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TFIIF Facilitates Dissociation of RNA Polymerase II from Noncoding RNAs That Lack a Repression Domain▽

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Noncoding RNAs (ncRNAs) have recently been found to regulate multiple steps in mammalian mRNA transcription. Mouse B2 RNA and human Alu RNA bind RNA polymerase II (Pol II) and repress mRNA transcription, using regions of the ncRNAs referred to as repression domains. Two other ncRNAs, mouse B1 RNA and human small cytoplasmic Alu (scAlu) RNA, bind Pol II with high affinity but lack repression domains and hence do not inhibit transcription. To better understand the interplay between ncRNAs that bind Pol II and their functions in transcription, we studied how Pol II binding and transcriptional repression are controlled by general transcription factors. We found that TFIIF associates with B1 RNA/Pol II and scAlu RNA/Pol II complexes and decreases their kinetic stability. Both subunits of TFIIF are required for this activity. Importantly, fusing a repression domain to B1 RNA stabilizes its interaction with Pol II in the presence of TFIIF. These results suggest a new role for TFIIF in regulating the interaction of ncRNAs with Pol II; specifically, it destabilizes interactions with ncRNAs that are not transcriptional repressors. These studies also identify a new function for ncRNA repression domains: they stabilize interactions of ncRNAs with Pol II in the presence of TFIIF.

mRNA transcription by RNA polymerase II (Pol II) is a complex process that is an integral control point for regulating eukaryotic gene expression. A wide assortment of regulatory factors are involved in controlling transcription, and many operate in response to specific stimuli and/or extracellular cues. These include promoter-specific activators and repressors, coregulatory proteins, and a variety of factors that remodel or covalently modify chromatin (9, 15, 21). In addition to these regulatory factors, Pol II requires a set of proteins, called the general transcription factors, for accurate transcription to occur at most genes; these proteins include TFIIA, TFIIB, TFIID, TFIIE, TFIIF, and TFIIH (26). These factors facilitate steps during transcription including, but not limited to, polymerase recruitment to the promoter, start-site selection, DNA melting, initiation, and elongation.

More recently, studies have identified noncoding RNAs (ncRNAs) that can control transcription by functioning in many of the capacities described above for protein transcriptional regulators. For example, ncRNAs have been shown to function in gene-specific activation or repression, coregulation, and chromatin modification and even as general factors (2, 8). Consequently, ncRNAs can act on a diverse array of steps in the transcription process. We previously identified two ncRNAs, mouse B2 RNA and human Alu RNA, that act as transcriptional repressors in response to heat shock (1, 19). The amounts of these ncRNAs in the nucleus increase upon heat shock. B2 RNA and Alu RNA bind directly to core Pol II and repress transcription by incorporating into complexes at promoters along with Pol II and rendering the polymerase transcriptionally inactive (6, 19, 28).

B2 and Alu RNAs are encoded by short interspersed elements (SINEs), which are retrotransposons scattered throughout the mouse and human genomes, respectively (12). Despite sharing a common biological function, B2 RNA and Alu RNA share little similarity in sequence or secondary structure (7, 24). Mice contain a second SINE in addition to B2, named B1. B1 SINEs can be transcribed into RNA, and levels of B1 RNA also increase upon heat shock (16, 17). Alu RNA is related to B1 RNA in sequence and secondary structure: it is a tandem repeat of two B1-like RNAs connected by an A-rich linker (22, 23). A portion of Alu RNA is processed into the shorter small cytoplasmic Alu (scAlu) RNA, which is composed of the 5′ B1-like element (18, 20). Biological roles for B1 RNA and scAlu RNA remain undiscovered (16). Our previous studies showed that both B1 and scAlu RNAs bind directly to Pol II with high affinity; however, neither one inhibits transcription in vitro (19). Therefore, high-affinity binding of an ncRNA to Pol II is not sufficient to cause transcriptional repression.

The specific ncRNA sequence and structural requirements needed for Pol II binding and transcriptional repression are becoming better understood. For example, two regions in Alu RNA mediate transcriptional repression and are referred to as repression domains: the A-rich linker (named the A region) that connects the two B1-like elements and the L region, a loosely structured region within the 3′ B1-like element (19). B1 and scAlu RNAs lack repression domains; however, when either of the Alu RNA repression domains was fused to B1 RNA, the resulting chimeric RNA was able to repress transcription in vitro (19).

Here we performed comparative studies of mouse and human SINE ncRNAs to understand how the binding of these ncRNAs to Pol II and their abilities to repress transcription are controlled by general transcription factors. We first asked

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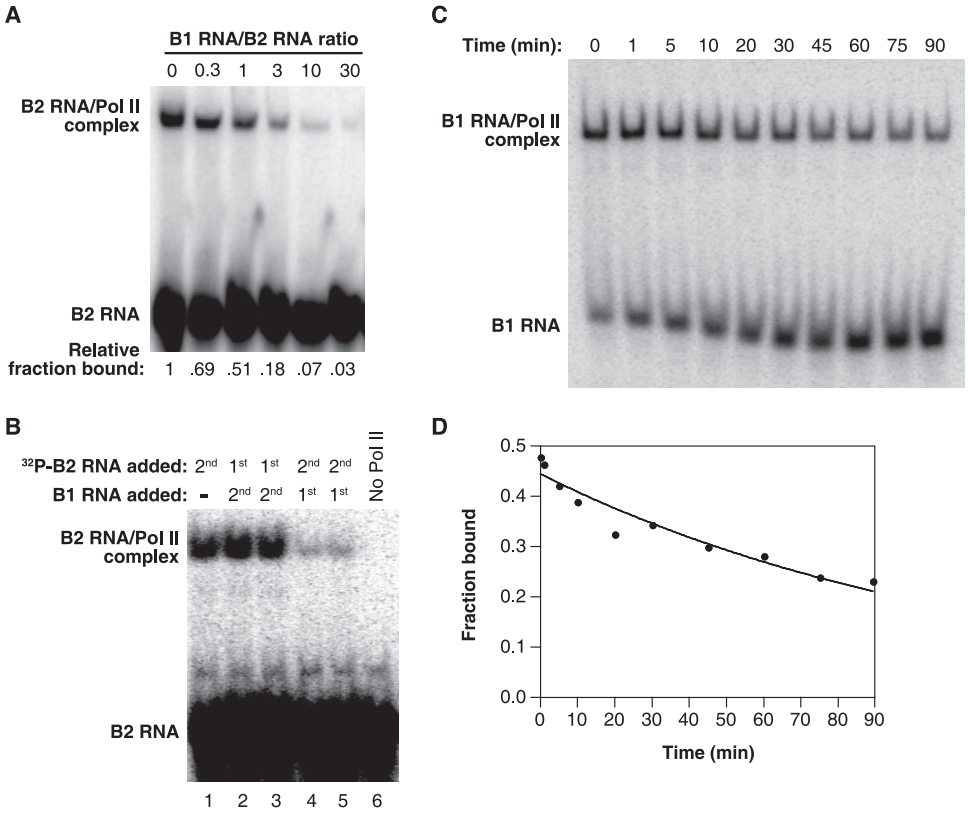

FIG. 1. B1 RNA and B2 RNA likely bind overlapping sites on Pol II. (A) B1 RNA and B2 RNA bind Pol II competitively. Purified human Pol II (2 nM) was incubated with ^{32}P-labeled B2 RNA (5 nM) and unlabeled B1 RNA (at the molar ratios indicated), and complexes were resolved by EMSA. The relative fraction of B2 RNA bound in the absence of unlabeled B1 RNA was set to 1 (lane 1). Two experiments were performed, and representative data are shown. (B) B1 RNA prebound to Pol II blocks the association of B2 RNA. ^{32}P-labeled B2 RNA and unlabeled B1 RNA were added to Pol II in the order indicated. RNAs added first were given 10 min to bind Pol II prior to the addition of the second RNA, after which the incubation was continued for an additional 15 min. Complexes were resolved by EMSA. Four experiments were performed, and representative data are shown. (C) B1 RNA/Pol II complexes are kinetically stable. ^{32}P-labeled B1 RNA/Pol II complexes were challenged with a 100-fold molar excess of unlabeled B1 RNA, and EMSA was used to measure the amount of ^{32}P-labeled B1 RNA/Pol II complex remaining over time. Two experiments were performed, and representative data are shown. (D) Shown are the data from C quantified and plotted as the fraction of B1 RNA bound to Pol II versus time and fit to a first-order exponential decay equation. The rate constant for the dissociation of the B1 RNA/Pol II complex is $1.5 \times 10^{-4} \pm 0.3 \times 10^{-4}$ s^{-1}, averaged from two independent experiments, with the error representing the range of the measurements.

whether B1 RNA and B2 RNA bind Pol II competitively. Despite finding that they compete with one another for binding to Pol II, B1 RNA could not prevent B2 RNA from repressing transcription. Therefore, we asked whether general transcription factors could affect the interaction between nonrepressor ncRNAs and Pol II. Further analysis revealed that TFIIF facilitated the dissociation of B1 RNA and scAlu RNA from Pol II. When repression domains from Alu RNA were fused to B1 RNA, the resulting chimeric ncRNAs remained stably bound to Pol II in the presence of TFIIF. These data support a new function for TFIIF: the ability to destabilize interactions between Pol II and ncRNAs that are not transcriptional repressors. Moreover, our results show that repression domains stabilize the interactions of ncRNAs with Pol II in the presence of TFIIF.

MATERIALS AND METHODS

Plasmid construction and RNA preparation. The construction of pUC-T7-B1, pUC-T7-B2, pUC-T7-scAlu, pUC-T7-B1-A, pUC-T7-B1-L, and pUC-T7-A rich was described elsewhere previously (1, 19). The RNAs were transcribed by T7 RNA polymerase and purified from denaturing gels as previously described (1). Immediately prior to use, RNAs were folded by heating samples at 90°C for 1 min in a buffer similar to that used for binding and transcription assays (10 mM HEPES [pH 7.9], 10 mM Tris [pH 7.9], 10% glycerol, 50 mM KCl, 1 mM dithiothreitol, 4 mM $MgCl_2$) and then transferring samples to 4°C.

In vitro transcription. Purified recombinant human TBP, TFIIB, and TFIIF and native Pol II were prepared as described previously (27). TFIIB (10 nM), TFIIF (2 nM), core Pol II (1 to 3 nM), and ncRNA (when present) were incubated together for 5 min at 30°C in 10 μl of buffer A (10 mM HEPES [pH 7.9], 10 mM Tris [pH 7.9], 10% glycerol, 50 mM KCl, 1 mM dithiothreitol, 50 μg/ml bovine serum albumin, 4 mM $MgCl_2$, and 15 units of RNA Guard [GE biosciences]). Two microliters of either a second ncRNA or buffer A was added, and the incubation was continued for an additional 10 min. In a separate tube, TBP (10 nM) was incubated with plasmid DNA (1 to 2 nM) containing the adenovirus major late promoter (AdMLP) core promoter (positions −53 to +10) fused to a 380-bp G-less cassette for 10 min at 30°C in 10 μl of buffer A. The contents of the two tubes were mixed, and preinitiation complexes were allowed to form for 10 min. A nucleotide mix was added, resulting in the following final concentrations: 625 μM ATP, 625 μM UTP, and 25 μM (5 μCi) [α-^{32}P]CTP. Transcription was allowed to proceed for 30 min. Transcripts were ethanol precipitated and resolved by 6% denaturing polyacrylamide gel electrophoresis. The 390-nucleotide (nt) G-less product was visualized by using phosphorimagery.

For the experiment shown in Fig. 3D, the bubble template was constructed by

annealing template and nontemplate strand oligonucleotides (Invitrogen) containing the AdMLP core promoter sequence from positions −40 to +40, with the sequence of the nontemplate strand changed to match that of the template strand from positions −9 to +3. Reaction mixtures contained either Pol II (2 nM) or Pol II-TFIIF (2 nM) in the presence or absence of ncRNAs (5 nM). Runoff transcription was performed with the following final concentrations of nucleotides: 625 μM ATP, 625 μM UTP, 625 μM GTP, and 25 μM (5 μCi) [α-^{32}P]CTP. The 40-nt product was resolved by 8% denaturing polyacrylamide gel electrophoresis and visualized by using phosphorimagery.

EMSAs. ^{32}P-labeled RNAs and purified human Pol II were incubated together in 20 μl of buffer A at 30°C for 20 min. Reaction mixtures were subjected to electrophoresis though 4% polyacrylamide gels containing 0.5× Tris-borate-EDTA buffer, 5% glycerol, and 5 mM magnesium acetate as previously described (6). The bands were visualized by phosphorimagery and quantitated by using Image J software. For the competition binding assays, ^{32}P-labeled B2 RNA was held constant at 5 nM, and unlabeled B1 RNA was titrated into reaction mixtures from 0.5 nM to 150 nM; Pol II (2 nM) was then added. For the blocking assays, the first RNA (10 nM) was incubated with Pol II (2 nM) for 10 min at 30°C prior to the addition of the second RNA (10 nM) for 15 min. An electrophoretic mobility shift assay (EMSA) was then performed as described above.

Dissociation rate assays. ^{32}P-labeled ncRNA (0.15 nM) and Pol II (2 nM) were incubated in buffer A with or without other proteins (as indicated) for 10 min at 30°C. A 100-fold excess of unlabeled ncRNA was then added, and after various times, reaction mixtures were loaded onto a native gel (described above). The data were visualized by using phosphorimagery and quantitated as the fraction bound (bound ncRNA/total ncRNA) by using Image J software. We analyzed only those experiments for which the total ncRNA counts remained relatively constant across the time course; therefore, RNA degradation was not a problem. Rate constants for dissociation shown in Fig. 1D and 3B and C were determined by fitting the data to a single exponential decay equation.

RESULTS

B1 RNA and B2 RNA compete with one another for binding to Pol II. Previous studies found that both B2 RNA and B1 RNA bind tightly to Pol II (K_D [equilibrium dissociation constant] of <2 nM) (6, 19). We have proposed that Pol II contains a high-affinity ncRNA docking site to which these ncRNAs bind (7). In accordance with this model, we predicted that B1 RNA and B2 RNA would bind Pol II competitively. To test this, highly purified human core Pol II was incubated with both unlabeled B1 RNA and ^{32}P-labeled B2 RNA, and complexes were then resolved by EMSAs. As shown in Fig. 1A, when the ratio of B1 RNA to B2 RNA increased, the amount of the B2 RNA/Pol II complex decreased. Moreover, when the molar ratio was 1:1, the fraction of B2 RNA bound to Pol II decreased by 50%, supporting previously reported findings that these RNAs bind Pol II with similar affinities (6, 19).

To complement the competition experiment (Fig. 1A), we asked whether prebinding B1 RNA to Pol II could block B2 RNA from binding (Fig. 1B). When unlabeled B1 RNA was prebound to Pol II, it almost completely blocked the association of the ^{32}P-labeled B2 RNA that was subsequently added (Fig. 1B, compare lane 1 to lanes 4 and 5). As a control, when ^{32}P-labeled B2 RNA was prebound to Pol II, the addition of unlabeled B1 RNA caused no decrease in the amount of the B2 RNA/Pol II complex observed (Fig. 1B, compare lane 1 to lanes 2 and 3). Together, the data in Fig. 1A and B show that the binding of B1 RNA or B2 RNA to Pol II is mutually exclusive: each ncRNA can block the association of the other. These findings are most consistent with B1 and B2 RNAs binding the same or an overlapping site on Pol II; however, an allosteric model in which the binding of one RNA causes a change in Pol II that precludes the association of the other cannot be excluded.

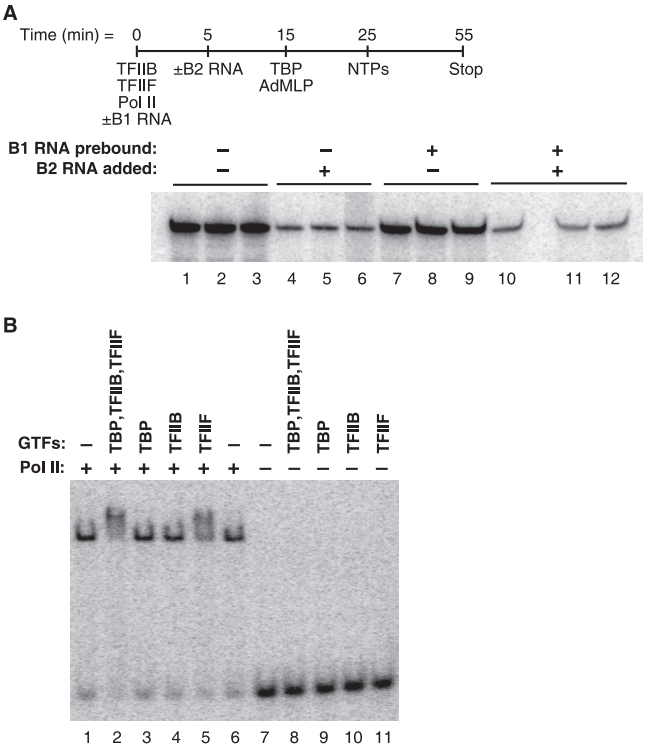

FIG. 2. B1 RNA cannot block transcriptional repression by B2 RNA in vitro. (A) B2 RNA represses transcription when added to reaction mixtures after B1 RNA is prebound to Pol II. ncRNAs (5 nM) were added to reaction mixtures as indicated. The 390-nt G-less transcript is shown. Two experiments were performed, and representative data are shown. NTPs, nucleotide triphosphates. (B) Of the proteins present in the minimal transcription system, only Pol II binds B1 RNA. ^{32}P-labeled B1 RNA (0.5 nM) and ^{32}P-labeled B1 RNA/Pol II (2 nM) complexes were incubated with human TBP, TFIIF, or TFIIB, as indicated, at the concentrations used for in vitro transcription. Bound and free RNAs were resolved by EMSA. Three experiments were performed, and representative data are shown. GTFs, general transcription factors.

The blocking experiment suggested that the B1 RNA/Pol II complex is kinetically stable. To test this directly, we measured the rate at which B1 RNA/Pol II complexes dissociate by adding an excess of unlabeled B1 RNA to complexes preformed with ^{32}P-labeled B1 RNA and determining the fraction of the complex that remained over time. As shown in Fig. 1C, the B1 RNA/Pol II complex is indeed kinetically stable, with approximately 50% of the complex still remaining after 90 min; the rate constant for dissociation is $1.5 \times 10^{-4} \pm 0.3 \times 10^{-4}$ s^{-1} (see plot of representative data in Fig. 1D).

TFIIF facilitates the dissociation of B1 RNA and scAlu RNA from Pol II. B2 RNA is a potent transcriptional inhibitor, with a 50% inhibitory concentration in the low nM range in vitro; in contrast, B1 RNA does not inhibit transcription in vitro despite tight binding to Pol II (6, 19). Since we found that B1 RNA blocks B2 RNA from binding Pol II and that the B1 RNA/Pol II complex is kinetically stable, we hypothesized that B1 RNA would prevent B2 RNA from functioning as a transcriptional repressor by blocking its association with Pol II. To test this, we used a reconstituted human transcription system consisting of TBP, TFIIB, TFIIF, and Pol II, which is the minimum set of

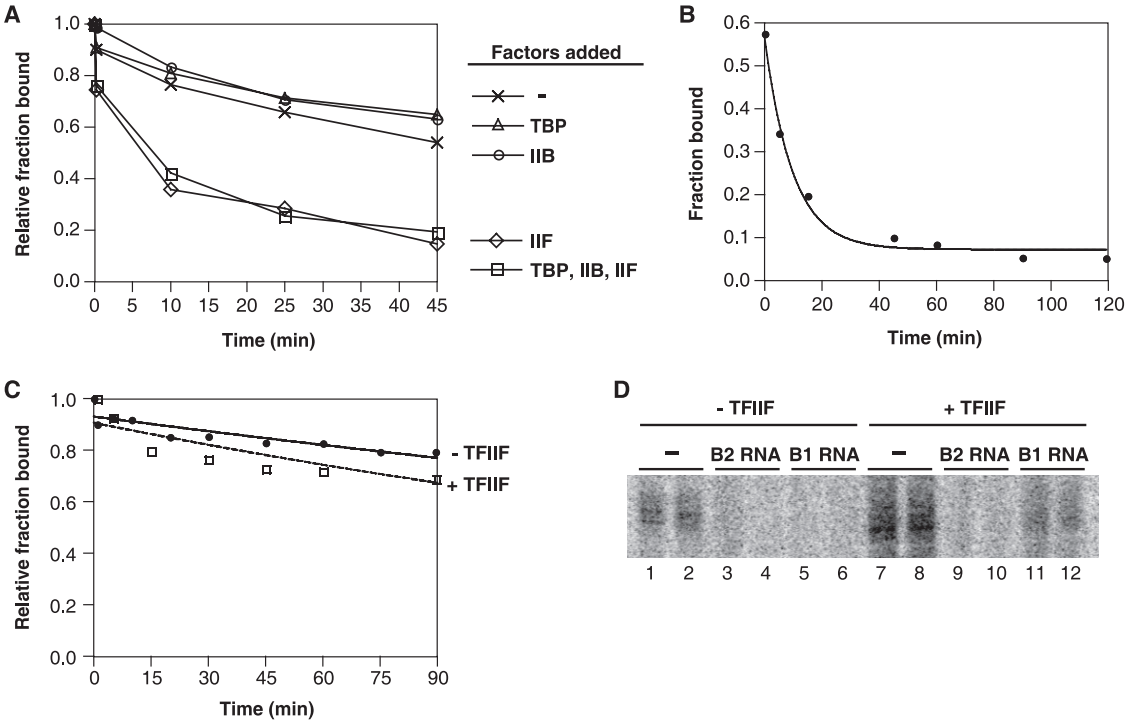

FIG. 3. TFIIF destabilizes the B1 RNA/Pol II complex. (A) Dissociation of the B1 RNA/Pol II complex was monitored over 45 min in the presence of TBP, TFIIB, TFIIF, or all three factors. Assays were performed as described in the legend of Fig. 1C. Three experiments were performed, and representative data are shown. (B) The rate constant for the dissociation of the B1 RNA/Pol II/TFIIF complex is $1.9 \times 10^{-3} \pm 1.0 \times 10^{-3}$ s^{-1}, averaged from three independent experiments, with the errors representing the standard deviations. Shown are representative data in which the fractions of B1 RNA bound to Pol II/TFIIF were quantified and fit to a single exponential decay equation. (C) TFIIF has little effect on the kinetic stability of the B2 RNA/Pol II complex. Shown are representative data in which the fractions of B2 RNA bound to Pol II (−TFIIF) or Pol II/TFIIF (+TFIIF) were quantified and fit to a single exponential decay equation. The rate constants for dissociation are $2.9 \times 10^{-5} \pm 0.1 \times 10^{-5}$ s^{-1} in the absence of TFIIF and $4.5 \times 10^{-5} \pm 1.9 \times 10^{-5}$ s^{-1} in the presence of TFIIF; each is the average of data from two experiments, with the error representing the range in the measurements. (D) B1 RNA inhibits transcription on a bubble template in the absence of TFIIF. ncRNAs (5 nM) were added to reaction mixtures containing Pol II in the presence and absence of TFIIF as indicated; the 40-nt transcript is shown. Two experiments were performed, and representative data are shown.

factors required to support promoter-specific basal transcription from the AdMLP (14). B2 RNA repressed transcription in this system (Fig. 2A, lanes 4 to 6), whereas B1 RNA did not (lanes 7 to 9). Surprisingly, B2 RNA still repressed transcription when added to reaction mixtures that contained B1 RNA prebound to Pol II (Fig. 2A, lanes 10 to 12). Therefore, although B1 RNA can block B2 RNA from binding Pol II in isolation, it cannot block transcriptional repression by B2 RNA. This result raised the possibility that other components of the transcription reaction control the rate of the dissociation of B1 RNA from Pol II.

To determine whether B1 RNA or the B1 RNA/Pol II complex can interact with other components of the transcription system, we analyzed complexes in native gels. When ^{32}P-labeled B1 RNA was incubated with TBP, TFIIB, TFIIF, or all three of these general factors together, the ncRNA was not shifted (Fig. 2B, lanes 8 to 11), whereas Pol II shifted the B1 RNA to a distinct band (lanes 1 and 6). When tested in combination with Pol II, TFIIF supershifted the B1 RNA/Pol II complex, whereas TBP and TFIIB did not (Fig. 2B, lanes 1 to 6). These results show that B1 RNA/Pol II/TFIIF ternary complexes form and suggest that they do so by Pol II simultaneously binding TFIIF and B1 RNA.

We next tested the effect of the general transcription factors on the kinetic stability of B1 RNA/Pol II complexes. We incubated ^{32}P-labeled B1 RNA with Pol II in the presence of TBP, TFIIB, and/or TFIIF and then added a large excess of unlabeled B1 RNA and monitored the fraction of ^{32}P-labeled B1 RNA bound to Pol II over time. We found that incubation with all three factors together destabilized B1 RNA/Pol II complexes (Fig. 3A). When each factor was tested individually, we discovered that TFIIF facilitated the dissociation of B1 RNA from Pol II, whereas TBP and TFIIB did not (Fig. 3A). We then performed experiments to measure the rate constant for dissociation of the B1 RNA/Pol II/TFIIF complex and found it to be $1.9 \times 10^{-3} \pm 1.0 \times 10^{-3}$ s^{-1} (Fig. 3B), which is approximately 12-fold greater than the rate constant for the dissociation of B1 RNA/Pol II complexes in the absence of TFIIF (Fig. 1D). In contrast, TFIIF had little effect on the rate at which B2 RNA/Pol II complexes dissociated (Fig. 3C). From these data, we conclude that TFIIF facilitates the dissociation of B1 RNA from Pol II.

Discovering this new role for TFIIF raised the question of whether B1 RNA might repress transcription in the absence of TFIIF. TFIIF is required for transcription at most promoters. Pol II by itself, however, can transcribe in vitro from a template containing a preformed transcription bubble (10); such a system provided the opportunity to probe transcriptional repres-

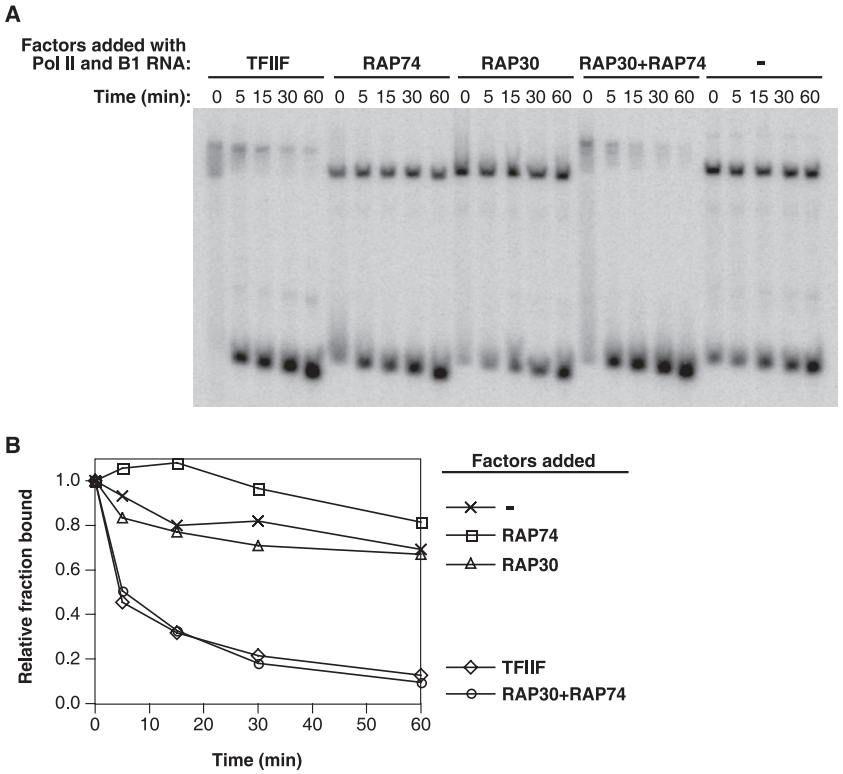

FIG. 4. Dissociation of B1 RNA/Pol II by TFIIF requires both subunits of TFIIF. (A) Both RAP30 and RAP74 are required to facilitate the dissociation of B1 RNA from Pol II. Dissociation assays were performed as described in the legend to Fig. 1C. Two experiments were performed, and representative data are shown. (B) Plot of the EMSA data shown in panel A.

sion by B1 RNA in the absence of TFIIF. We created an AdMLP template containing a mismatched sequence from positions −9 to +3, thereby generating a heteroduplex that mimicked the transcription bubble. As shown in Fig. 3D, B1 RNA repressed transcript synthesis by Pol II alone on the bubble template, and this repression was partially relieved in the presence of TFIIF. In contrast, TFIIF did not relieve repression by B2 RNA under these conditions.

TFIIF is a heterotetramer comprised of two RAP30 subunits and two RAP74 subunits. Both of these subunits are thought to be required for transcription in general and are absolutely required for transcription from the AdMLP in vitro (3, 4, 25). To determine whether either RAP30 or RAP74 alone could facilitate the dissociation of B1 RNA from Pol II, we expressed and purified the individual subunits. Monitoring of the stability of B1 RNA/Pol II complexes over time in the presence of RAP30 or RAP74 revealed that neither subunit alone facilitated the dissociation of B1 RNA/Pol II complexes (Fig. 4A and B). Importantly, adding RAP30 and RAP74 together resulted in the facilitated dissociation of B1 RNA/Pol II similar to that observed with TFIIF that was purified as a heterotetramer. Moreover, adding RAP30 and RAP74 together to B1 RNA and Pol II resulted in a supershifted complex, whereas neither subunit alone caused a supershift. Hence, it is the transcriptionally active form of TFIIF containing both subunits that can specifically associate with the B1 RNA/Pol II complex and facilitate its dissociation.

scAlu RNA is similar to B1 RNA in sequence and secondary structure, and these RNAs share important functional properties: both bind Pol II, neither represses transcription, and neither contains a repression domain (19). We therefore asked whether TFIIF would affect the scAlu RNA/Pol II complex similarly to the B1 RNA/Pol II complex. As shown in Fig. 5, scAlu RNA/Pol II complexes are kinetically stable, with a half-time for dissociation of approximately 1 h. TFIIF both supershifted the scAlu RNA/Pol II complex and decreased its kinetic stability. Hence, TFIIF has a general ability to dissociate Pol II from ncRNAs that lack repression domains (e.g., B1 RNA and scAlu RNA).

Adding a repression domain from Alu RNA to B1 RNA makes B1 RNA resistant to facilitated dissociation by TFIIF. We previously found that incorporating either of two repression domains from Alu RNA (the A region or the L region) into B1 RNA turned it into a transcriptional repressor (19). We hypothesized that the two chimeric ncRNAs, B1-A RNA and B1-L RNA, would bind Pol II stably in the presence of TFIIF. To test this, we formed complexes between Pol II and B1-A RNA, B1-L RNA, or B1 RNA in the absence and presence of TFIIF. After challenge with the respective unlabeled RNA in excess, we monitored the fraction of bound ^{32}P-labeled RNA over time. As shown in Fig. 6, chimeric RNAs containing B1 RNA and either the L or the A repression domain from Alu RNA were resistant to facilitated dissociation by TFIIF. We conclude that the presence of a repression domain causes an ncRNA to remain more stably bound to Pol II in the presence of TFIIF, and this property is an important

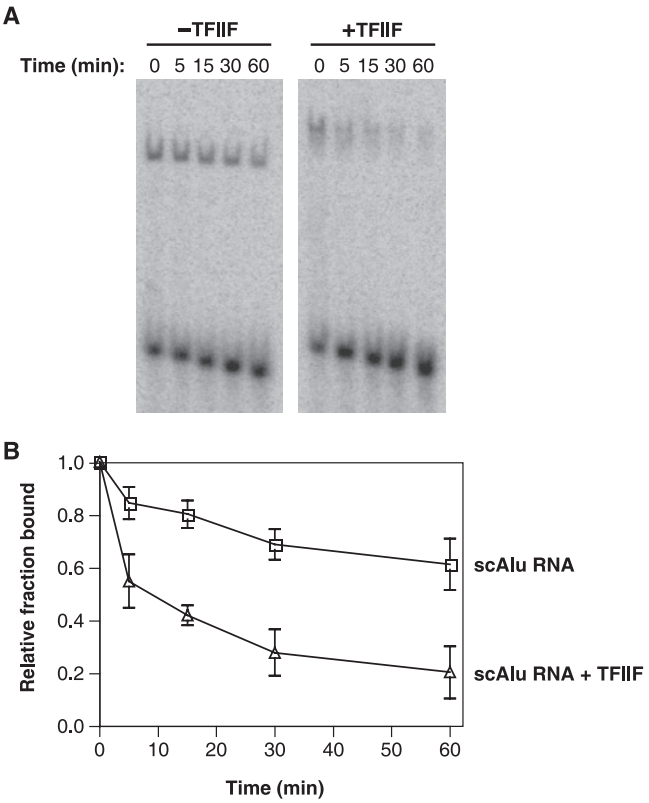

FIG. 5. TFIIF facilitates the dissociation of scAlu RNA from Pol II. (A) TFIIF supershifts and destabilizes the scAlu RNA/Pol II complex. Dissociation assays were performed as described in the legend to Fig. 1C. Two experiments were performed, and representative data are shown. (B) The relative fraction of ncRNA bound was quantified, averaged between two experiments, and plotted against time. The error bars represent the ranges in the measurements.

aspect of the mechanism by which ncRNAs repress transcription.

We further asked whether a physical linkage of a repression domain to B1 RNA was required to stabilize the interaction between B1 RNA and Pol II in the presence of TFIIF. For these experiments, we used the A region, which is a single-stranded A-rich sequence from Alu RNA that we previously showed could neither repress transcription nor bind to Pol II in isolation (19). We incubated B1 RNA and the isolated A region with Pol II in the absence and presence of TFIIF and measured the dissociation of complexes over time. The isolated A region did not stabilize the B1 RNA/Pol II/TFIIF complex (Fig. 6).

DISCUSSION

Here we describe a new function for TFIIF: it has the ability to destabilize complexes between Pol II and ncRNAs that are not transcriptional repressors. We found that B1 RNA, which does not repress transcription in vitro, forms a kinetically stable complex with Pol II. B1 RNA competes with the transcriptional repressor B2 RNA for binding to Pol II and can block the association of B2 RNA with Pol II, indicating that these two ncRNAs bind to the same or overlapping sites on the

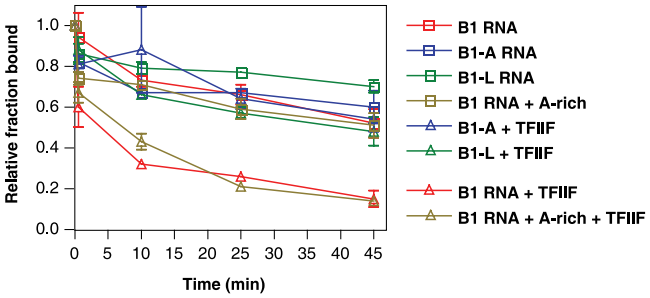

FIG. 6. The addition of a repression domain from Alu RNA onto B1 RNA stabilizes its interaction with Pol II in the presence of TFIIF. Dissociation assays with Pol II or Pol II/TFIIF were performed as described in the legend to Fig. 1C using the following four ncRNAs: B1 RNA, B1-A RNA, B1-L RNA, and B1 RNA plus the isolated A region. The relative fraction of ncRNA bound was quantified, averaged between two experiments, and plotted against time. The error bars represent the ranges in the measurements.

polymerase. In spite of this competition in binding, B1 RNA was unable to protect Pol II from B2 RNA-mediated repression in a minimal in vitro transcription system. This suggested that a component(s) present in the minimal transcription system controlled the association of B1 RNA with Pol II, which led to the discovery that TFIIF facilitates the dissociation of Pol II from B1 RNA and the related scAlu RNA. This activity required both RAP30 and RAP74; hence, it is the biologically active form of TFIIF that removes ncRNAs from Pol II. Finally, we found that the addition of either of two different ncRNA repression domains onto B1 RNA attenuated the TFIIF-facilitated dissociation from Pol II. Together, these results reveal a new function for TFIIF: facilitating the removal of tightly bound nonrepressive ncRNAs from Pol II. This activity would allow TFIIF, which associates with Pol II in cells, to prevent nonrepressor RNAs from occupying the ncRNA docking site on Pol II such that specific repressor ncRNAs such as B2 RNA and Alu RNA can bind the polymerase and inhibit transcription.

The observation that B1 RNA and B2 RNA compete with one another for binding Pol II supports our previous proposal that human Pol II has a docking site that binds specific ncRNAs with high affinity. The first RNA that we found to bind with high affinity and specificity to Pol II, a synthetic oligonucleotide consisting entirely of guanosines (rG-oligo) (13), also competes with B2 RNA for binding to the polymerase (6). The observation that both B1 RNA and rG-oligo compete with B2 RNA for binding to the polymerase argues strongly that these RNAs all bind overlapping sites on the polymerase. It is, however, possible that these RNAs bind different sites on Pol II and that each one causes a conformational change in the polymerase that excludes the binding of the other. We imagine that other cellular ncRNAs will be found to bind to the high-affinity ncRNA docking site on Pol II and control transcription, perhaps using a diversity of mechanisms.

The finding that TFIIF can control the interaction between Pol II and nonrepressor ncRNAs raises a number of intriguing questions. First, how does TFIIF facilitate the dissociation of B1 RNA and scAlu RNA from Pol II? TFIIF does not simply compete with the RNAs for binding Pol II because it can

supershift both B1 RNA/Pol II and scAlu RNA/Pol II complexes. TFIIF could interact directly with B1 RNA to remove it from Pol II; however, our EMSAs did not detect such an interaction. Alternatively, TFIIF could confer a structural change in Pol II that results in the destabilization of the contacts between B1 RNA and the polymerase; in this model, contacts between B2 RNA and Pol II would remain stable. Future structural and mechanistic studies will be necessary to fully understand the mechanism by which TFIIF destabilizes complexes between Pol II and ncRNAs that lack repression domains.

Importantly, repression domains stabilize the binding of ncRNAs to Pol II in the presence of TFIIF. This demonstrates a new function for these domains. Similar rates of dissociation from Pol II were observed for B1, B1-A, and B1-L RNAs (Fig. 6), showing that the additional contacts between Pol II and a repression domain do not substantially change complex stability in the absence of TFIIF. Our previous studies indicated that repression domains inhibit transcription by interfering with contacts between Pol II and DNA in complexes at promoters (28). Perhaps specific sequences within repression domains mediate this mechanism of transcriptional inhibition, while others confer resistance to TFIIF-facilitated dissociation. Although B1 RNA does not contain a repression domain, we found that it represses transcription by Pol II alone on a bubble template. How the mechanism of repression in this system relates to the mechanism of repression by B2 and Alu RNAs assembled in complete preinitiation complexes remains unclear. Importantly, however, the finding that TFIIF partially alleviates repression by B1 RNA on a bubble template is consistent with the model that TFIIF functions to destabilize complexes between Pol II and ncRNAs that are not repressors of transcription from preinitiation complexes.

It is feasible that inside cells, TFIIF functions to remove nonrepressor ncRNAs that bind tightly to Pol II (e.g., B1 RNA and scAlu RNA) and, in addition, may evict other RNAs that spuriously bind Pol II. The observation that the biologically active form of TFIIF having both the RAP30 and RAP74 subunits is needed to dissociate B1 RNA from Pol II in vitro supports this idea. Pol II has multiple nucleic acid binding sites: a DNA channel for binding its template, an RNA channel for allowing the exit of its product RNA, and an ncRNA docking site. Previously, TFIIF was shown to prevent Pol II from making nonproductive contacts with DNA (5, 11); we now propose that TFIIF also evolved to control the association of RNAs with the ncRNA docking site on Pol II. Specifically, TFIIF could prevent B1 RNA, the levels of which rise upon heat shock, from binding to Pol II in heat-shocked cells so that B2 RNA can bind and function as a transcriptional repressor.

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REFERENCES

1. **Allen, T. A., S. Von Kaenel, J. A. Goodrich, and J. F. Kugel.** 2004. The SINE-encoded mouse B2 RNA represses mRNA transcription in response to heat shock. Nat. Struct. Mol. Biol. **11:**816–821.
2. **Barrandon, C., B. Spiluttini, and O. Bensaude.** 2008. Non-coding RNAs regulating the transcriptional machinery. Biol. Cell **100:**83–95.
3. **Burton, Z. F., M. Killeen, M. Sopta, L. G. Ortolan, and J. Greenblatt.** 1988. RAP30/RAP74: a general initiation factor that binds to RNA polymerase II. Mol. Cell. Biol. **8:**1602–1613.
4. **Chang, C.-H., C. F. Kostrub, and Z. F. Burton.** 1993. RAP30/74 (transcription factor IIF) is required for promoter escape by RNA polymerase II. J. Biol. Chem. **268:**20482–20489.
5. **Conaway, J. W., and R. C. Conaway.** 1990. An RNA polymerase II transcription factor shares functional properties with Escherichia coli sigma 70. Science **248:**1550–1553.
6. **Espinoza, C. A., T. A. Allen, A. R. Hieb, J. F. Kugel, and J. A. Goodrich.** 2004. B2 RNA binds directly to RNA polymerase II to repress transcript synthesis. Nat. Struct. Mol. Biol. **11:**822–829.
7. **Espinoza, C. A., J. A. Goodrich, and J. F. Kugel.** 2007. Characterization of the structure, function, and mechanism of B2 RNA, an ncRNA repressor of RNA polymerase II transcription. RNA **13:**583–596.
8. **Goodrich, J. A., and J. F. Kugel.** 2009. From bacteria to humans, chromatin to elongation, and activation to repression: the expanding roles of noncoding RNAs in regulating transcription. Crit. Rev. Biochem. Mol. Biol. **44:**3–15.
9. **Kadonaga, J. T.** 2004. Regulation of RNA polymerase II transcription by sequence-specific DNA binding factors. Cell **116:**247–257.
10. **Keene, R. G., and D. S. Luse.** 1999. Initially transcribed sequences strongly affect the extent of abortive initiation by RNA polymerase II. J. Biol. Chem. **274:**11526–11534.
11. **Killeen, M. T., and J. F. Greenblatt.** 1992. The general transcription factor RAP30 binds to RNA polymerase II and prevents it from binding nonspecifically to DNA. Mol. Cell. Biol. **12:**30–37.
12. **Kramerov, D. A., and N. S. Vassetzky.** 2005. Short retroposons in eukaryotic genomes. Int. Rev. Cytol. **247:**165–221.
13. **Kugel, J. F., and J. A. Goodrich.** 2002. Translocation after synthesis of a four-nucleotide RNA commits RNA polymerase II to promoter escape. Mol. Cell. Biol. **22:**762–773.
14. **Kugel, J. F., and J. A. Goodrich.** 2003. In vitro studies of the early steps of RNA synthesis by human RNA polymerase II. Methods Enzymol. **370:**687–701.
15. **Li, B., M. Carey, and J. L. Workman.** 2007. The role of chromatin during transcription. Cell **128:**707–719.
16. **Li, T., J. Spearow, C. M. Rubin, and C. W. Schmid.** 1999. Physiological stresses increase mouse short interspersed element (SINE) RNA expression in vivo. Gene **239:**367–372.
17. **Liu, W. M., W. M. Chu, P. V. Choudary, and C. W. Schmid.** 1995. Cell stress and translational inhibitors transiently increase the abundance of mammalian SINE transcripts. Nucleic Acids Res. **23:**1758–1765.
18. **Maraia, R. J., C. T. Driscoll, T. Bilyeu, K. Hsu, and G. J. Darlington.** 1993. Multiple dispersed loci produce small cytoplasmic Alu RNA. Mol. Cell. Biol. **13:**4233–4241.
19. **Mariner, P. D., R. D. Walters, C. A. Espinoza, L. F. Drullinger, S. D. Wagner, J. F. Kugel, and J. A. Goodrich.** 2008. Human Alu RNA is a modular transacting repressor of mRNA transcription during heat shock. Mol. Cell **29:**499–509.
20. **Matera, A. G., U. Hellmann, M. F. Hintz, and C. W. Schmid.** 1990. Recently transposed Alu repeats result from multiple source genes. Nucleic Acids Res. **18:**6019–6023.
21. **Naar, A. M., B. D. Lemon, and R. Tjian.** 2001. Transcriptional coactivator complexes. Annu. Rev. Biochem. **70:**475–501.
22. **Schmid, C. W.** 1998. Does SINE evolution preclude Alu function? Nucleic Acids Res. **26:**4541–4550.
23. **Schmid, C. W., and W. R. Jelinek.** 1982. The Alu family of dispersed repetitive sequences. Science **216:**1065–1070.
24. **Sinnett, D., C. Richer, J. M. Deragon, and D. Labuda.** 1991. Alu RNA secondary structure consists of two independent 7 SL RNA-like folding units. J. Biol. Chem. **266:**8675–8678.
25. **Tan, S., T. Aso, R. C. Conaway, and J. W. Conaway.** 1994. Roles for both the RAP30 and RAP74 subunits of transcription factor IIF in transcription initiation and elongation by RNA polymerase II. J. Biol. Chem. **269:**25684–25691.
26. **Thomas, M. C., and C. M. Chiang.** 2006. The general transcription machinery and general cofactors. Crit. Rev. Biochem. Mol. Biol. **41:**105–178.
27. **Weaver, J. R., J. F. Kugel, and J. A. Goodrich.** 2005. The sequence at specific positions in the early transcribed region sets the rate of transcript synthesis by RNA polymerase II in vitro. J. Biol. Chem. **280:**39860–39869.
28. **Yakovchuk, P., J. A. Goodrich, and J. F. Kugel.** 2009. B2 RNA and Alu RNA repress transcription by disrupting contacts between RNA polymerase II and promoter DNA within assembled complexes. Proc. Natl. Acad. Sci. U. S. A. **106:**5569–5574.