**Transcription Factors TFII F, ELL, and Elongin Negatively Regulate SII-induced Nascent Transcript Cleavage by Non-arrested RNA Polymerase II Elongation Intermediates**

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B. Jean Elmendorf‡§, A. Shilatifard¶, Qin Yan‡§, Joan Weliky Conaway‡§‡§‡‡, and Ronald C. Conaway‡§‡§

From the ‡Program in Molecular and Cell Biology, Oklahoma Medical Research Foundation, Oklahoma City, Oklahoma 73104, the ¶Department of Biochemistry and Molecu lar Biology, University of Oklahoma Health Sciences Center, Oklahoma City, Oklahoma 73190, the ‡Edward A. Doisy Department of Biochemistry, St. Louis University School of Medicine, St. Louis, Missouri 63104, and the **Howard Hughes Medical Institute, Oklahoma Medical Research Foundation, Oklahoma City, OK 73104

TFII F, ELL, and Elongin belong to a class of RNA polymerase II transcription factors that function similarly to activate the rate of elongation by suppressing transient pausing by polymerase at many sites along DNA templates. SII is a functionally distinct RNA polymerase II elongation factor that promotes elongation by reactivating arrested polymerase. Studies of the mechanism of SII action have shown (i) that arrest of RNA polymerase II results from irreversible displacement of the 3'-end of the nascent transcript from the polymerase catalytic site and (ii) that SII reactivates arrested polymerase by inducing endonucleolytic cleavage of the nascent transcript by the polymerase catalytic site thereby creating a new transcript 3'-end that is properly aligned with the catalytic site and can be extended. SII also induces nascent transcript cleavage by paused but non-arrested RNA polymerase II elongation intermediates, leading to the proposal that pausing may result from reversible displacement of the 3'-end of nascent transcripts from the polymerase catalytic site. On the basis of evidence consistent with the model that TFII F, ELL, and Elongin suppress pausing by preventing displacement of the 3'-end of the nascent transcript from the polymerase catalytic site, we investigated the possibility of cross-talk between SII and transcription factors TFII F, ELL, and Elongin. These studies led to the discovery that TFII F, ELL, and Elongin are all capable of inhibiting SII-induced nascent transcript cleavage by non-arrested RNA polymerase II elongation intermediates. Here we present these findings, which bring to light a novel activity associated with TFII F, ELL, and Elongin and suggest that these transcription factors may expedite elongation not only by increasing the forward rate of nucleotide addition by RNA polymerase II, but also by inhibiting SII-induced nascent transcript cleavage by non-arrested RNA polymerase II elongation intermediates.

Biochemical studies of transcription by RNA polymerase II have led to the identification and purification of a collection of transcription factors that promote elongation through direct interactions with transcribing polymerase. These elongation factors fall into two functional classes based on their mechanisms of action.

One class includes TFII F (1–5), ELL (6–8), and Elongin (9–11), which are all capable of activating the overall rate of elongation by RNA polymerase II by suppressing transient pausing by the enzyme. Previous studies have shown that bacterial and eukaryotic RNA polymerases are susceptible to pausing for varying lengths of time at each step of nucleotide addition. Because the duration of pausing is often greater than the rate of phosphodiester bond formation ($k_{cat}$), it has been proposed that elongating RNA polymerases cycle between active and inactive conformations at each step of nucleotide addition (1, 12–18). TFII F, ELL, and Elongin are all capable of increasing the rate of elongation by RNA polymerase II at extremely low ribonucleoside triphosphate concentrations when the time required for nucleotide addition can vary from seconds to minutes, suggesting that these transcription factors increase the rate of elongation not by decreasing $k_{cat}$, but by decreasing the fraction of time polymerase spends in an inactive conformation (7, 8, 11, 19).

The other class of RNA polymerase II elongation factors includes members of the SII family of transcription factors. SII promotes elongation by reacting RNA polymerase II that has become arrested at a variety of impediments including specific DNA sequences referred to as intrinsic arrest sites (20–24). Unlike paused RNA polymerase II, which resumes transcription even in the absence of elongation factors, arrested RNA polymerase II is in an inactive state and resumes transcription only with assistance from SII.

Evidence from a variety of studies suggests that transcriptional arrest and pausing are mechanistically related and result from aberrant backward movement of RNA polymerase II on the DNA with concomitant displacement of the 3'-end of the nascent transcript from the polymerase catalytic site, an event that is either spontaneously reversible in the case of pausing or not in the case of arrest (24). Current evidence is consistent with the model that SII reactivates arrested RNA polymerase II by inducing a polymerase-associated endoribonuclease activity, which cleaves the nascent transcript 7–10 nucleotides upstream of the displaced 3'-end (21, 25), thus creating a new 3'-end that is properly aligned with the enzyme's catalytic site and can be reextended. Notably, SII also promotes nascent
transcript cleavage by paused but non-arrested RNA polymerase II elongation complexes; in this case, transcript cleavage typically occurs in two nucleotide increments (25, 26).

The mechanism(s) by which TFIIF, ELL, and Elongin suppress transient pausing by transcribing RNA polymerase II are not completely understood. In a previous study, Reines and co-worker (17) observed that TFIIF can decrease the rate at which RNA polymerase II falls into arrest, raising the possibility that TFIIF can inhibit backsliding of polymerase and consequent displacement of the 3′-end of the nascent transcript from the polymerase catalytic site. In addition, we previously observed (i) that TFIIF, ELL, and Elongin can all dramatically increase the ability of RNA polymerase II to bind to and extend DNA primers in a DNA template-directed reaction and (ii) that TFIIF mutations that reduce TFIIF elongation activity also reduce this TFIIF activity, consistent with the idea that the mechanisms by which TFIIF, ELL, and Elongin promote extension of DNA 3′-ends and suppress transient pausing by RNA polymerase II may be related (27).1 As suggested by Salzman and co-workers, the DNA template-directed addition of ribonucleotides to the 3′-ends of DNA by RNA polymerase II may occur in a reaction that mimics formation of the RNA polymerase II ternary elongation complex (28). In this case, RNA polymerase II would bind to the 3′-hydroxyl terminus of DNA, just as the enzyme binds to the 3′-end of an elongating RNA molecule; the polymerase catalytic site would then add ribonucleotides to the DNA 3′-hydroxyl terminus as if it were the 3′-end of a nascent transcript. Accordingly, our observation that TFIIF, ELL, and Elongin can promote extension by RNA polymerase II of DNA primers is consistent with the model that these transcription factors may facilitate proper positioning of the 3′-ends of these DNA primers in the polymerase catalytic site and, by extension, that these transcription factors may suppress transient pausing by polymerase by facilitating proper positioning of the 3′-end of nascent transcripts in the enzyme’s catalytic site.

In light of this model and because SII-induced cleavage of nascent transcripts by paused or arrested RNA polymerase II elongation intermediates is believed to occur when the 3′-end of nascent transcripts becomes misaligned with the polymerase catalytic site, we investigated the possibility of cross-talk between SII and transcription factors TFIIF, ELL, and Elongin. These studies led to the discovery that TFIIF, ELL, and Elongin are all capable of inhibiting SII-induced cleavage of transcripts by non-arrested RNA polymerase II elongation intermediates. Here we present these findings, which bring to light a novel activity associated with TFIIF, ELL, and Elongin and suggest that these transcription factors may expedite elongation by RNA polymerase II not only by increasing the forward rate of nucleotide addition by polymerase but also by inhibiting SII-induced nascent transcript cleavage by non-arrested RNA polymerase II elongation intermediates.

**EXPERIMENTAL PROCEDURES**

**Materials**—Unlabeled ultrapure ribonucleoside 5′-triphosphates, 3′-O-MeGTP,2 and [α-32P]CTP (>3000 Ci/mmol) were purchased from Amersham Pharmacia Biotech. Dinucleotide CpA, araCTP, polyvinyl alcohol (type II), and Sephadex G-50 (catalog number G-50-150) were obtained from Sigma. Acetylated bovine serum albumin and recombinant placental ribonuclease inhibitor were from Promega. Empty 4-ml spin columns were purchased from 5 Prime → 3 Prime, Inc., Boulder, CO. Preparation of DNA Templates for Transcription—The 310-base pair DNA fragment containing the AdML promoter was obtained by digestion of the plasmid pDN-AdML (29) with EcoRI and NdeI and by purification of the EcoRI-NdeI fragment by agarose gel electrophoresis. The oligo(dC)-tailed pCPHS220 DNA template was prepared as described (30).

Preparation of RNA Polymerase II and Transcription Factors—RNA polymerase II (31) and TFIIH (rat δ, SP 5-PW fraction; (32)) were purified from rat liver nuclear extracts as described previously. Recombinant yeast TBP (AcA 44 fraction; (33)), recombinant TFIIA (34), and recombinant TFIIH (35) were expressed in *Escherichia coli* and purified as described. Recombinant human TFIIF was prepared as described (36), except that the 56-kDa subunit was expressed in *E. coli* strain BL21(DE3)-pLysS. Recombinant human ELL was expressed in *E. coli* and purified from preparative SDS-polyacrylamide gels as described (8). The recombinant 3-subunit Elongin ABC complex was expressed in *E. coli* and purified as described (19).

Recombinant human SII was expressed in *E. coli* and purified as described. Experimental Procedures: Regulation of SII Elongation Activity. FIG. 1. TFIIF and ELL antagonize SII-induced nascent transcript cleavage by non-arrested RNA polymerase II elongation complexes. A, AdML promoter sequence in the vicinity of the transcriptional start site. +1 indicates the position of the in vivo start site. The site of initiation of transcripts initiated with the dinucleotide CpA is indicated above the template sequence. B, Mg2+-dependent nascent transcript cleavage activity of the SII used in these studies. Paused elongation complexes were prepared as described under “Experimental Procedures.” Lanes 1 and 11, purified ternary complexes. Complexes were incubated with ~2.5 ng (lane 2), ~5 ng (lanes 3 and 4), ~10 ng (lanes 5 and 6), ~20 ng (lanes 7 and 8), or ~40 ng (lanes 9 and 10) of SII at 28 °C for 10 min. In the reaction shown in lanes 12 and 14, complexes were incubated with ~500 ng of SII with (lane 12) or without (lane 14) 6 mM MgCl2 for 20 min at 28 °C. In the reactions shown in Lanes 13 and 15, complexes were incubated with 70 μM 3′-O-MeGTP for 20 min at 28 °C with (lane 13) or without (lane 15) 6 mM MgCl2. C, TFIIF and ELL antagonize SII-dependent transcript cleavage in a dose-dependent manner. Purified ternary transcription complexes (lanes 1 and 11) were incubated for 10 min at 28 °C with 25 ng of SII without (lanes 2, 3, 12, and 13) or with increasing amounts of TFIIF (~100 ng, lanes 4 and 5; ~1 μg, lanes 6 and 7; ~2 μg, lanes 8 and 9) or ELL (~1 ng, lanes 10 and 15; ~5 ng, lanes 16 and 17; or ~10 ng, lane 18).
follows. The pT7–7Met expression vector encoding the full-length human SII open reading frame was obtained from Dr. Caroline Kane, (Berkeley, CA) and expressed in E. coli BL21 (pLysS) cells as described (37). SII was purified by chromatography on consecutive phosphocellulose and TSK phenyl 5-PW columns. Briefly, cell lysates were centrifuged 30 min at 31,000 × g, and the supernatant was applied to a phosphocellulose column (Whatman P-11) pre-equilibrated in 40 mM Hepes-NaOH (pH 7.9), 1 mM EDTA, 1 mM DTT, 10% (v/v) glycerol, and 0.1 mM KCl. SII was eluted with the same buffer containing 0.33 mM KCl. Active fractions were pooled and precipitated with 60% (NH₄)₂SO₄. The pellet was dissolved in 100 mM Hepes-NaOH (pH 7.9), 0.1 mM DTT, and 0.1 M KCl. SII was purified by chromatography on consecutive phosphocellulose and TSK phenyl 5-PW high pressure liquid chromatography columns (75 × 7.5 mm; Bio-Rad) pre-equilibrated in 100 mM Hepes-NaOH, pH 7.9, 0.1 mM DTT, 5% (v/v) glycerol, and 1.5 M (NH₄)₂SO₄. SII was eluted with a 36-ml linear gradient from 1.5 M to 0.1 M (NH₄)₂SO₄ in the same buffer.

Preparation of Paused RNA Polymerase II Elongation Complexes—For a single assay of promoter-specific transcription, preinitiation complexes were assembled at the AdML promoter at 28 °C by a 30-min preincubation of 30 μl of reaction mixtures containing 20 mM Hepes-NaOH (pH 7.9), 20 mM Tris-HCl (pH 7.9), 50 mM KCl, 0.1 mM EDTA, 1 mM DTT, 5 mM MgCl₂, 0.5 mM MgCl₂ bovine serum albumin, 2% (w/v) polyvinyl alcohol, 3% (v/v) glycerol, six units of recombinant placental ribonuclease inhibitor, −10 ng of the EcoRI to NdeI fragment from pDN-AdML, −5 ng of recombinant yeast TBP, −10 ng of recombinant TFIIB, −20 ng of recombinant TFIIE, and 0.01 unit of RNA polymerase II. Except where indicated otherwise, transcription reactions were performed at 28 °C for 30 min in the presence of 200 μM CTP, 0.5 μM (α-32P)CTP, 15 μM ATP, 15 μM UTP, and 70 μM 3'-O-MeGTP.

Transcription reactions were scaled up as necessary for each experiment. Paused RNA polymerase II elongation complexes were purified by applying ~200–250 μl of each transcription reaction mixture to two consecutive 3-ml Sephadex G-50 spin columns prepacked in 4-ml empty spin columns and pre-equilibrated in 20 mM Hepes-NaOH (pH 7.9), 20 mM Tris-HCl (pH 7.9), 50 mM KCl, 0.1 mM EDTA, 1 mM DTT, 0.5 mg/ml bovine serum albumin, 2% (w/v) polyvinyl alcohol, and 3% (v/v) glycerol, in the presence or absence of 5 mM MgCl₂. The columns were spun for 5 min at 2000 × g in a swinging bucket rotor, and 30 μl of eluant/reaction mixture was used in further experiments. Reactions were stopped by the addition of an equal volume of 8.0 M urea containing 0.025% (v/v) bromophenol blue and 0.025% (v/v) xylene cyanol FF. Transcripts were analyzed by electrophoresis through polyacrylamide gels containing 25% acrylamide, 3% bis-acrylamide, 5 mM Tris base, 89 mM boric acid, and 2 mM EDTA. Transcripts were quantitated using a Molecular Dynamics PhosphorImager.

RESULTS AND DISCUSSION

To begin to explore the possibility of cross-talk between elongation factor SII and transcription factors that suppress transient pausing by RNA polymerase II, we investigated the effect of TFIIF and ELL on SII-induced nascent transcript cleavage by RNA polymerase II elongation intermediates that had synthesized 16-nucleotide-long transcripts in a basal transcription system reconstituted with recombinant general initiation factors TBP, TFIIB, TFIIE, and TFIIF and purified RNA polymerase II and TFIIF from rat liver.
polymerase II preinitiation complexes were assembled at the AdML promoter by preincubation of polymerase and the general initiation factors with a DNA fragment containing the AdML promoter. Radioactively labeled transcripts were synthesized in the presence of 200 μM of the initiating dinucleotide CpaA, which directs most transcription initiation from position −1 relative to the normal AdML transcriptional start site (Fig. 1A), 15 μM ATP, 15 μM UTP, 0.5 μM [α-32P]CTP, and 70 μM 3′-O-MeGTP, which prevents most transcription beyond the first G residue of the AdML transcript at position +15. RNA polymerase II elongation complexes were purified free of ribonucleoside triphosphates by two consecutive Sephadex G-50 spin columns and treated with elongation factor SII. As shown in Fig. 1B and consistent with previous results (21–23), SII induced cleavage of nascent transcripts by paused RNA polymerase II elongation intermediates in a dose-dependent manner (lanes 2–10). Similarly, SII induced cleavage of RNA polymerase II elongation complexes that had synthesized 15-nucleotide-long transcripts and had paused after incorporating the first A residue immediately preceding the first G residue of the AdML transcript at position +15. RNA polymerase II elongation complexes were transcriptionally active because they could incorporate the RNA chain-terminating nucleotide 3′-O-MeG when Mg2+ was added to reaction mixtures (compare lanes 11, 13, and 15).

To test the effects of TFIIF and ELL on SII-induced nascent transcript cleavage, purified RNA polymerase II elongation complexes containing 16-nucleotide 3′-O-MeG-terminated transcripts were incubated with a fixed level of SII with or without increasing concentrations of TFIIF and ELL. As shown in Fig. 1C, SII-induced nascent transcript cleavage by non-arrested RNA polymerase II elongation intermediates was effectively inhibited by levels of TFIIF and ELL that potently activated the rate of elongation by polymerase under our reaction conditions (data not shown). In addition, the inhibitory effects of ELL (Fig. 2) and TFIIF (data not shown) in these reactions could be overcome by increasing the levels of SII in the presence of fixed ELL and TFIIF concentrations, suggesting that SII, TFIIF, and ELL function competitively in regulation of nascent transcript cleavage.

ELL and TFIIF are both capable of stimulating the rate of elongation by RNA polymerase II in the presence of extremely low levels of ribonucleoside triphosphates (Refs. 7, 8, 11 and data not shown). To address the possibility that the observed ELL- and TFIIF-dependent inhibition of SII-induced nascent transcript cleavage by non-arrested RNA polymerase II elongation intermediates was due to ELL- or TFIIF-stimulated reextension of cleaved nascent transcripts in the presence of low levels of residual ribonucleoside triphosphates contaminating gel-filtered preparations of paused elongation complexes, ELL and TFIIF were added back to SII-treated elongation complexes. In these experiments, paused RNA polymerase II elongation complexes containing 16-nucleotide, 3′-O-MeG-terminated transcripts were treated with SII for 5 min and then incubated with ELL, TFIIF, or ribonucleoside triphosphates. As shown in Fig. 3, addition of ELL (lanes 1, 3–5) or TFIIF (lanes 6–8) did not promote reextension of cleaved transcripts. In contrast, cleaved transcripts could be reextended by addition of ribonucleoside triphosphates to reaction mixtures, confirming that RNA polymerase II elongation complexes incubated with SII were transcription-competent at the time ELL and TFIIF were added to reactions (compare lanes 1 and 2).

The results shown in Fig. 3 are consistent with the model that TFIIF and ELL function by blocking SII-induced nascent transcript cleavage and not by promoting reextension of cleaved transcripts. As a further test of this model, we performed the experiment shown in Fig. 4. In this experiment, we took advantage of the observation that, because of its ability to promote nascent transcript cleavage, SII can reactivate elongation complexes paused following incorporation of a chain-terminating nucleotide by promoting removal of the chain-terminating nucleotide from the 3′-ends of nascent transcripts. Purified RNA polymerase II elongation complexes that had synthesized 16-nucleotide, 3′-O-MeG-terminated transcripts were incubated with ATP, UTP, GTP, and 20-nucleotide transcripts terminated with araC. Paused elongation complexes containing 16-nucleotide, 3′-O-MeG-terminated transcripts were prepared and purified in the presence of MgCl2 as described under “Experimental Procedures” and incubated for 10 min at 28 °C with 50 μM ATP, 50 μM UTP, 50 μM GTP, and 50 μM araC (NTP chase) in the presence or absence of ~10 ng of SII, ~500 ng of TFIIF, or ~5 ng of ELL.

![Diagram](image-url)
with 3 mM sodium pyrophosphate (PPi) for 3 min at 28 °C without (lane 2) or with ~25, 50, 250, 500 ng of TFIIF (lanes 3–6, respectively). Lanes 8–11 complexes were incubated for 3 min at 28 °C with 5 ng of ELL, ~15 ng of SII, or 1 mM PPi as indicated in the figure.

terminated transcripts that could be extended in the presence of SII was substantially decreased when reactions also contained either TFIIF (lane 5) or ELL (lane 9), consistent with the model that TFIIF and ELL are capable of inhibiting SII-induced nascent transcript cleavage by non-arrested RNA polymerase II elongation complexes.

On the basis of evidence that SII induces nascent transcript cleavage by polymerases that have backtracked along the DNA template and lost proper contact with the 3’-end of transcripts, our observation that TFIIF and ELL block SII-induced nascent transcript cleavage raised the possibility that TFIIF and ELL might function by helping to maintain the 3’-end of nascent transcripts in the RNA polymerase II catalytic site. If this model is correct, TFIIF and ELL might be expected to stimulate not only the rate of nucleotide addition by RNA polymerase II but also the reverse reaction, pyrophosphorolysis. Indeed, Hawley and co-worker (22) previously demonstrated that TFIIF can stimulate the rate of pyrophosphorolysis by RNA polymerase II elongation complexes that had synthesized 185–350-nucleotide-long transcripts. Consistent with their findings, we observe that both TFIIF and ELL can stimulate the rate of pyrophosphorolysis by RNA polymerase II elongation complexes containing 16-nucleotide, 3’-O-MeG-terminated transcripts (Fig. 5).

Because the experiments presented above were all performed with RNA polymerase II elongation complexes that had initiated transcription from the AdML promoter in the presence of the general initiation factors, we wanted to confirm that TFIIF and ELL inhibit SII-induced nascent transcript cleavage through direct interactions with these elongation complexes and not indirectly through the action of one or more of the general initiation factors present in transcription reactions. To accomplish this, we took advantage of the oligo(dC)-tailed pCpGR220 DNA template (30). Because the first non-template strand (dT) residue is 136 base pairs downstream of the oligo(dC) tail, RNA polymerase II will initiate transcription from the oligo(dC) tail and synthesize transcripts of ~136 nucleotides in the presence of ATP, CTP, and GTP. In these experiments, paused RNA polymerase II elongation complexes that had synthesized ~136 nucleotide transcripts were purified by two consecutive Sephadex G-50 spin columns and treated with SII or 3 mM PPi, in the presence or absence of TFIIF, ELL, and Elongin. As shown in Fig. 6, TFIIF, ELL, and Elongin are all capable not only of inhibiting SII-dependent nascent transcript cleavage but also of stimulating the rate of pyrophosphorolysis by paused RNA polymerase II elongation intermediates in the absence of the general initiation factors, suggesting that these activities are intrinsic to elongation factors that suppress transient pausing by RNA polymerase II. In the experiment of Fig. 6A, amounts of ELL and Elongin proteins corresponding to a molar ratio relative to SII of ~0.1:1 were sufficient to inhibit SII-induced transcript cleavage, whereas an amount of TFIIF corresponding to a molar ratio relative to SII of ~1.8:15 was needed. As shown in Fig. 6B, the same relative levels of ELL,
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Elongin, and TFIIF were needed to stimulate the rate of pyrophosphorylation. At the present time, we do not know whether the apparent difference in the specific activities of ELL, Elongin, and TFIIF reflects a difference in their intrinsic abilities to modulate the activities of RNA polymerase II or whether it reflects a difference in the fraction of active molecules present in our preparations of elongation factors.

In conclusion, in this report we present evidence that transcription factors TFIIF, ELL, and Elongin, which have all been shown previously to activate the rate of elongation by RNA polymerase II in vitro, are also capable of potently inhibiting SII-induced cleavage of nascent transcripts by non-arrested RNA polymerase II elongation intermediates. In addition, we present evidence that, like TFIIF, transcription factors ELL and Elongin are capable of strongly stimulating the rate of pyrophosphorylation by non-arrested RNA polymerase II elongation intermediates. Because SII-induced cleavage of nascent transcripts by RNA polymerase II elongation intermediates is believed to occur when the 3'-end of transcripts becomes misaligned with the polymerase catalytic site, our findings raise the possibility that TFIIF, ELL, and Elongin inhibit SII-induced transcript cleavage at least in part by helping to maintain the 3'-end of transcripts in the enzyme's catalytic site. If this model is correct, then inhibition of SII-induced nascent transcript cleavage by TFIIF, ELL, and Elongin could be a manifestation of their intrinsic elongation factor activity. Notably, in the experiments presented here we observe a correlation between the TFIIF, ELL, and Elongin levels required to inhibit SII-induced nascent transcript cleavage and to stimulate pyrophosphorylation, which is the reverse reaction of nucleotide addition and RNA transcript elongation. Whether TFIIF, ELL, and Elongin inhibit SII-induced transcript cleavage by controlling the orientation of the 3'-ends of nascent transcripts or by simply interacting with the RNA polymerase II elongation complex and sterically blocking its interaction with SII, however, remains to be determined. Nevertheless, our findings bring to light a novel activity associated with TFIIF, ELL, and Elongin and suggest that these transcription factors could expedite elongation not only by increasing the forward rate of nucleotide addition by RNA polymerase II but also by inhibiting SII-induced nascent transcript cleavage by non-arrested RNA polymerase II elongation intermediates.

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