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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 an 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 photocopy of 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 blockade (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 β’ 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 pYS1 (provided by Dr. S. Natori, Tokyo University), respectively, and purified as described (19). SII 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 HiTrap 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 Staphylococcus aureus (21, 22) and washed three times with reaction buffer (60 mM KCl, 20 mM Tris-HCl, pH 7.9, 3 mM Hepes-NaOH, pH 7.9, 0.5 mM EDTA, 2% (w/v) polyvinyl alcohol, 0.2 mg/ml acetylated bovine serum albumin, 3% (v/v) glycerol). SII and MgCl₂ were added, and the reaction was incubated for 1.5 min at 28 °C. After washing three times with reaction buffer, [α-32P]CTP (20 μCi, 3000 Ci/mmol), 400 μM GTP, 100 μM UTP or 4-thio-UMP, and 7 mM MgCl₂ were added and incubated for 1 h at 28 °C. Reactions were stopped with SDS and treated with proteinase K. RNA was precipitated with ethanol and electrophoresed on 5% polyacrylamide, 50% urea gels.

SII Binding and Cross-linking—Elongation complexes bearing labeled RNA were washed three times and resuspended in cross-linking buffer (40 mM Tris-HCl, pH 7.9, 60 mM KCl, 0.5 mM EDTA). These complexes were liberated from S. aureus by digestion with ribonuclease
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 two largest subunits of RNA polymerase II as seen previously (2, 3). SII treatment results in full read-through of site Ia; Fig. 1A, lane 4 versus lane 2. Comparison with a ladder of UMP-containing transcripts differing in length by one nucleotide suggests that the 4-thio-UMP-containing transcripts are comprised primarily of RNAs of 203, 204, and 205 nucleotides in length, containing three, four, and five 4-thio-UMP residues, respectively (data not shown). 4-Thio-UMP-containing transcripts were also less effectively extended in the presence of all four natural NTPs (33 versus 10% read-through of site Ia; Fig. 1B, lane 3 versus lane 5). The decreased 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 increasing likelihood that these complexes become arrested.

Cross-linking of SII and RNA Polymerase II Subunits to the Nascent RNA—UV irradiation of these 4-thio-UMP-containing complexes resulted in the cross-linking of nascent RNA to the two largest subunits of RNA polymerase II as seen previously (2, Ref. 12, Fig. 2, lanes 1 and 2). When a fraction containing recombinant, full-length, human SII was allowed to bind the complexes in the absence of Mg2+ prior to irradiation, a polypeptide corresponding to the size of SII (48 kDa, including mass of cross-linked RNA) was also photoaffinity-labeled (Fig. 2, lane 1). Neither SII nor RNA polymerase II subunits were labeled if the complexes were not subjected to UV exposure (Fig. 2, lane 5). Similarly, when UTP was substituted for 4-thio-UTP in the reaction, photoaffinity labeling was not observed for either SII or RNA polymerase II subunits (Fig. 2, lane 6). The cross-linked products were sensitive to proteinase K (10 μg for

![Fig. 1. Position of 4-thio-UMP in nascent transcript.](image-url)

**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.

T1 (200 units/reaction) at 28 °C for 15 min, followed by centrifugation for 2 min at 4 °C to remove the cells. This treatment removed bases 1–180 from the nascent RNA (23). SII or ΔSII was added to the supernatant, and the reaction was incubated at 28 °C for 15 min. Reactions were transferred to a polystyrene microtiter plate (Corning), which was placed in a Pyrex dish filled with water and UV-illuminated for 30 min at 4 °C on a Photodyne model 3–3500 transilluminator (12). Proteins were precipitated in 10% trichloroacetic acid and electrophoresed on polyacrylamide-SDS gels as indicated. Gels were fixed in 0.56% (v/v) methanol, 1% (v/v) acetic acid, 10% (v/v) glycerol for 30 min, dried, and subjected to autoradiography and analysis on a Fuji phosphoimaging system.

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 half of the RNA polymerase II molecules becoming arrested at site Ia (positions 205, 206, and 207, where 1 is the transcription start site) (Fig. 1A; Ref. 5). SII treatment results in full read-through of site Ia. An intermediate in read-through is an elongation complex bearing an RNA shortened by 7–9 nucleotides at the 3′-end (5). When arrested complexes are washed free of NTPs, the shortened transcript and the cleaved oligonucleotide can be observed (4). We took advantage of this intermediate to incorporate a radioactive marker and a photoactive nucleotide analogue, 4-thio-UMP, into RNA. In the presence of [α-32P]CTP and GTP, complexes bearing the 198-base cleaved transcript could be extended to G200 with a single 32P atom in the transcript between bases 198 and 199 (Fig. 1, A and B, lane 1). In the presence of 100 μM UTP, the RNA is extended to positions U204, U205, and U206 (Fig. 1B, lane 2; Ref. 4). Although 4-thio-UTP can serve as a substrate in this reaction, it is utilized less efficiently than UTP and results in slightly shorter transcripts (Fig. 1B, lane 4 versus lane 2).
SII Contacts RNA in the RNA Polymerase II Complex

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 addition (27) suggests that the SII:RNA contact may be of some functional or architectural importance to the catalytic center of the RNA polymerase II elongation complex. In addition to a possible allosteric rearrangement of RNA polymerase II upon 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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