NusA interferes with interactions between the nascent RNA and the C-terminal domain of the α subunit of RNA polymerase in *Escherichia coli* transcription complexes

(photocrosslinking/nucleotide analogs/Gln111 roadblock)

KEBIN LIU* AND MICHELLE M. HANNA*†‡

*Department of Botany and Microbiology and †Department of Chemistry and Biochemistry, University of Oklahoma, Norman, OK 73019

Communicated by Peter von Hippel, University of Oregon, Eugene, OR, February 16, 1995

ABSTRACT The effects of NusA on the RNA polymerase contacts made by nucleotides at internal positions in the nascent RNA in *Escherichia coli* transcription complexes were analyzed by using the photocrosslinking nucleotide analog 5-[(4-azidophenacyl) thio]-UMP. It was placed at nucleotides between +6 and +15 in RNA transcribed from the phage λP_R promoter. Crosslinks of analog in these positions in RNAs which contained either 15, 28, 29, or 49 nt were examined. Contacts between the nascent RNA and proteins in the transcription complex were analyzed as the RNA was elongated, by placing the crosslinker nearest the 5' end of the RNA 10, 23, 24, or 44 nt away from the 3' end. The β or β' subunit of polymerase, and NusA when added, were contacted by RNA from 15 to 49 nt long. When the upstream crosslinker was 24 nt from the 3' end of the RNA (29-nt RNA), α was also contacted in the absence of NusA. The addition of NusA prevented RNA crosslinking to α . When the crosslinker was 44 nt from the 3' end (49-nt RNA), α crosslinks were still observed, but crosslinks to β or β' and NusA were greatly diminished. RNA crosslinking to α , and loss of this crosslink when NusA was added, was observed in the presence of NusB, NusE, and NusG and when transcription was carried out in the presence of an *E. coli* S100 cell extract. Peptide mapping localized the RNA interactions to the C-terminal domain of α .

RNA polymerase holoenzyme from *Escherichia coli* contains four subunits with stoichiometry $\beta\beta'\alpha_2\sigma$. After initiation of transcription, σ is released, and the elongation factor NusA can bind to polymerase to modulate elongation (1). Other factors, including NusE and NusG, can interact with the polymerase directly (reviewed in ref. 2), and NusB can interact indirectly through NusE (3). Analysis of protein–nucleic acid interactions in *E. coli* transcription complexes has produced several models for the structures of elongation complexes (reviewed in refs. 4–6). We have analyzed the contacts made by the nascent RNA by incorporating photocrosslinking ribonucleotide analogs into ternary transcription complexes, and RNA crosslinks have been localized to β and β' (7–10) and to NusA (11). No interactions between the α subunit and the RNA were observed.

Specific interactions between the α subunit and proteins or the DNA in initiation complexes at some promoters are required for activation of these promoters (reviewed in refs. 12–16). We report here that α also interacts with the nascent RNA in elongation complexes, and we show that NusA binding alters α –RNA interactions in such a way that α –RNA crosslinks are no longer observed. These experiments involved placement of the photocrosslinking nucleotide analog 5-[(4-azidophenacyl) thio] uridine 5'-monophosphate (5-APAS-UMP) (7, 17–19) at internal positions in the RNA during

transcription from the λP_R promoter. Once incorporated at nucleotides between +6 and +15 in the transcript, the crosslinkers were moved away from the 3' end of the RNA by forming RNA 15, 28, 29, or 49 nt long. RNA crosslinks to the individual polymerase subunits and NusA were examined.

MATERIALS AND METHODS

Immobilized Transcription Complexes Containing Analog in the First 15 nt of RNA. All experiments utilized DNA containing the λP_R promoter, and after transcription complex formation, samples were irradiated and analyzed as described (11). 5'-Biotinylated DNA fragment was attached to streptavidin-MagneSphere paramagnetic particles (40 μ g; Promega). ApApC (150 μ M) and 50 nM RNA polymerase were added and incubated at 37°C for 2 min. Then 40 μ M ATP, 5 μ M UTP, 150 μ M 5-APAS-UTP, and 5 μ M [α -³²P]GTP were added and the reaction was incubated at 37°C for 5 min to form analog-tagged RNA that was 15 nt long (Fig. 1). The beads were washed and the 15-nt RNA was elongated to 28 nt by addition of 100 μ M CTP, GTP, and UTP or to 29 by addition of 100 μ M CTP, GTP, UTP and 3'-OMe-ATP, followed by incubation at 37°C for 15 min. The reaction mixture was divided and aliquots were incubated at 37°C for 2 min with or without NusA (200 nM).

RNA 49 nt Long with Analog and Radioactive Label Only in the First 15 nt. Complexes containing 15-nt RNA were prepared as described above, except that the DNA contained an *Eco*RI site at +63 relative to the P_R transcription initiation site. Gln111 [an *Eco*RI restriction endonuclease with a glutamine substitution at residue 111 (20), provided by Paul Modrich, Duke University] was added so that the molar ratio of Gln111 to DNA was 4:1. The mixture was incubated at 37°C for 40 min. ATP, CTP, GTP, and UTP (100 μ M each) were added and the reaction was incubated at 37°C for 10 min. The reaction mixture was divided and aliquots were incubated at 37°C for 2 min with or without NusA (200 nM).

Crosslinking of RNA in the Presence of Nus Factors or S100 Cell Extract Proteins. DNA (5 nM) was incubated with Gln111 (20 nM) and then ATP and CTP (100 μ M each), [α -³²P]GTP (100 μ M), UTP (5 μ M), and 5-APAS-UTP (150 μ M) were added. This produced 49-mer RNA with analog and radioactive label throughout. The reaction mixture was incubated at 37°C for 10 min and divided into three aliquots, which were incubated at 37°C for 2 min without NusA, with 200 nM NusA, or with 200 nM NusA and 1 μ M NusB, NusE, and NusG. Transcription complexes containing several lengths of RNA, including the 194-nt released RNA formed by transcription to the t_R terminator, were prepared by incubation of 25 nM

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

Abbreviations: NTD α and CTD α , N- and C-terminal domains of the α subunit; 5-APAS-UMP, 5-[(4-azidophenacyl) thio] uridine 5'-monophosphate.

‡To whom reprint requests should be addressed.

FIG. 1. Crosslinking of α and NusA to the nascent RNA. (A) RNA sequence from the λ $P_{R'}$ promoter. Positions containing 5-APAS-UMP are indicated with larger bold letters. (B) RNA synthesized in photocrosslinking reactions. The autoradiograms of 15% polyacrylamide/urea gels are shown. Analog was present only between positions +6 and +15 in all RNAs. The 15-nt RNA (lanes 1–4) was extended without additional analog or radioactive label to 28 nt (lanes 5–8) or 29 nt (lanes 9–12). For preparation of RNA 49 nt long, RNA was elongated from the 15-mer by use of a Gln111 roadblock at +63 (lanes 13–16). In all reactions, NusA was added after transcription was completed. (C) Proteins crosslinked to RNA of different lengths. Protein–RNA complexes from SDS/10% polyacrylamide gels were transferred to nitrocellulose membranes. RNA polymerase and NusA were located by silver staining of the membranes. Autoradiograms of the membranes are shown. Lanes 1–16 involved reactions in which the analog was positioned only in the first 15 nt of the transcript. For lanes 17–20, reaction mixtures contained 49-nt RNA with analog throughout and were treated with ribonuclease T1 prior to electrophoresis.

DNA, 20 μ M ATP, 20 μ M 3'-OMe-ATP, 40 μ M CTP, 20 μ M [α - 32 P]GTP, 5 μ M UTP, 150 μ M 5-APAS-UTP, and 50 nM RNA polymerase at 37°C for 10 min, with or without 200 nM NusA, NusB, NusE, and NusG. S100 cell extracts were prepared from *E. coli* strain AD7333 as described (21). Transcription complexes (20 μ l) containing 49-mer RNA with 5-APAS-UMP and radioactive label throughout were prepared, and 2 μ l of S100 cell extract (12 μ g of protein) was added for 2 min at 37°C. All reaction mixtures were irradiated and treated with ribonuclease T1, as described (11).

Proteolytic Mapping of the RNA-Binding Domain in the α Subunit. Complexes containing the 49-nt RNA with analog and label throughout were prepared. After crosslinking and ribonuclease T1 digestion of the RNA, the protein band corresponding to the α subunit crosslinked to the nascent RNA was located by autoradiography and excised from the gel. The gel piece was washed and treated with hydroxylamine (22). The gel piece was then equilibrated three times in 1 ml of 60 mM Tris-HCl, pH 6.8/3% (wt/vol) SDS/5% (vol/vol) 2-mercaptoethanol at 37°C for 30 min each, crushed, and loaded onto an SDS/10% polyacrylamide gel, adjacent to protein molecular weight standards. After electrophoresis, the proteins were electrophoretically transferred to a nitrocellulose membrane. The membrane was silver-stained and subjected to autoradiography.

RESULTS

DNA templates containing the λ $P_{R'}$ promoter were attached to magnetic beads. Transcription was initiated with ApApC

and 15-nt RNA was formed by addition of [α - 32 P]GTP, 5-APAS-UTP, UTP, and ATP (Fig. 1A). Photocrosslinking analogs were located at +5, +10, +11, +14, and +15, and [α - 32 P]GMP at +4, +7, +8, and +9. Elongation of the 15-nt RNA to 28, 29, or 49 nt was done in the absence of additional analog. The 28-mer was made by leaving out one nucleotide. The 29-mer was made by incorporation of an RNA chain terminator at +29, and the 49-mer was made by using a Gln111 transcription roadblock. Gln111 is an *EcoRI* enzyme containing an amino acid substitution at position 111 (20). It binds to an *EcoRI* site, but it does not cleave, and when bound, it functions to stop *E. coli* RNA polymerase just upstream of the Gln111 so that the 3' end of the RNA is 14 nt upstream from the *EcoRI* site (23). After irradiation of the transcription complexes, the RNA can be crosslinked to a protein through any of the positions containing analog, and the proteins contacted can be identified by denaturing electrophoresis and autoradiography. Only proteins which have become covalently attached to the RNA through irradiation will be radioactively labeled.

Crosslinks Between the Nascent RNA and α or NusA. The contacts made by photocrosslinking nucleotide analogs placed 6–15 nt from the 5' end of the RNA from the λ $P_{R'}$ promoter are shown in Fig. 1. RNA 15, 28, 29, or 49 nt long was synthesized by placing the most upstream crosslinker 10, 23, 24, or 44 nt from the 3' end of the RNA. After synthesis of the RNA, the reaction mixtures were split and NusA was added to one half. The reaction containing the 15-nt RNA also contained RNAs 10, 11, and 14 nt long (Fig. 1B, lanes 1–4), which correspond to positions in the RNA at which sequential UMPs are encoded. The 15-nt RNA contacted primarily the β and β' subunits of the core polymerase, both in the presence and in the absence of NusA (Fig. 1C, lane 3). A small amount of crosslinking to the σ subunit was also observed. This may be due to the 10-mer, or it may be due to some smaller RNAs in the reaction mixture, which would not be visible on this gel. When added, some crosslinking of NusA by the 15-nt RNA was also detected (lane 4).

When the RNA was elongated to 28 nt by moving the crosslinker at +6 to 23 nt from the 3' end of the RNA, β and/or β' were still contacted. When NusA was present, >50% of the RNA crosslinking was to NusA (Fig. 1C, lanes 5–8). Crosslinking to NusA was not accompanied by an equivalent decrease in labeling of β or β' , and no crosslinks to α were observed. Movement of the crosslinker by only one additional nucleotide, to 24 nt from the 3' end of the RNA, resulted in crosslinking to α (Fig. 1C, lane 11). When NusA was added, crosslinking to α almost disappeared, whereas significant crosslinking to NusA was observed (Fig. 1C, lane 12). Further movement of the upstream crosslinker to 44 nt from the 3' end of the RNA again resulted in crosslinking to β and/or β' and α (Fig. 1C, lane 15), although labeling of β and/or β' was significantly less than with the 28- and 29-mers. Addition of NusA again virtually eliminated the α -RNA crosslinks (Fig. 1C, lane 16). When the 49-mer was labeled throughout with analog, crosslinkers were located at nine positions in the region of the RNA 25–44 nt from the 3' end. In this case, α was heavily labeled, and NusA eliminated the α -RNA crosslinks (Fig. 1C, lanes 17–20).

Crosslinks to α and NusA in the Presence of other Nus Factors and S100 Cell Extract Proteins. Complexes were prepared which contained either the 49-nt RNA or several RNAs of lengths up to 194 nt. When multiple lengths of RNA were present, only β and/or β' and α were crosslinked to the RNA in the absence of NusA (Fig. 2, lane 3). When NusA, NusB, NusE, and NusG were present at 200 nM, α -RNA crosslinks were eliminated and NusA–RNA interactions were observed. No crosslinking to NusB, NusE, or NusG was observed at these Nus factor concentrations, and no decrease in the RNA crosslinks to β and/or β' was observed (lane 4).

FIG. 2. Protein-RNA contacts in the presence of NusA, NusB, NusE, and NusG. For lanes 1–4, reaction mixtures contained multiple lengths of RNA, up to 194 nt, with analog throughout. When present (lanes 2 and 4) NusA, NusB, NusE, and NusG were added at 200 nM at the start of transcription. For lane 5, the 49-nt RNA substituted throughout with analog was synthesized by use of the Gln111 roadblock; NusA was present at 200 nM, and NusB, NusE, and NusG were present at 1 μ M. For lanes 6 and 7, the 49-nt RNA substituted throughout with analog was incubated in the presence of S100 extract proteins. Lanes 1–7 are the autoradiograms of the nitrocellulose membranes resulting from transfer of proteins from SDS/10% polyacrylamide gels. Lanes 8 and 9, silver-stained membrane corresponding to reactions in lanes 6 and 7.

In complexes containing the 49-nt RNA (as in Fig. 1C, lane 17–20), when NusA was present at 200 nM and all other Nus factors were present in 1 μ M, similar results were obtained (Fig. 2, lane 5). In the presence of *E. coli* S100 proteins, crosslinking to α was still observed (lane 7). Addition of NusA diminished this crosslinking (data not shown). An extract protein which migrated between NusB and NusG was radioactively labeled in extracts, but this labeling occurred even in the absence of irradiation (Fig. 2, lane 6). Low levels of photocrosslinking of about 7 or 8 other extract proteins also occurred.

Crosslinks Between RNA and α Involve the C-Terminal Domain of α (CTD α). The α subunit contains a single hydroxylamine cleavage site located between amino acids 209 and 210. The α subunit was crosslinked to the 49-nt RNA, which contained analog and radioactive label throughout the transcript, the RNA was treated with ribonuclease T1, and the α subunit was isolated from a denaturing gel (Fig. 3, lane 1). After isolation of the labeled α subunit and treatment with hydroxylamine, two protein bands were observed on a gel, corresponding to the N-terminal domain (NTD α) and CTD α , and only the smaller band, corresponding to CTD α , was radioactively labeled (Fig. 3, lane 2).

DISCUSSION

The α subunit of *E. coli* RNA polymerase has at least two functions in transcription. The first is a role in assembly of the polymerase, which requires NTD α (24). The second involves interaction with a variety of molecules involved in promoter activation, and this requires CTD α (12, 25). We show here that CTD α interacts with the nascent RNA in elongation complexes and that NusA interferes with this interaction, concomitant with RNA crosslinking to NusA. NusA does not crosslink to purified RNA in the absence of the ternary transcription complex, however (11). The interaction of the RNA with α probably occurs at some point during transcription of all promoters, as we have observed α -RNA crosslinking with eight different templates (data not shown). The specific lengths of RNA that interact with α may differ for each

FIG. 3. Mapping of the domain on α which crosslinks to RNA. The α subunit was crosslinked to the 49-nt RNA and the complex was digested with ribonuclease T1. A portion of the reaction mixture was run in a denaturing gel, and the labeled α subunit was excised from the gel. After treatment of the gel piece with hydroxylamine, it was again analyzed on a denaturing gel (lane 2), adjacent to an aliquot of the untreated reaction mixture (lane 1). The proteins were transferred to nitrocellulose, and the membrane was silver-stained and then exposed to x-ray film. Only CTD α (14 kDa) was radioactively labeled (lane 2). The position of the NTD α (23 kDa) is indicated. Positions of other molecular size markers that were run on the same gel are indicated on the left.

template, however, because of different RNA secondary structures or conformations of the polymerase. In every case, addition of NusA decreased the RNA crosslinks to α .

On the template used in these studies, contacts with the α subunit did not begin until crosslinker was 24 nt from the 3' end of the RNA (Fig. 1). Contacts with α were still observed when the crosslinker was 44 nt from the 3' end, at which point crosslinks to the β and/or β' subunits had greatly diminished. The crosslinking pattern was identical for the 49-mer complex even after nucleotides and the Gln111 roadblock protein were removed prior to crosslinking (data not shown). These data suggest that CTD α may lie near the point at which the nascent RNA exists the transcription complex, consistent with other studies showing that α is positioned at the upstream side of the initiation complex (13). Loss of the α -RNA crosslinks when NusA was added suggests that NusA may alter the path taken by the nascent RNA or interact with α . Although we have determined that on this template α contacts begin when the crosslinker is 24 nt from the 3' end of the RNA, this is unlikely to be true of all transcription complexes. Rather, it is likely that contacts may differ for complexes in different stages of the translocation cycle. Movements of RNA and DNA in the transcription complex are not uniform (13, 26). Thus, on a different template, the transition to α crosslinking may occur at a different position.

Over a decade ago, photocrosslinking studies revealed that β , β' and σ subunits all contacted the 5' end of the nascent RNA at different points during transcription (27, 28). Since then, other studies with 5' probes have been carried out, and crosslinks to the α subunit were never reported (reviewed in ref. 8). We believe that the 5' end of the RNA may contact α but that the methods used in these studies did not allow for detection of this interaction. We have modified the crosslinking protocols, and these modifications now allow us to detect RNA interactions with proteins of any size and to carry out crosslinking in crude extracts. By treating the complexes with ribonuclease T1 and transferring the protein-RNA complexes to nitrocellulose, we can now analyze protein-RNA crosslinks without the interference of long RNAs, which comigrate with α in many gels. Although it is unclear whether the 5' end of the RNA contacts α , it is clear that CTD α does contact internal

positions in the RNA (Fig. 3). The RNA which was crosslinked to the α subunit which was isolated for hydroxylamine cleavage was labeled throughout with crosslinker and radioactive label. Therefore, contacts made by any region of the RNA containing crosslinker could be mapped. Our results suggest that the RNA crosslinks only to CTD α . Similar crosslinking experiments using a polymerase lacking CTD α (truncated at aa 235) produced no RNA crosslinking to the truncated subunit containing only NTD α . Pausing and Rho-independent termination are no longer enhanced by the addition of NusA to polymerase lacking CTD α (unpublished work).

These results suggest, but certainly do not prove, that the RNA-binding domain for polymerase may be less than the 80–100 nt suggested from the earlier work with crosslinkers placed only at the 5' end of the RNA. The RNAs made in the earlier studies with the 5' probe had the potential to form several stable RNA hairpins in the longer RNAs. If these hairpins formed, the 5' end of the RNA may have been pulled back toward the 3' end of the RNA, causing crosslinking to polymerase by longer RNAs. In the experiments described here, crosslinking of β or β' and NusA is minimal with the 49-mer, where the crosslinker is 44 nt from the 3' end of the RNA (Fig. 1C). This may indicate that at this position, on this template, the RNA is near the exit point from the transcription complex. When the crosslinkers were moved 90–100 nt from the 3' end of the RNA, even RNA crosslinking to α was not observed (data not shown). Thus, when the crosslinker was only about 50 nt beyond the point of α crosslinking, and when the RNA was still tethered to the polymerase, α was not crosslinked.

Does this crosslinking to α occur because α forms part of a binding site for the nascent RNA, or does crosslinking result simply because the RNA in the 49-mer complex has now left the surface of the polymerase and is free to interact with any protein in the vicinity? The decrease in the relative labeling of NusA as the RNA was elongated from 28 nt to 49 nt seems to argue against nonspecific interactions caused by the RNA, which is tethered to the polymerase, "flopping" in solution and contacting any protein within reach of the crosslinking group. NusA is heavily labeled by internal regions in the 49-nt RNA (Fig. 1, lane 20), but only slightly by the crosslinkers between +6 and +15 (Fig. 1, lane 16). Elongation of the RNA to $\approx$ 100 nt eliminates the α -RNA crosslinks, suggesting that RNA crosslinks are not due to a nonspecific nucleic acid binding site on α . In addition, the Gln111 protein, which contacts the downstream end of the polymerase, is not crosslinked to the RNA even when the RNA is almost 200 nt long (data not shown), when the RNA could reach the front of the transcription complex.

What is the effect of other transcription factors and *E. coli* proteins on α and NusA interactions with the nascent RNA? Crosslinking to α occurred even in the presence of S100 extract proteins, with only seven or eight other proteins in the extract being crosslinked to the RNA (Fig. 2, lane 7). Addition of NusA to the extract eliminated the α crosslink, with concomitant crosslinking of RNA to NusA (data not shown), just as was observed with the purified proteins. NusB, NusE, and NusG did not alter the effect of NusA on α crosslinking at any concentration tested (200 nM to 2 μ M). When NusB, NusE, and NusG were present at the same concentration at which NusA was crosslinked (200 nM), they were not crosslinked to the RNA, even when RNA as long as 194 nt was present (Fig. 2, lane 4). The proteins used for these experiments supported N-protein-mediated antitermination at the concentrations used (data not shown). Even when concentrations of NusB, NusE, and NusG were raised to 1 μ M, only NusA was significantly crosslinked in the 49-mer complex (Fig. 2, lane 5). Using the apparent equilibrium association constant (K_a) for NusE-polymerase binding, 10^6 M $^{-1}$ (29), we calculate that at 1 μ M NusE and 50 nM polymerase, the polymerase should be

approximately half-saturated with NusE. We can detect crosslinking to NusA when it is present at only 50 nM, at a 1:1 ratio to RNA polymerase. Using $K_a = 3 \times 10^7$ M $^{-1}$ for NusA interaction with the elongation complex (1), we estimate that at these concentrations only about a third of the transcription complexes should contain NusA. At 1 μ M NusB, any NusE should be associated with NusB, since $K_a = 10^7$ M $^{-1}$ for the NusB-NusE interaction (3). Although a binding constant for NusG with the elongation complex has only been estimated, its binding to the core has also been shown to occur, although weakly, in the absence of N (29). It is difficult to determine to what extent NusB, NusE, and NusG are interacting with transcription complexes in our experiments by utilizing binding constants determined by affinity chromatography, sucrose gradient cosedimentation, or other methods which involve isolation of complexes. Photocrosslinking is more sensitive than any of these methods and can be used to identify even transient or weak interactions that might not survive some isolation methods. Only when the concentrations of NusB, NusE, and NusG were raised to 2 μ M was a low level of RNA crosslinking to NusB and NusG detectable (data not shown). Perhaps these proteins do associate with RNA in intact termination complexes with Rho or antitermination complexes with N.

RNA interactions with α , and the influence of NusA on this interaction, are somewhat surprising in light of existing genetic data. Two mutations that suppress mutations in the gene for NusA have been reported, and these fall in the *rpoB* and *rpoC* genes, which encode the β and β' subunits of RNA polymerase (30, 31). If NusA is binding to β and/or β' , and if the RNA interacts both with β , β' and NusA, one might expect a decrease in β or β' contacts with the RNA upon addition of NusA. We do not observe such a decrease. However, NusA could be binding in such a way that the RNA is more shielded from solvent, causing the crosslinking yield to increase. Thus, NusA could bind directly to β and/or β' without a decrease in β and/or β' labeling. Could the binding site for NusA include α ? To date, all published mutations in *rpoA*, the gene encoding the α subunit have altered activation phenotypes, not altered elongation phenotypes. However, one mutation in α which suppresses some Nus factor mutations, including *nusA1*, has been isolated by David Friedman (personal communication). This mutation falls in CTD α . Although the data presented here do not prove a direct interaction between NusA and α , we recently have determined that the deletion of CTD α eliminates NusA function in enhancement of pausing (unpublished work). Increasing NusA concentrations results in NusA-RNA crosslinking, but NusA function in pausing and termination is not regained. Thus, the CTD α may be involved in stabilizing the interaction of NusA with the polymerase and may be required for some functions of NusA. Many additional experiments must be done to fully understand the relationship of NusA to α , and the role that α -RNA interactions may play in transcription elongation. However, it is clear that the nascent RNA does contact α during transcription in the absence of NusA. How NusA functions to block the α -RNA interaction and what relationship this may have to pausing still remain to be determined.

We thank Dr. Juan Zhang for preparation of 5-APAS-UTP and Yuying Zhang for preparation of the NusA expression vector used for NusA purification. This work was supported by Grant RO1 GM47493 from the National Institutes of Health to M.M.H.

1. Gill, S. C., Weitzel, S. E. & von Hippel, P. H. (1991) *J. Mol. Biol.* **220**, 307–324.
2. Das, A. (1993) *Annu. Rev. Biochem.* **62**, 893–930.
3. Mason, S. W., Li, J. & Greenblatt, J. (1992) *J. Mol. Biol.* **223**, 55–66.
4. Eric, D. A., Yager, T. D. & von Hippel, P. H. (1992) *Annu. Rev. Biophys. Biomol. Struct.* **21**, 379–415.

5. Chan, C. L. & Landick, R. (1994) in *Transcription: Mechanisms and Regulation*, eds. Conaway, R. C. & Conaway, J. W. (Raven, New York), pp. 297–321.
6. Chamberlin, M. J. (1991) *The Harvey Lectures* (Wiley-Liss, New York), Vol. 88, pp. 1–21.
7. Dissinger, S. & Hanna, M. M. (1990) *J. Biol. Chem.* **265**, 7662–7668.
8. Meares, C. F. (1991) *Acct. Chem. Res.* **24**, 183–190.
9. Hanna, M. M., Zhang, Y., Reidling, J. C., Thomas, M. J. & Jou, J. (1993) *Nucleic Acids Res.* **21**, 2073–2079.
10. Zhang, Y. & Hanna, M. (1994) *J. Bacteriol.* **176**, 1787–1789.
11. Liu, K. & Hanna, M. M. (1995) *J. Mol. Biol.* **247**, 547–558.
12. Ishihama, A. (1993) *J. Bacteriol.* **175**, 2483–2489.
13. Ross, W., Gosink, K. K., Salomon, K. I., Zou, C., Ishihama, A., Severinov, K. & Gourse, R. L. (1993) *Science* **262**, 1407–1413.
14. Tao, K., Fujita, N. & Ishihama, A. (1993) *Mol. Microbiol.* **7**, 859–864.
15. Blattner, E. E., Ross, W., Tang, H., Gourse, R. L. & Ebright, R. H. (1994) *Cell* **78**, 889–896.
16. Chen, Y., Ebright, Y. W. & Ebright, R. H. (1994) *Science* **265**, 90–92.
17. Hanna, M. M. (1989) *Methods Enzymol.* **180**, 383–409.
18. Hanna, M. M., Dissinger, S., Williams, B. D. & Colston, J. E. (1989) *Biochemistry* **28**, 5814–5820.
19. Dissinger, S. & Hanna, M. M. (1991) *J. Mol. Biol.* **219**, 11–25.
20. King, K., Benkovic, S. & Modrich, P. (1989) *J. Biol. Chem.* **264**, 11807–11815.
21. Goda, Y. & Greenblatt, J. (1985) *Nucleic Acids Res.* **13**, 2569–2582.
22. Saris, C. J. M., van Eanbergen, J., Jenks, B. G. & Bloemers, H. P. J. (1983) *Anal. Biochem.* **132**, 54–67.
23. Pavco, P. A. & Steege, D. A. (1990) *J. Biol. Chem.* **265**, 9960–9969.
24. Igarashi, K., Fujita, N. & Ishihama, A. (1991) *J. Mol. Biol.* **218**, 1–6.
25. Ishihama, A. (1992) *Mol. Microbiol.* **6**, 3283–3288.
26. Nudler, E., Goldfarb, A. & Kashlev, M. (1994) *Science* **265**, 793–796.
27. Grachev, M. A. & Zaychikov, E. F. (1981) *FEBS Lett.* **130**, 23–26.
28. Hanna, M. M. & Meares, C. F. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 4238–4242.
29. Mason, S. W. & Greenblatt, J. (1991) *Genes Dev.* **5**, 1504–1512.
30. Ito, K., Egawa, K. & Nakamura, Y. (1991) *J. Bacteriol.* **173**, 1492–1501.
31. Sparkowski, J. & Das, A. (1992) *Genetics* **130**, 411–428.