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Communication

Elongation Factor SII Contacts the 3′-End of RNA in the RNA Polymerase II Elongation Complex*

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Elongation factor SII (also known as TFIIS) is a RNA polymerase II binding protein that allows bypass of template arrest sites by activating a nascent RNA cleavage reaction. Here we show that SII contacts the 3′-end of nascent RNA within an RNA polymerase II elongation complex as detected by photoaffinity labeling. Photocross-linking was dependent upon the presence of SII, incorporation of 4-thio-UMP into RNA, and irradiation and was sensitive to treatment by RNase and proteinase. A transcriptionally active mutant of SII lacking the first 130 amino acids was also cross-linked to the nascent RNA, but SII from Saccharomyces cerevisiae, which is inactive in concert with mammalian RNA polymerase II, failed to become photoaffinity labeled. SII-RNA contact was not detected after a labeled oligoribonucleotide was released from the complex by nascent RNA cleavage, demonstrating that this interaction takes place between elongation complex-associated but not free RNA. This shows that the 3′-end of RNA is near the SII binding site on RNA polymerase II and suggests that SII may activate the intrinsic RNA hydrolysis activity by positioning the transcript in the enzyme’s active site.

RNA polymerase II in the arrested conformation cannot extend an RNA chain in the presence of all four NTPs. Arrested complexes retain transcription potential, however, since they can be reactivated for RNA chain extension by elongation factor SII. SII can bind RNA polymerase II in vitro, and SII binding is thought to be the activating event for a ribonuclease activity within RNA polymerase II (reviewed in Ref. 1). This ribonuclease removes a small number of nucleotides from the 3′-end of the nascent RNA prior to its re-extension through the blockage (2–4) in a reiterative cleavage and resynthesis process, allowing an RNA polymerase II molecule multiple attempts at chain extension through an obstacle (1, 5, 6).

Genetic evidence suggests that SII has an in vivo role related to RNA polymerase II function which involves the binding of SII to RNA polymerase II’s largest subunit (7, 8). Although SII binds to purified RNA polymerase II, it has been difficult to isolate elongation complex-associated SII, presumably because the lifetime of the interaction is very short (9, 10).

Full-length SII does not bind nucleic acids at physiological ionic strength (11). Specific internal and extensive N-terminal deletion mutants of SII bind DNA, RNA, and DNA:RNA hybrids in gel mobility shift assays, suggesting that cryptic nucleic acid binding activity is a property of SII which may be revealed only after SII associates with an elongation complex (11). However, the functional significance of SII’s nucleic acid binding activity in the context of RNA cleavage and the stimulation of elongation is unknown.

Contacts between RNA and the largest two RNA polymerase II subunits have been elucidated by cross-linking radiolabeled RNAs synthesized in the presence of photoactive nucleotide analogues (12–15). Similarly, the α and β subunits of Escherichia coli RNA polymerase can be cross-linked to nascent RNA (see Ref. 16 and references therein).

Since full-length SII may bind nucleic acids preferentially in the context of an elongation complex, we set up a system to trap SII on the complex and detect an interaction between SII and the nascent RNA. We report here that full-length SII, but not transcriptionally inactive SII, can be cross-linked to the nascent RNA via a photoactive nucleotide situated at the 3′-end of the transcript. This contact may be important in the elongation function of SII.

MATERIALS AND METHODS

Proteins and Reagents—RNA polymerase II and basal transcription factors were isolated from rat liver (17) or purified from E. coli (for TFIIB) (18) as described. Recombinant human and yeast SII were expressed from pT7–Met (provided by Dr. C. Kane, University of California, Berkeley) and pYSE1 (provided by Dr. S. Natori, Tokyo University), respectively, and purified as described (19). ΔSII was expressed from pET22b (+) /ΔTFIIS (provided by Dr. C. Kane, University of California, Berkeley) and purified by nickel affinity chromatography as described (20).

Fast protein liquid chromatography-purified nucleoside triphosphates were purchased from Pharmacia Biotech Inc. [α-32P]CTP was obtained from Amersham Corp. RNase A and T1 were obtained from United States Biochemical Corp. Proteinase K was from Promega (Madison, WI).

4-Thio-UTP was synthesized and purified by DEAE-cellulose or Hi-Trap Q (Pharmacia) chromatography as described (12).

3′-End Labeling of RNA and Incorporation of 4-Thio-UMP in the Elongation Complex—Transcription was reconstituted with partially purified initiation factors and RNA polymerase II on pAdTerm-2 as described (21). Ternary complexes (approximately 25 fmol/reaction) containing a 14-nucleotide transcript were synthesized in the presence of 7 mM MgCl2 and ATP, UTP, and CTP (20 μM each). Transcripts were extended by adding heparin to 10 μg/ml and all four NTPs to 800 μM (each). After 20 min, elongation complexes were immunoprecipitated with anti-RNA IgG (D44) and fixed to nitrocellulose. 4-Thio-UTP was incorporated and purified with DEAE-cellulose or Hi-Trap Q (Pharmacia) chromatography as described (12).

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RESULTS AND DISCUSSION

Incorporation of 4-Thio-UMP by Rat Liver RNA Polymerase II—The template used in these reactions contains the core adenovirus major late promoter and a segment of the human histone H3.3 gene containing an intrinsic arrest site called Ia. Transcription in the absence of SII results in approximately 10% read-through of site Ia (Fig. 2, lane 3). When SII was added to the supercomplex, 50–70% of site Ia RNA were extended to G200 with a single 32P atom in the transcript between bases 198 and 199 (Fig. 1, A, lane 3 versus lane 5). The decrease in read-through efficiency could result from slower addition of nucleotides to the 4-thio-UMP-containing 3′-end of the nascent transcript and/or an arrest site in the nascent transcript. Site Ia RNA—4-thio-UMP-containing transcripts are shown cross-linked with proteins in the absence of added SII. Complexes in lanes 3 and 4 were treated with RNase A (10 μg) and proteinase K (PRK, 20 μg), respectively, following UV irradiation. Complexes in lane 5 were treated as those in lane 1 but were not irradiated with UV. Complexes in lane 6 contained RNA synthesized using UTP rather than 4-thio-UTP (4SU). Positions of prestained molecular mass markers (Bio-Rad): myosin (203 kDa), β-galactosidase (118 kDa), bovine serum albumin (86 kDa), ovalbumin (51.6 kDa), carbonic anhydrase (34.1 kDa), soybean trypsin inhibitor (29 kDa), and lysozyme (19.2 kDa) are indicated at left. Positions of individual cross-linked polypeptides and free RNA are indicated at right.

4-Thio-UMP-containing transcripts differing in length by one nucleotide could be extended to G200 with a single 32P atom in the transcript between bases 198 and 199 (Fig. 1, A, lane 3 versus lane 5). The decrease in read-through efficiency could result from slower addition of nucleotides to the 4-thio-UMP-containing 3′-end of the nascent transcript and/or an arrest site in the nascent transcript. Site Ia RNA—4-thio-UMP-containing transcripts are shown cross-linked with proteins in the absence of added SII. Complexes in lanes 3 and 4 were treated with RNase A (10 μg) and proteinase K (PRK, 20 μg), respectively, following UV irradiation. Complexes in lane 5 were treated as those in lane 1 but were not irradiated with UV. Complexes in lane 6 contained RNA synthesized using UTP rather than 4-thio-UTP (4SU). Positions of prestained molecular mass markers (Bio-Rad): myosin (203 kDa), β-galactosidase (118 kDa), bovine serum albumin (86 kDa), ovalbumin (51.6 kDa), carbonic anhydrase (34.1 kDa), soybean trypsin inhibitor (29 kDa), and lysozyme (19.2 kDa) are indicated at left. Positions of individual cross-linked polypeptides and free RNA are indicated at right.

**Fig. 1.** Position of 4-thio-UMP in nascent transcript. A, sequence of the 3′-end of Ia RNA. Site of SII-activated cleavage is indicated (arrow). *indicates the position of the 32P label. ′ indicates 3′-ends of Ia-arrested RNAs (positions 205, 206, and 207). Positions of 4-thio-U are underlined (positions 201–205). B, autoradiograph of RNAs. [α-32P]CTP-labeled G200 complexes (lane 1) were extended with 100 μM UTP (lanes 2 and 3) or 100 μM 4-thio-UTP (lanes 4 and 5). Complexes in lanes 3 and 5 received an additional 10 min of incubation following the addition of 800 μM each of ATP, UTP, GTP, and CTP. The position of the 260-nucleotide marker RNA is indicated at left. Positions of different RNAs are indicated at right. RO, runoff.

**Fig. 2.** Photoaffinity labeling of SII and RNA polymerase II polypeptides. RNA polymerase II elongation complexes bearing 32P-containing RNA were assembled, labeled, and irradiated as described in the text in the presence (lanes 1 and 3–6) or absence (lane 2) of recombinant human SII (TSK-phenyl-5PW, 1.25 μg). Proteins were denatured and resolved on an 8% SDS-polyacrylamide gel. Lane 2 shows cross-linked polypeptides in the absence of added SII. Complexes in lanes 3 and 4 were treated with RNase A (10 μg) and proteinase K (PRK, 20 μg), respectively, following UV irradiation. Complexes in lane 5 were treated as those in lane 1 but were not irradiated with UV. Complexes in lane 6 contained RNA synthesized using UTP rather than 4-thio-UTP (4SU). Positions of prestained molecular mass markers (Bio-Rad): myosin (203 kDa), β-galactosidase (118 kDa), bovine serum albumin (86 kDa), ovalbumin (51.6 kDa), carbonic anhydrase (34.1 kDa), soybean trypsin inhibitor (29 kDa), and lysozyme (19.2 kDa) are indicated at left. Positions of individual cross-linked polypeptides and free RNA are indicated at right.
In summary, SII-RNA cross-linking was containing RNA, UV irradiation failed to produce photoaffinity-basal transcription factors were mixed with 4-thio-UMP-con- crossed RNA (Fig. 2, excess) (Fig. 3, polypeptide of identical mobility cross-linked with comparable chromatography to apparent homogeneity (4). We found that a poly peptide, which was unable to activate RNA cleavage in the mammalian RNA polymerase II complex, 1 was added, no SII cross-linking was observed, as was the case for a buffer-only control (Fig. 3, lane 3). We further confirmed the identity of the putative SII polypeptide by testing as a substrate a smaller version of SII lacking the first 130 amino acid residues (ΔSII). This deletion mutant can activate nascent RNA cleavage and stimulate read-through of the Ia site (20). This truncated form of the protein cross-linked to the nascent RNA, and the protein: RNA conjugate displayed a correspondingly faster electrophoretic mobility (27 kDa, including the mass of the RNA) on an SDS-polyacrylamide gel (Fig. 4, lane 1 versus lane 3). ΔSII was cross-linked to RNA with approximately one-half the efficiency of the full-length molecule.

Current models for SII function suggest that this elongation factor binds the RNA polymerase II complex and activates nascent RNA cleavage and arrest site read-through in a Mg2+-dependent fashion. Thereafter, SII is probably released from the complex (9, 10). Here, we have trapped SII on the elongation complex by withholding MgCl2, thereby preventing the cleavage and removal of the 32P- and 4-thio-UMP-containing 3′-end of the nascent RNA. Under cleavage conditions, i.e. when Mg2+ was included in the binding reaction prior to UV irradiation, photoaffinity labeling of RNA polymerase II subunits decreased by greater than 90%, and photoaffinity labeling of SII was no longer observed (Fig. 4, lane 4). Thus, the detection of the RNA:SII contact is a function of the presence of the RNA in an elongation complex prior to its removal by the cleavage reaction and again demonstrates the specificity of cross-linking.

To our knowledge, this is the first report of the direct detection of a biochemical interaction between full-length SII and RNA or a specific, active RNA polymerase II elongation complex. Earlier studies of RNA contacts with RNA polymerase II subunits employed a similar technique with HeLa nuclear extracts, which presumably contained SII (e.g. Ref. 12). The failure to detect this interaction could be related to the low efficiency of SII cross-linking, the low in vivo abundance of SII compared with these reactions, the presence of Mg2+ in the reaction, and/or the more random placement of 4-thio-UMP nucleotides at positions distant from the 3′-end of the molecule. The experiments presented here were also performed using RNA polymerase II complexes located at or near an arrest site at which SII-activated cleavage is favored over most template

1 W. Powell, data not shown.
positions.

GreA (24) and GreB (25) are structurally distinct elongation factors from E. coli that are functionally analogous to SII. Both factors promote read-through of arrest sites by RNA polymerase and stimulate nascent RNA cleavage by the enzyme (25). Like SII, GreB relieves the arrested condition by inducing cleavage of large oligonucleotides from nascent RNA (25). GreA functions differently since it must be present before arrest to stimulate read-through and the RNA is shortened by a dinucleotide increment (25). Similar to what we observe here, GreA has recently been shown to contact the nascent transcript in an E. coli elongation complex (26).

The contact of SII and RNA in the RNA polymerase II elongation complex is likely to be functionally important because: 1) it is specific for complex-associated RNA, 2) GreA makes a similar contact in the E. coli RNA polymerase complex, and 3) previous work using SII fragments suggests the existence of a cryptic nucleic acid binding capability in the full-length molecule (11). The close proximity or perhaps identity of the active site for SII-activated RNA cleavage and that for nucleotide binding, SII may be directly involved in the positioning of the RNA within the active site before cleavage. This may help explain how SII dramatically enhances the rate of RNA polymerase II's RNA cleavage reaction. It is tempting to speculate that SII's contact with the region of the transcript to be removed by cleavage may also facilitate the removal of the oligonucleotide from the RNA product site. The possibility remains, however, that the contact between SII and the 3' end of the RNA detected here may simply reflect a close relationship between SII's docking site on RNA polymerase II and the enzyme's active site. Nonetheless, this work places SII in close proximity to the catalytic center of the enzyme, and it is an important step toward defining the geometry of the active site of elongating RNA polymerase II.

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