol.*, **175**, 2483–2489.
- Ito, K., Egawa, K. and Nakamura, Y. (1991) Genetic interaction between the beta' subunit of RNA polymerase and the arginine-rich domain of *Escherichia coli* nusA protein. *J. Bacteriol.*, **173**, 1492–1501.
- Jin, D.J., Cashel, M., Friedman, D.I., Nakamura, Y., Walter, W.A. and Gross, C.A. (1988) Effects of rifampicin resistant rpoB mutations on antitermination and interaction with nusA in *Escherichia coli*. *J. Mol. Biol.*, **204**, 247–261.
- Kassavetis, G. and Chamberlin, M. (1981) Pausing and termination of transcription within the early region of bacteriophage T7 DNA *in vitro*. *J. Biol. Chem.*, **256**, 2777–2786.
- Kimura, M., Fujita, N. and Ishihama, A. (1994) Functional map of the alpha subunit of *Escherichia coli* RNA polymerase: deletion analysis of the amino-terminal assembly domain. *J. Mol. Biol.*, **242**, 107–114.
- Kingston, R.E. and Chamberlin, M.J. (1981) Pausing and attenuation of *in vitro* transcription in the rrnB operon of *E. coli*. *Cell*, **27**, 523–531.
- Landick, R. and Yanofsky, C. (1987) Isolation and structural analysis of the *Escherichia coli* trp leader paused transcription complex. *J. Mol. Biol.*, **196**, 363–377.
- Lee, D.N., Phung, L., Stewart, J. and Landick, R. (1990) Transcription pausing by *Escherichia coli* RNA polymerase is modulated by downstream DNA sequences. *J. Biol. Chem.*, **265**, 15145–15153.
- Liu, K. and Hanna, M.M. (1995a) NusA contacts nascent RNA in *E. coli* transcription complexes. *J. Mol. Biol.*, **247**, 547–558.
- Liu, K. and Hanna, M.M. (1995b) NusA interferes with interactions between the nascent RNA and the C-terminal domain of the alpha subunit of *E. coli* transcription complexes. *Proc. Natl Acad. Sci. USA*, **92**, 5012–5016.
- Maizels, N. (1973) The nucleotide sequence of the lactose messenger ribonucleic acid transcribed from the UV5 promoter mutant of *E. coli*. *Proc. Natl Acad. Sci. USA*, **70**, 3585–3589.
- Nudler, E., Goldfarb, A. and Kashlev, M. (1994) Discontinuous mechanism of transcription elongation. *Science*, **265**, 793–796.
- Nudler, E., Kashlev, M., Nikiforov, V. and Goldfarb, A. (1995) Coupling between transcription termination and RNA polymerase inchworming. *Cell*, **81**, 351–357.
- Pavco, P.A. and Steege, D.A. (1990) Elongation by *Escherichia coli* RNA polymerase is blocked *in vitro* by a site-specific DNA binding protein. *J. Biol. Chem.*, **265**, 9960–9969.
- Ring, B. and Roberts, J. (1994) Function of a nontranscribed DNA strand site in transcription elongation. *Cell*, **78**, 317–324.
- Roberts, J.W. (1988) Phage lambda and the regulation of transcription termination. *Cell*, **52**, 5–6.
- Ross, W., Gosink, K.K., Salomon, K.I., Zou, C., Ishihama, A., Severinov, K. and Gourse, R.L. (1993) A third recognition element in bacterial promoters: DNA binding by the alpha subunit of RNA polymerase. *Science*, **262**, 1407–1413.
- Russo, F.D. and Silhavy, T.J. (1992) Alpha: the Cinderella subunit of RNA polymerase. *J. Biol. Chem.*, **267**, 14515–14518.
- Schmidt, M.C. and Chamberlin, M.J. (1984) Amplification and isolation of *Escherichia coli* NusA protein and studies of its effects on *in vitro* RNA chain elongation. *Biochemistry*, **23**, 197–203.
- Schmidt, M.C. and Chamberlin, M.J. (1987) NusA protein of *Escherichia coli* is an efficient transcription termination factor for certain terminator sites. *J. Mol. Biol.*, **195**, 809–818.
- Sparkowski, J. and Das, A. (1992) Simultaneous gain and loss of functions caused by a single amino acid substitution in the beta subunit of *Escherichia coli* RNA polymerase: suppression of nusA and rho mutations and conditional lethality. *Genetics*, **130**, 411–428.
- Tang, H., Severinov, K., Goldfarb, A. and Ebright, R.H. (1995) Rapid RNA polymerase genetics: one-day, no-column preparation of reconstituted recombinant *Escherichia coli* RNA polymerase. *Proc. Natl Acad. Sci. USA*, **92**, 4902–4906.
- Wang, D., Meier, T.I., Chan, C.L., Feng, G., Lee, D.N. and Landick, R. (1995) Discontinuous movements of DNA and RNA in RNA polymerase accompany formation of a paused transcription complex. *Cell*, **81**, 341–350.
- Whalen, W. and Das, A. (1990) Action of an RNA site at a distance: Role of the nut genetic signal in transcription antitermination by phage lambda N gene product. *New Biol.*, **2**, 975–991.
- Yager, T.D. and von Hippel, P.H. (1987) Transcript elongation and termination. In Neidhardt, F.C. (ed.), *Escherichia coli and Salmonella typhimurium*. American Society for Microbiology, Washington DC, pp. 1241–1275.
- Yang, X.J. and Roberts, J.W. (1989) Gene Q antiterminator proteins of *Escherichia coli* phages 82 and lambda suppress pausing by RNA polymerase at a rho-dependent terminator and at other sites. *Proc. Natl Acad. Sci. USA*, **86**, 5301–5305.
- Yang, X.J., Hart, C.M., Grayhack, E.J. and Roberts, J.W. (1987) Transcription antitermination by phage lambda gene Q protein requires a DNA segment spanning the RNA start site. *Genes Dev.*, **1**, 217–226.
- Yarnell, W.S. and Roberts, J.W. (1992) The phage lambda gene Q transcription antiterminator binds DNA in the late gene promoter as it modifies RNA polymerase. *Cell*, **69**, 1181–1189.
- Zaychikov, E., Denisova, L. and Heumann, H. (1995) Translocation of the *Escherichia coli* transcription complex observed in the registers 11 to 20: 'jumping' of RNA polymerase and asymmetric expansion and contraction of the transcription bubble. *Proc. Natl Acad. Sci. USA*, **92**, 1739–1743.
- Zhang, Y. and Hanna, M. (1994) NusA changes the conformation of *Escherichia coli* RNA polymerase at the binding site for the 3' end of the nascent RNA. *J. Bacteriol.*, **176**, 1787–1789.
- Zhang, Y. and Hanna, M.M. (1995) Expression and functional characterization of *E. coli* NusA and lambda Q as glutathione S-transferase fusion proteins. *Protein Expression and Purification*, **6**, 625–631.
- Zou, C., Fujita, N., Igarashi, K. and Ishihama, A. (1992) Mapping the cAMP receptor protein contact site on the alpha subunit of *Escherichia coli* RNA polymerase. *Mol. Microbiol.*, **6**, 2599–605.

Received on August 7, 1995; revised on September 29, 1995