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Contacts between mammalian RNA polymerase II and the template DNA in a ternary elongation complex

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ABSTRACT

Elongation complexes of RNA polymerase II, RNA-DNA-enzyme ternary complexes, are intermediates in the synthesis of all eukaryotic mRNAs and are potential regulatory targets for factors controlling RNA chain elongation and termination. Analysis of such complexes can provide information concerning the structure of the catalytic core of the RNA polymerase and its interactions with the DNA template and RNA transcript. Knowledge of the structure of such complexes is essential in understanding the catalytic and regulatory properties of RNA polymerase. We have prepared and isolated complexes of purified RNA polymerase II halted at defined positions along a DNA template, and we have used deoxyribonuclease I (DNAse I) to map the interactions of the polymerase with the DNA template. DNAse I footprints of three specific ternary complexes reveal that the enzyme-template interactions of individual elongation complexes are not identical. The size of the protected region is distinct for each complex and varies from 48 to 55 bp between different complexes. Additionally, the positioning of the protected region relative to the active site varies in different complexes. Our results suggest that RNA polymerase II is a dynamic molecule and undergoes continual conformational transitions during elongation. These transitions are likely to be important in the processes of transcript elongation and termination and their regulation.

INTRODUCTION

In order to understand regulatory mechanisms controlling transcript elongation, it would be useful to refer to a physical portrait of RNA polymerase II during transcription. Such information can be obtained by halting the RNA polymerase at specific sites along a transcription unit as has been done for the bacterial RNA polymerase (1–4). Structural analysis of RNA polymerase II in such transcription complexes has been hampered by the necessity to include multiple accessory factors required for promoter binding and initiation. The presence of many proteins in the initiation complex has made the assignment of contacts due to the polymerase itself difficult. Changes in the structure of the initiation complex during the transition from promoter binding to early elongation indicate that the complex is altered by the loss of transcription factors or structural changes within the protein components (5–7). Additional changes in the complexes' contacts with the DNA template have been found for transcription complexes which are committed to elongation (8, 9).

Transcription by RNA polymerase II is subject to significant regulation at all stages of transcription from initiation to elongation and termination. A large number of genes are regulated by premature blocks to elongation. Some of these sites are recognized by purified RNA polymerase II (10), and the block to elongation can be signalled by a combination of a T-run in the non-transcribed strand and a bend in the DNA template (11). Read through at some of these sites can be promoted by at least one protein, the elongation factor SII (10, for review) This protein interacts directly (12–14) and transiently (14) with RNA polymerase II during elongation in vitro. This result suggests that SII may effect a structural change in the polymerase itself. Recently it also has been shown that SII promotes hydrolysis of the nascent transcript within the ternary complex, a reaction which is more complex than a simple catalytic reversal of the polymerization reaction (15–17).

The structure of RNA polymerase II in elongation complexes can be directly examined by the use of 3′ extended templates (18). With these templates, purified RNA polymerase can accurately initiate and elongate in the absence of any accessory factors. Thus, structural features of the purified enzyme in the ternary complex can be examined. With this template, we have positioned mammalian RNA polymerase II at three specific sites along a DNA template. Each specific complex has been isolated, and the conformation of each elongation intermediate has been studied. Each of the three complexes is distinct in its conformation, and thus each of the three complexes presents a different physical target for the action of elongation regulatory factors.

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EXPERIMENTAL PROCEDURES

Reagents

RNA polymerase II was purified from calf thymus by a modification (19) of the procedure described by Hodo and Blatti (20) which resulted in >90% pure RNA polymerase II. The preparation was determined to contain 25% active molecules (18). The following reagents were purchased from the sources indicated: HPLC purified ribonucleoside triphosphates (Pharmacia/LKB); [α-^{32}P]CTP >400 Ci/mmol (Amersham); [α-^{32}P]GTP >3000 Ci/mmol (NEN); [γ-^{32}P]ATP >7000 Ci/mmol (NEN); Ribonuclease A and Ribonuclease T1 (Boehringer Mannheim); Terminal deoxynucleotidyl transferase (Ratliff Biochemicals, Los Alamos, NM); Exonuclease VII (BRL); Deoxyribonuclease I (Worthington Biochemicals). Restriction endonucleases were purchased from either New England BioLabs or Boehringer Mannheim and were used according to the manufacturer's instructions. T4 polynucleotide kinase was purchased from BRL and was used according to the manufacturer's instructions.

Buffers

Buffers used included: *Transcription buffer* (70 mM Tris-OAc pH 8.0, 20% (v/v) Glycerol, 60 mM NH_4Cl, 6 mM MgOAc, 5 mM Spermidine, 0.15 mM dithiothreitol); *DNase I Diluent* (transcription buffer with 10 mM $CaCl_2$, 10 μg/ml acetylated BSA); *DNase I Stop Load Buffer* (3.5 mg/ml sonicated salmon sperm DNA, 50 mM EGTA, 0.1% xylene cyanol, 0.1% bromophenol blue); *RNA/DNA elution buffer* (10 mM Tris Cl pH 7.8, 2 mM EDTA, 0.1% SDS); *TBE buffer* (89 mM Tris base, 89 mM boric acid, 2.5 mM EDTA); *Formamide load buffer* (80% deionized formamide, 1× TBE, 0.1% xylene cyanol, 0.1% bromophenol blue); *Urea load buffer* (7 M Urea, 10 mM EDTA, 0.5% SDS, 0.05% xylene cyanol, 0.05% bromophenol blue).

DNA Template

The template pCpGR220 with a 5′ end labelled non-template strand was prepared from the plasmid pGR220 (21) in the following manner. The plasmid pGR220 was digested with SmaI and the 5′ phosphates were removed with calf intestinal phosphatase. For DNase I footprinting, the 5′ end of the non-template strand was ^{32}P labelled using [γ-^{32}P]ATP and T4 polynucleotide kinase. Unincorporated [γ-^{32}P]ATP was removed by centrifugal gel filtration using Sephadex G-50 (18) followed by one phenol:chloroform (1:1) extraction and ethanol precipitation. An extension of deoxycytidylate residues was added to the 3′ ends of the template using terminal deoxynucleotidyl transferase (18). The template, pCpGR220, was digested with XbaI to remove the end not adjoining the transcribed sequence. A second digestion with Asp718 generates a fragment of suitable size for DNase I footprinting. This fragment is termed pCpGR220*S when it contains the ^{32}P end label or pCpGR220S when not labelled.

The digestion with SmaI is essential prior to the addition of the 3′ extensions which direct initiation by the polymerase. The sequence at the site of initiation produced by this restriction enzyme permits the polymerase to engage in 'normal elongation,' displacing its nascent transcript and reannealing the duplex DNA during transcription (22). If 3′ extensions are added to ends produced by most other restriction enzymes tested (22, and unpublished results), the polymerase does not displace its transcript. Instead, a long RNA:DNA hybrid is formed during transcription and the non-template strand is displaced (22). These aberrant elongation reactions do form stable ternary elongation complexes, but their relationship to complexes which form during normal elongation is questionable. The necessity of using SmaI limits the type of manipulation that can be done with the template as discussed below in 'Footprinting the Template Strand'.

Preparation and walking of ternary complexes

Homogeneous populations of ternary complexes paused at specific sites along the DNA template were formed by a modification of the RNA polymerase walking procedure developed by Levin *et al.* (1). The enzyme to DNA ratio was empirically determined to optimize complex formation. The molar concentrations used are 1.2 nM RNA polymerase II and 10 nM template (in molecules). For DNase I footprinting stable ternary complexes with a transcript of 135nt (G135) were prepared by incubating RNA polymerase II with pCpGR220*S for 5 min at 37°C in transcription buffer containing 800 μM ATP and GTP and 100 μM CTP. This allowed the formation of a 135nt transcript blocked from further elongation by lack of UTP. The unincorporated NTPs are removed by passage of the ternary complexes through three consecutive Sephadex G-50 gel filtration spin columns (1). G135 complexes were walked to U138 by the addition of 10 μM UTP or to G143 by the addition of 10 μM each of UTP, CTP, and GTP and incubated at 37°C for 1 min. Simultaneously, stable ternary complexes were prepared as controls with labelled RNA transcripts on the template pCpGR220S with a substitution of [α-^{32}P]CTP for the 100 μM CTP. The walks were completed in the same manner described above. Transcripts were analyzed by polyacrylamide gel electrophoresis (PAGE) on a denaturing 6% gel (30:0.8, acrylamide:bisacrylamide, 7 M urea, 0.8 mm thick, 25 cm long) in 1×TBE (89 mM Tris base, 89 mM boric acid, 2.5 mM EDTA).

DNase I footprinting of ternary complexes

The separately formed and isolated elongation intermediates G135, U138 and G143 were footprinted with DNase I. First the complexes were digested with exonuclease VII to remove the 3′ dC extensions and then with DNAse I to generate a footprint. The complexes were separated from the free DNA by nondenaturing PAGE and the bands were visualized by autoradiography. The complexed DNA was eluted from gel slices and analyzed by denaturing PAGE. The ternary complexes were formed on pCpGR220*S as described above in 200 μl reaction volumes; 15 U of exonuclease VII were added to the reactions after 2 min and incubation was continued an additional 3 min to allow the removal of the 3′ dC extensions. DNase I was added to complexes halted at G135, U138 or G143 to a final concentration of 0.001 μg/ml and the reactions were incubated for 1.5 min at 30°C. The digestion was stopped by the addition of 1/10th volume of DNase I Stop/Load buffer and the samples were immediately loaded onto 4% nondenaturing acrylamide gels (30:0.8 acrylamide:bisacrylamide, 0.7 mm thick, 23 cm long) in 1× TBE. Due to the large volume of the reaction, ~75 ml aliquots were loaded into each of several 2 cm wide wells. Electrophoresis was carried out at room temperature with 220 volts until the xylene cyanol had migrated 8 cm from the well (2.5–3 hrs). This electrophoresis step effectively separates the ternary complexes from the free DNA (1, 23).

As a control for complex purity, as assessed by transcript homogeneity, each experiment was conducted in parallel with

ternary complexes formed on pCpGR220S, as described above. These ternary complexes containing ^{32}P labelled transcripts were subjected to the same treatments as the 5′ end labelled pCpGR220*S ternary complexes, except that they were not digested with DNase I.

The complexes, both DNA labelled and RNA labelled, were identified by autoradiography and eluted from gel slices in 200 μl RNA/DNA elution buffer. The nucleic acids were precipitated with ethanol and resuspended in formamide loading buffer. Immediately before loading onto the gel, the samples were heated for 3 min at 93°C and chilled on ice. Electrophoresis was carried out in 6% acrylamide gels (19:1 acrylamide:bisacrylamide, 0.3 mm thick, 40 cm long) containing 7 M Urea in 1× TBE at 1700 volts. Gels were dried onto 3 MM Whatman paper and were exposed to film with intensifying screens at −70°C.

Chemical sequencing ladders were prepared by the methods of Maxam and Gilbert (24).

Footprinting the template strand

The technical aspects of preparing an effective unidirectional 3′ extended template have so far precluded footprinting of the template strand. Such footprints require specific labelling of the template strand as well as formation and isolation of ternary complexes containing RNA polymerase molecules positioned at specific locations. Templates were prepared which were first digested with either Asp 718 or EcoO105 and then labelled with polynucleotide kinase and [γ-^{32}P]ATP prior to the SmaI digestion described above. The kinase reaction labelled both the template and non-template strands. Following SmaI digestion, which removed the ^{32}P from the non-template strand, 3′ extensions were added to both ends. In order to retain label on the 5′ end of the template strand, such prepared templates necessarily contained 3′ extensions at both ends. RNA polymerase II molecules can bind to such 3′ extension:duplex junctions and initiate transcription. Nucleotide deprivation (the absence of UTP) would be predicted to prevent the formation of stable ternary complexes containing long transcripts originating from these labelled ends. However, any initiation from these ends would be expected to generate transcripts hybridized to the DNA. Multiple complexes were always observed in nondenaturing PAGE following ternary elongation complex formation with template-strand labelled DNA templates. None of these complexes gave a reproducible footprint with DNase I.

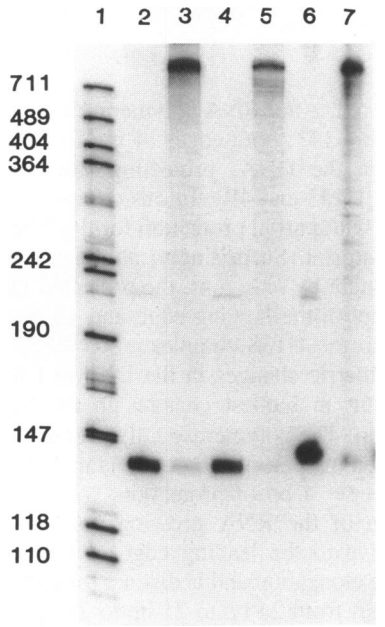

Figure 1. Preparation and analysis of halted elongation complexes G135, U138, and G143. Halted elongation complexes were formed on pCpGR220 and transcripts were analyzed on a denaturing polyacrylamide gel as described in Methods. The activity of the halted elongation complexes was determined by addition of NTPs to 800μM to allow elongation to resume. Lane 1: DNA size markers; lane 2: RNA from a G135 complex labelled with α[^{32}P]CTP; lane 3: RNA after elongation of the G135 complex; lane 4: RNA from a U138 complex 3′ end labelled with α[^{32}P]UTP; lane 5: RNA after elongation of the U138 complex; lane 6: RNA from a G143 complex 3′ end labelled with a[^{32}P]GTP; lane 7: RNA after elongation of the G143 complex.

RESULTS

Rationale

The objective of these studies was to obtain structural information concerning purified RNA polymerase II and how it contacts the DNA template in a normal elongation complex. We previously reported that transcripts in a single specific elongation complex were apparently single stranded to within at least 3 nt of the growing point (21). In this study we have analyzed additional elongation complexes by employing the same synthetic DNA template on which purified RNA polymerase II initiates efficiently and can then be halted at specific sites for lack of a particular NTP substrate. Each of the elongation complexes described is homogeneous and remains stable and active for the duration of the analysis, and thus we assume that these halted complexes represent actual intermediates in the normal elongation process.

Formation and analysis of elongation complexes

The template pCpGR220 was utilized to form elongation complexes halted at several defined sites. By performing transcription with subsets of ribonucleotides (see Methods), we isolated specific elongation complexes containing RNA transcripts of either 135 nt, 138 nt, or 143 nt (Fig. 1). These elongation complexes are termed G135, U138, and G143 respectively. Analysis of the RNAs in each of these complexes shows that they are quite pure, and the majority (90%) of the complexes are active as shown by their ability to continue elongation (Fig. 1).

Analysis of gel isolated elongation complexes

Non-denaturing polyacrylamide gel electrophoresis was used to separate each elongation complex from free DNA prior to analysis of the contacts between RNA polymerase II and the DNA template. Elongation complexes with either the RNA or the DNA labelled were electrophoresed on non-denaturing gels, and the positions of the complexes labelled in either the RNA or the DNA were coincident (Fig. 2). Complexes A and B contained identical RNA products; the migration difference is likely due to the binding of exonuclease VII to a fraction of the complexes. This enzyme was used to remove the 3′ extension from the template following transcription. When other methods were used to remove the extension, only one complex was observed on these gels.

The band containing the A complexes was excised and the RNA components were analyzed by denaturing PAGE (Fig. 3). The RNA was homogeneous for each complex with no contamination

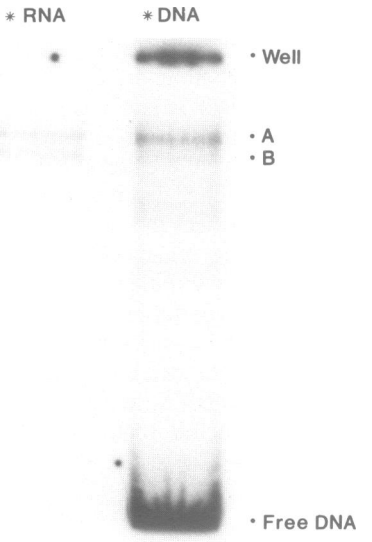

Figure 2. Isolation of halted elongation complexes by non-denaturing PAGE. The complexes were formed as described in Methods on either pCpGR220S, for labelling the RNA, or pCpGR220*S, to form DNA labelled complexes. The example shown is a G135 complex. The position of the wells, complexes (A & B), and the free DNA are indicated.

Figure 3. Analysis of RNA transcripts excised from non-denaturing gels. The upper band for each complex as visualized in Figure 2 was excised from the non-denaturing gel and the RNA eluted as described in Methods. Eluted RNAs were electrophoresed on a denaturing 6% polyacrylamide gel. The complex from which the RNA was eluted is indicated above the autoradiogram. All transcripts are of the expected sizes.

from the G135 complex in either the U138 or G143 complexes. The homogeneity of these RNA transcripts demonstrates that structural studies on elongation complexes isolated in this manner will reflect the structure of only a single complex.

DNAse I footprinting of the non-template strand of elongation complexes

Using the three individual and separate elongation complexes that were shown to be stable, active, and isolable in homogeneous form, we probed the polymerase-DNA contacts with DNAse I (Fig. 4). The DNAse I footprints of the three separate complexes were distinctly different in several respects. Surprisingly, the upstream and downstream boundaries of RNA polymerase II did not move in concert relative to the template as the RNA increased in length. Instead, the region of the template protected by RNA polymerase II increases upstream on the non-template strand when the polymerase elongates downstream from G135 to U138 (Fig. 4A, lanes G135 and U138). The footprint then moves significantly downstream, with both the upstream and downstream boundaries of the polymerase advancing, when the polymerase transcribes from U138 to G143 (Fig. 4A, lanes U138 and G143).

In the G135 complex, the DNA template is protected from +113 to +161, a total of 48 bp, with the 3 bp on each edge of this region being only partially protected (Fig. 4A, lane G135, and 4B). When the polymerase adds 3 additional nucleotides as it transcribes to position U138, the protected region of the DNA moves backwards relative to the G135 complex (Fig. 4A lane U138 and 4B), giving a total of 55 bp protection. On the upstream edge, this protection is only partial but distinct from +104 to +116, while on the downstream edge the footprint definitely protects two less bps to +159 even while the final 3bp are only partially protected. After RNA polymerase II elongates a further 5 nt to position G143, both edges of the footprint have moved downstream on the DNA, protecting from +120 to +171 (Fig. 4A, lane G143 and 4B). In this complex, the polymerase protects 51 bp with partial protection for the 7 bp on the leading edge of the footprint. Surprisingly, although the transcript has increased in length by only 5 nt, the protected DNA region has advanced 16 bp on the lagging edge and 12 bp on the leading edge relative to the U138 complex.

These asymmetric changes in the DNAse I footprint during elongation result in modest changes in the total size of the protected region. The polymerase fully protects 42+/− 2 bp of the DNA. When the partial protection is included, this distance extends to 51+/− 4 bp of protection.

The position of the RNA growing point within the DNA footprint relative to the leading edge of the polymerase also changes during elongation and is distinct for each complex. This distance changes from 26 bp to 21 bp to 28 bp respectively for the G135, U138, and G143 complexes (Fig. 4B). These results also suggest that the ternary complex is a dynamically changing structure of multiple conformations.

DISCUSSION

We have used specifically halted elongation complexes of RNA polymerase II to investigate its interactions with the nascent transcript and the template DNA. By monitoring the stability and activity of the halted elongation complexes, we were able to

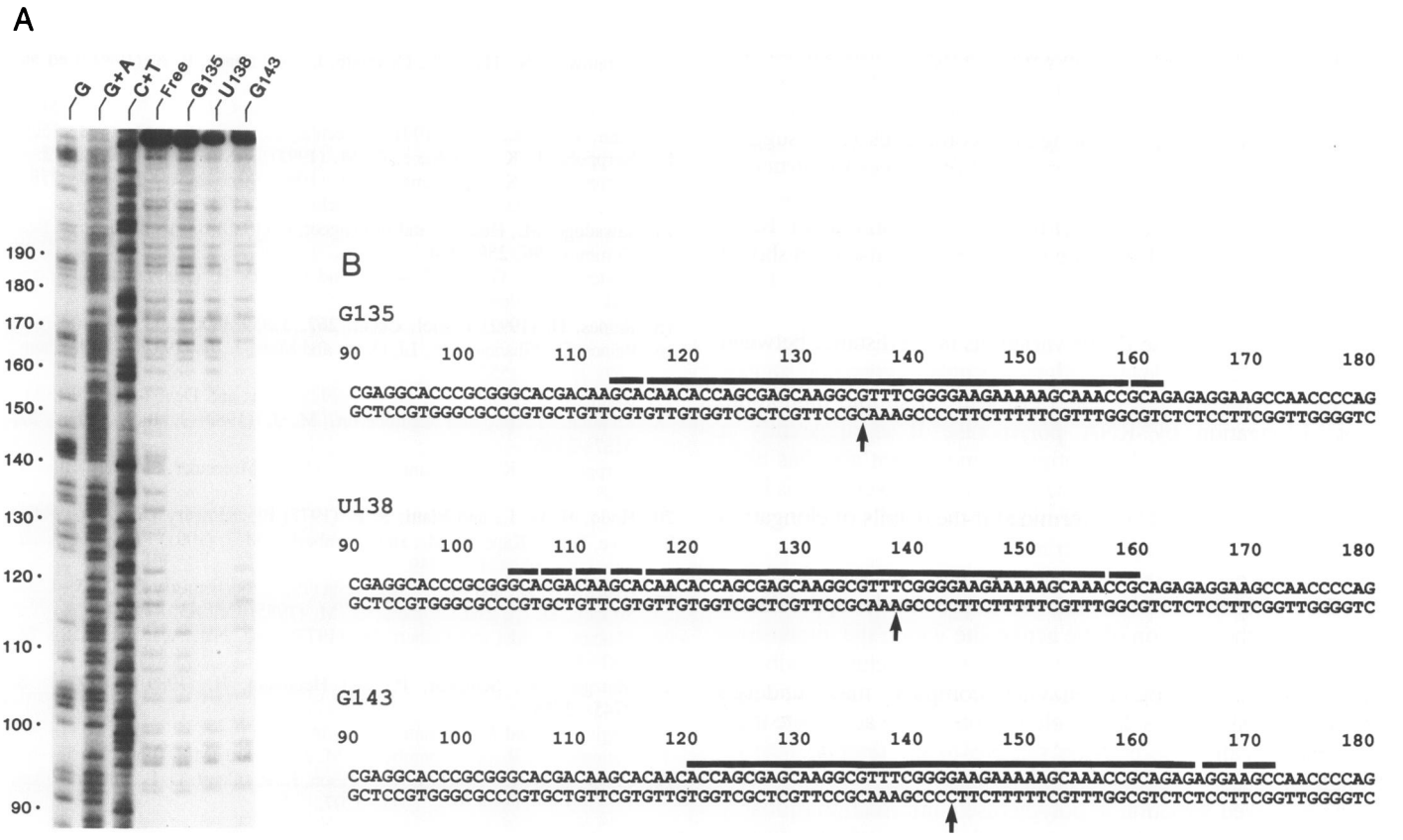

Figure 4. DNAse I footprinting on the non-template strand of the halted elongation complexes. **(A)** Complexes were formed on pCpGR220*S, exonuclease VII treated, DNAse I treated, isolated by non-denaturing PAGE, excised and eluted. The DNA was analyzed on a denaturing 6% polyacrylamide gel. Lanes G, G+A, and C+T: chemical sequencing reactions. Lane Free: DNAse I treated pCpGR220*S in the absence of RNA polymerase II. The digestion patterns for the halted elongation complexes G135, U138, and G143 are indicated. DNAse I cleavage positions are numbered relative to the 5′ end of the non-template strand. **(B)** Diagrammatic representation of the protected regions of the non-template strand. The position of the 3′ end of the transcript is indicated by an arrow. The isolation and footprinting of the three separate complexes was performed two times with identical results.

confirm that they have the properties expected of normal elongation intermediates. Thus the polymerase-DNA interactions reported here should reflect structures of the elongating enzyme. It is possible that once halted, the conformation of the polymerase changes away from its native state. However, each of the complexes does remain active throughout the analysis, and thus we conclude that the active enzyme is not constrained to a single conformation.

These results are the first reported DNAse I footprints of elongation complexes containing purified RNA polymerase II, and they show that the polymerase changes its contacts with the DNA template during elongation. Among the three complexes examined, several features of the footprint were distinctly different: the size of the footprint varied, the position of the 3′ growing point of the RNA relative to the leading edge of the footprint varied, and the position of the protected regions did not change in concert with the addition of nucleotides to the lengthening transcript. The asymmetric changes in the footprint were seen both upstream and downstream of the active site of polymerization.

These results are not dependent on initiation from a 3′ extended template. An earlier study examined two elongation complexes that were formed by promoter specific initiation in nuclear extracts and then isolated (9). These two complexes also were significantly different in the size of the protected region following DNAse I digestion. Further, the two complexes also contained a variable distance between the leading edge of the footprint and the active site of the polymerase (9). These results with nuclear extracts suggest that the footprint variations reported here with the purified enzyme are an accurate reflection of what occurs during elongation regardless of whether the enzyme has initiated from a promoter or a 3′ extended template.

These results share some similarities and differences from the results seen for *E.coli* RNA polymerase. The similarities lie in the flexibility of the structure of the elongation complexes when positioned at different sequences along the template (2−4). However, unlike what is seen in these complexes formed with RNA polymerase II, footprints of sequentially positioned bacterial elongation complexes maintain a fixed downstream boundary as elongation continues although the upstream boundary compresses (2−4). Both boundaries change for the bacterial polymerase at periodic positions along the template. Too few complexes have been examined with RNA polymerase II to evaluate whether or not there is a periodic change in structure. However, the leading edge of the footprints with the bacterial polymerase has not yet been observed to retreat upstream as the transcript increases in length.

Template sequences probably directly influence the structure of the elongating complex. In a separate series of experiments (25), elongation complexes of *E.coli* RNA polymerase were

studied which were positioned over identical template sequences although each complex contained a transcript of different length. In this case, the DNAse I footprints appeared identical for the individual complexes. These results, and similar results with vaccinia RNA polymerase elongation complexes (26), suggest that the template sequence (or structure) influences the structure of the polymerase during elongation. Such template influences are important in intrinsic termination by mammalian RNA polymerase II (11), and template sequences have also been shown to be influential in regulating termination by *E.coli* RNA polymerase (27, 28).

For RNA polymerase II, the variations in the distance between the active site and the leading edge of template protection suggest that the polymerase does not translocate in simple monotonic steps. Elongation by RNA polymerase II is apparently a discontinuous process. Discontinuous movement also has been reported for *E.coli* RNA polymerase (2). However, at this level of resolution, it cannot be determined if the details of elongation are the same for both polymerases.

Discontinuous elongation indicates far more stuctural flexibility of RNA polymerase than has been previously appreciated. Variation in the location of the active site within the dimensions of the footprint suggests at least two forms of structural flexibility. The entire multisubunit enzyme complex may undergo conformational changes during elongation, or the active site itself may move within the enzyme relative to the template during elongation.

The observed variation in polymerase conformation probably reflects a basic element of transcript elongation. A structurally flexible polymerase raises many possibilities for transcription regulation. Specifically during elongation when the enzyme reaches a site that signals it to stop, the polymerase may take on an alternate conformation that is stable yet incapable of further elongation. A factor that could alter this conformation might alleviate the elongation block and allow continuation of transcription. SII protein promotes such readthrough presumably by interacting with stalled elongation complexes to promote cleavage of the nascent transcript prior to the resumption of elongation (15–17). Alternatively, polymerases blocked during elongation might be acted upon by termination factors which promote RNA release perhaps by modifying the conformation of a putative transcript binding site on the polymerase (21). Clearly the distinctive structures assumed by the ternary complex at different positions along the template during transcription might make the complexes more or less vulnerable to specific types of factors. Structural analyses of many elongation complexes positioned at naturally occurring pauses as well as in the readily transcribed sequences described here might highlight important features involved in the elongation control of such complexes.

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REFERENCES

1. Levin, J. R., Krummel, B., and Chamberlin, M. J. (1987) J. Mol. Biol. **196**, 85–100.
2. Krummel, B., and Chamberlin, M. J. (1989) Biochemistry **28**, 7829–7842.
3. Krummel, B., and Chamberlin, M. J. (1992) J. Mol. Biol. **225**, 221–237.
4. Krummel, B., and Chamberlin, M. J. (1992) J. Mol. Biol. **225**, 239–250.
5. Cai, H., and Luse, D. S. (1987) J. Biol. Chem. **262**, 298–304.
6. Van Dyke, M. W., Roeder, R. G., and Sawadogo, M. (1988) Science **241**, 1335–1338.
7. Buratowski, S., Hahn, S., Guarente, L., and Sharp, P. A. (1989) Cell **56**, 549–561.
8. Cai, H., and Luse, D. S. (1987) Molecular and Cell. Biol. **7**, 3371–3379.
9. Linn, and Luse, D. S. (1991) Molecular and Cell. Biol. **11**, 1508–1522.
10. Kerppola, T. K., and Kane, C. M. (1991) FASEB J. **5**, 2833–2842.
11. Kerppola, T. K., and Kane, C. M. (1990) Biochemistry **29**, 269–278.
12. Natori, S. (1982) Molec. Cell. Biochem. **46**, 173–187.
13. Sawadogo, M., Huet, J., and Fromageot, P. (1980) Biochem. Biophys. Res. Commun. **96**, 258–264.
14. Sluder, A. E., Greenleaf, A. L., and Price D. H. (1989) J. Biol. Chem. **264**, 8963–8969.
15. Reines, D. (1992) J. Biol. Chem. **267**, 3795–3800.
16. Reines, D., Ghanouni, P., Li, Q.-q., and Mote, J., Jr. (1992) J. Biol. Chem. **267**, 15516–15522.
17. Izban, M. G., and Luse, D. S. (1992) Genes and Dev. **6**, 1342–1356.
18. Kadesch, T. R., and Chamberlin, M. J. (1982) J. Biol. Chem. **27**, 3187–3196.
19. Kerppola, T. K., and Kane, C. M. (1988) Molecular and Cell. Biol. **8**, 4389–4394.
20. Hodo, H. G. I., and Blatti, S. P. (1977) Biochemistry **16**, 2334–2343.
21. Rice, G. A., Kane, C. M., and Chamberlin, M. J. (1991) Proc. Nat. Acad. Sci. USA **88**, 4245–4249.
22. Dedrick, R. L., and M. J. Chamberlin (1985) Biochemistry **24**, 2245–2253.
23. Straney, D. C., and Crothers, D. M. (1985) Cell **43**, 449–459.
24. Maxam, A. M., and Gilbert, W. (1977) Proc. Nat. Acad. Sci. USA **74**, 560–564.
25. Metzger, W., Schickor, P., and Heumann, H. (1989) EMBO J. **8**, 2745–2754.
26. Hagler, J., and S. Shuman (1992), in press.
27. Reynolds, R. R., and Chamberlin, M. J. (1991) J. Mol. Biol. **224**, 53–63.
28. Cheng, S. W. C., Lynch, E. C., Leason, K. R., Court, D. L., and Friedman, D. (1991) Science **254**, 1205–1207.