Structure and Function of RNA Polymerase II Elongation Factor ELL

IDENTIFICATION OF TWO OVERLAPPING ELL FUNCTIONAL DOMAINS THAT GOVERN ITS INTERACTION WITH POLYMERASE AND THE TERNARY ELONGATION COMPLEX

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The human ELL gene on chromosome 19p13.1 undergoes frequent translocations with the trithorax-like MLL gene on chromosome 11q23 in acute myeloid leukemia. Recently, the human ELL gene was shown to encode an RNA polymerase II elongation factor that activates elongation by suppressing transient pausing by polymerase at many sites along the DNA. In this report, we identify and characterize two overlapping ELL functional domains that govern its interaction with RNA polymerase II and the ternary elongation complex. Our findings reveal that, in addition to its elongation activation domain, ELL contains a novel type of RNA polymerase II interaction domain that is capable of negatively regulating polymerase activity in promoter-specific transcription initiation in vitro. Notably, the MLL-ELL translocation results in deletion of a portion of this functional domain, and ELL mutants lacking sequences deleted by the translocation bind RNA polymerase II and are fully active in elongation, but fail to inhibit initiation. Taken together, these results raise the possibility that the MLL-ELL translocation could alter ELL-RNA polymerase II interactions that are not involved in regulation of elongation.

Eukaryotic messenger RNA synthesis is a complex biochemical process governed by the concerted action of a diverse collection of general transcription factors that control the activity of RNA polymerase II at both the initiation and elongation stages of transcription (1–3). At least five general initiation factors (TFIIB, TFIID, TFIIE, TFIIF, and TFIIIH)1 have been identified in eukaryotic cells and found to promote selective binding of RNA polymerase II to promoters and to support a basal level of transcription (1, 2). In addition, five general elongation factors (P-TEFb, SII, TFIIF, Elongin (SIII), and ELL)2 have been defined biochemically and found to increase elongation factors (P-TEFb, SII, TFIIF, Elongin (SIII), and basal level of transcription (1, 2). In addition, five general binding of RNA polymerase II to promoters and to support a identified in eukaryotic cells and found to promote selective

1 The abbreviations used are: TF, transcription factor; ALL, acute lymphoblastic leukemia; AdML, adenovirus major late promoter; TBP, TATA box-binding protein.
2 Of the general elongation factors, P-TEFb and SII promote elongation by preventing RNA polymerase II from arresting transcription prematurely. P-TEFb catalyzes the conversion of early, arrest-prone elongation complexes into productive elongation complexes (6, 7); SII protects RNA polymerase II from arrest at a variety of transcriptional impediments, including specific DNA sequences that act as intrinsic arrest sites and some nucleoprotein complexes and DNA bound drugs (8). The remaining general elongation factors, TFIIF (9), Elongin (SIII) (10, 11), and ELL (12), all appear to increase the overall rate of elongation by RNA polymerase II by suppressing transient pausing by polymerase at many sites along the DNA template.

Recently, Elongin (SIII) and ELL have been implicated in the development of human cancers. Elongin (SIII) is a potential target for regulation by the product of the von Hippel-Lindau tumor suppressor gene (13, 14), which is mutated in the majority of clear-cell renal carcinomas and in families with von Hippel-Lindau disease, a rare genetic disorder that predisposes individuals to a variety of cancers including clear-cell renal carcinomas, hemangioblastomas and hemangiomas, and pheochromocytomas (15). The ELL (qeleven-nineteen lysine-rich leukemia) gene on chromosome 19p13.1 was first identified as one of several genes that undergo chromosomal translocations with the MLL (mixed lineage leukemia) gene on chromosome 11q23 in a variety of different leukemias (16, 17). The MLL gene encodes a ~4000-amino acid protein that contains N-terminal A-T hook DNA binding and methyltransferase-like domains and a C-terminal region that resembles the product of the Drosophila trithorax gene (18–20). The chimeric genes generated by MLL translocations all encode proteins that contain the same N-terminal portion of MLL, including its A-T hook DNA binding and methyltransferase-like domains (21). The product of the chimeric MLL-ELL gene includes all but the first 45 amino acids of the 621-amino acid ELL protein (16, 17).

While the precise roles of MLL-fusion proteins in the development of leukemia are not clear, substantial evidence suggests that the MLL translocation partners make a critical contribution to the disease process. First, different leukemic phenotypes correlate with specific translocations and thus with specific fusion partners; for example, a t(4;11)(q21;q23) translocation, which generates an MLL-AF4 fusion, is found in acute lymphoblastic leukemias (ALL) and preB cell ALL (preB-ALL) (20, 22–25), whereas t(11;19)(q23;p13.1) (MLL-ELL) and t(9;11)(p22;q23) (MLL-AF9) translocations are associated with acute myeloid leukemias (16, 17, 25, 26). Second, targeted disruption of the MLL gene in mice causes defects in hox gene expression and segmentation, but is not sufficient for development of the leukemic phenotype (27). Finally, replacement of the normal MLL gene with an MLL-AF9 chimera, but not with

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an MLL-myc chimera, leads to acute myeloid leukemia in mice (28). Based on these observations, a thorough understanding of the functional properties of ELL and the other MLL fusion partners is likely to be important for understanding the roles of MLL-containing chimeras in leukemogenesis.

As part of our effort to understand how ELL regulates the activity of the RNA polymerase II elongation complex, we sought to identify and characterize ELL sequences important for its function. These studies led to the discovery that, in addition to its elongation activation domain, ELL contains a novel functional domain that is capable of negatively regulating RNA polymerase II activity in promoter-specific transcription initiation in vitro and that can be disrupted without affecting ELL elongation activity. Remarkably, the MLL-ELL translocation results in deletion of a portion of this domain, and ELL mutants lacking sequences deleted by the translocation bind RNA polymerase II and are fully active in elongation, but do not inhibit promoter-specific initiation. Here we present these findings, which bring to light new features of the ELL protein and its ability to control the activity of RNA polymerase II.

**EXPERIMENTAL PROCEDURES**

**Materials—**Unlabeled ultrapure ribonucleoside 5′-triphosphates were purchased from Pharmacia Biotech, Inc. [α-32P]CTP (650 Ci/ mmol) was obtained from Amersham Corp. Bovine serum albumin (Fraction V) was from ICN Immunobiologicals. Guanidine hydrochloride (Sequana grade) was purchased from Pierce Chemical Co. Heparin and isopropyl β-D-thiogalactoside were obtained from Sigma. Recombinant placentical ribonuclease inhibitor (RNasin) was from Promega.

Low melting temperature agarose was purchased from CLON-TECH. Phenylmethylsulfonyl fluoride (Pentex fraction V) was from ICN Immunobiologicals. Guanidine hydrochloride (Sequana grade) was purchased from Pierce Chemical Co. Heparin and isopropyl β-D-thiogalactoside were obtained from Sigma. Recombinant placentical ribonuclease inhibitor (RNasin) was from Promega.

Low melting temperature agarose was purchased from CLON-TECH. Phenylmethylsulfonyl fluoride was from Sigma and was dissolved in dimethyl sulfoxide to 1 M. Polyvinyl alcohol (average molecular weight 30,000–70,000) was obtained from Sigma and was dissolved in water to 20% (w/v) and centrifuged at 100,000 × g for 30 min prior to use.

**DNA Templates for Transcription—**pDN-AdML (29) and pCPGR220S/PX (30) plasmid DNA were isolated from Escherichia coli using the Trition-lysozyme method (31). Plasmid DNA was banded twice in CsCl-ethidium bromide density gradients. Oligo(dC)-tailed pDNA templates were prepared as previously described (29). A restriction fragment prepared by digestion of pDN-AdML DNA with EcoRI and NdeI was used as template in runoff transcription assays. The fragment was purified from 1.5% low melting temperature agarose gels using GELase (Epicentre Technologies) according to the manufacturer's instructions.

**Expression and purification of ELL—**ELL and ELL mutants were expressed in E. coli using the M13mpET bacteriophage expression system (12). A 50-ml culture of E. coli strain JM109(DE3) was grown to an OD600 of 0.3 in Luria broth containing 2.5 mM MgCl2 at 37 °C with gentle shaking. Cells were then infected with M13mpET carrying a cDNA encoding N-terminal 6-histidine-tagged ELL or ELL mutants at a multiplicity of infection of 20. After 3.5 h at 37 °C, cells were shifted to 30 °C, induced with 1 mM isopropyl β-D-thiogalactoside, and incubated for an additional 12 h at 30 °C. Cells were collected by centrifugation at 2000 × g for 15 min at 4 °C. The cell pellet was resuspended in 7 ml of ice-cold 30 mM Tris-HCl (pH 8.0), 1 mM EDTA, and 20% (v/v) sucrose and kept on ice for 10 min. Cells were collected by centrifugation at 6000 × g for 10 min at 4 °C. The cell pellet was resuspended in 7 ml of ice-cold water and kept on ice for 30 min. Osmotically shocked cells were collected by centrifugation at 6000 × g for 10 min at 4 °C and resuspended in 7 ml of ice-cold 20 mM Tris-HCl (pH 7.9), 10 mM imidazole (pH 7.9), 0.5 mM NaCl, 0.2 mM EDTA, 1 mM diithiothreitol, 0.5 mM phenylmethylsulfonyl fluoride, and 0.1 mg/ml lysozyme and kept on ice for 30 min. After one cycle of freeze-thaw, the suspension was centrifuged at 100,000 × g for 30 min at 4 °C. Incubation bodies were solubilized by resuspension in 5 ml of ice-cold 50 mM Tris-HCl (pH 8.0) containing 6 M guanidine hydrochloride. The resuspended and solubilized suspension was centrifuged at 100,000 × g for 30 min. Histidine-tagged ELL or ELL mutant proteins were purified from the supernatant by Ni2+-nitritotriacetic acid-agarose affinity chromatography using ProBond™ metal-binding resin (Invitrogen). Ni2+ chromatography was performed at 4 °C. 5 ml of supernatant was applied to a 1-ml packed bed volume of Ni2+-agarose pre-equilibrated in buffer A (20 mM Tris-HCl (pH 7.9), 10 mM imidazole (pH 7.9), 0.5 mM phenylmethylsulfonyl fluoride, and 6 mM guanidine hydrochloride). The Ni2+ column was washed with buffer A containing 40 mM imidazole (pH 7.9), and ELL protein was eluted with buffer A containing 300 mM imidazole (pH 7.9). To prepare ELL protein for transcription assays, fractions containing guanidine-solubilized ELL were dialyzed for 2 h against 40 mM Hepes-NaOH (pH 7.9), 100 mM KCl, 50 μM ZnSO4, and 10% (v/v) glycerol. The final concentrations of the solubilized and renatured ELL and ELL mutants were estimated by comparison to silver-stained protein standards.

**Preparation of RNA Polymerase II and Transcription Factors—**RNA polymerase II (32) and TFIIH (rat δ, TSK DEAE-5 FW fraction) (33) were purified as described from rat liver nuclear extracts. Recombinant yeast TBP (34) and rat TFIIH (rat α (α) (35)) were expressed in E. coli and purified as described. Recombinant TFII F was prepared as described (36), except that the 56-kDa subunit was expressed in E. coli strain BL21(DE3)-pLysS. Recombinant TFII F was purified as described (37) from E. coli strain JM109(DE3) co-infected with M13mpET-RAP30 and M13mpET-RAP74.

**Assay of Runoff Transcription—**All reaction mixtures were 60 μl. As indicated in the figure legends, preinitiation complexes were assembled by preincubation of ~20 ng of template DNA (EcoRI to NdeI fragment from pDN-AdML), ~10 ng of recombinant TFII F, ~10 ng of recombinant TFII F, ~7 ng of recombinant TFII F, ~40 ng of TFII H, ~20 ng of recombinant TBP, ~0.01 unit of RNA polymerase II, and 8 units of RNasin. The reaction mixture was incubated at 28 °C in 20 mM Hepes-NaOH (pH 7.9), 20 mM Tris-HCl (pH 7.9), 60 mM KCl, 2 mM diithiothreitol, 0.5 mg/ml bovine serum albumin, 2% (v/v) polyvinyl alcohol, and 3% (v/v) glycerol for 30 min at 28 °C. Transcription was initiated by addition of MgCl2 and ribonucleoside triphosphates as indicated in the figure legends and carried out for the times indicated. After incubation of reaction mixtures at 28 °C for the times indicated in the figure legends, runoff transcripts were analyzed by electrophoresis through 6% polyacrylamide gels containing 7 M urea and 1 × TBE (20 mM Tris borate, 1 mM EDTA). For some experiments, transcription was quantitated using a Molecular Dynamics PhosphorImager.

**Oligo(dC)-tailed Template Assay of Elongation by RNA Polymerase II—**Pulse-chase assays were carried out essentially as described (12). ~0.01 unit of RNA polymerase II and ~100 ng of oligo(dC)-tailed pCPGR220S/PX were incubated at 28 °C in 20 mM Hepes-NaOH (pH 7.9), 30 mM Tris-HCl (pH 7.9), 65 mM KCl, 50 μM ZnSO4, 0.2 mM diithiothreitol, 0.5 mg/ml bovine serum albumin, 2% (v/v) polyvinyl alcohol, 3% (v/v) glycerol, 3 units of RNasin, 7 mM MgCl2, 50 μM ATP, 50 μM GTP, 1.8 μM CTP, and 10 μCi of [α-32P]CTP for the times indicated in the figure legends. Transcripts were analyzed by electrophoresis through 6% polyacrylamide gels containing 7 M urea and 1 × TBE.

**Preparation of mAb 8WG16—**Monoclonal antibody 8WG16 (38) was purified from ascites fluid by adsorption to Affi-Gel protein A-agarose (Bio-Rad) according to the manufacturer's instructions. The 8WG16 hybridoma was a generous gift from N. Thompson and R. Burgess (University of Wisconsin-Madison).

**RESULTS**

**ELL Can Both Stimulate Elongation and Inhibit Promoter-specific Initiation by RNA Polymerase II—**We previously demonstrated that ELL is capable of activating the rate of elongation by RNA polymerase II in vitro during both promoter-dependent and promoter-independent transcription (12). In experiments further investigating the effect of ELL on transcription by RNA polymerase II, we made the surprising discovery that ELL is also capable of potently inhibiting initiation. As described below, further analysis revealed that ELL inhibits initiation at least in part by binding to RNA polymerase II and blocking its interaction with the TATA factor and TFII B at the promoter.

The effect of ELL on transcription by RNA polymerase II was investigated using the AdML promoter and a transcription system reconstituted, in the presence and absence of ELL, with recombinant TBP, TFII B, TFII E, TFII F, and purified RNA polymerase II and TFII H from rat liver. In the pulse-chase experiment of Fig. 1, preinitiation complexes were assembled at the AdML promoter by preincubation of linearized pDN-AdML DNA (29) with TBP, TFII B, TFII E, TFII F, and RNA polymerase II. Transcription was initiated by addition of ATP, GTP, UTP, and a low concentration of [α-32P]CTP, which
is sufficient for synthesis of short, highly radioactive transcripts. After 5 min, short transcripts were chased for varying times following addition of a 50-fold excess of nonradioactive CTP. Consistent with our previous results (12), addition of ELL to transcription reactions after initiation, during the chase phase of the reaction, led to a significant increase in the rate of accumulation of full-length runoff transcripts and, thus, to an increase in the rate of elongation by RNA polymerase II (Fig. 1, compare lanes 1–5 and lanes 6–10). In contrast, addition of ELL to transcription reactions before assembly of the preinitiation complex led to a significant reduction in the rate of elongation by RNA polymerase II (Fig. 1, compare lanes 6–10). Furthermore, even though the yield of full-length runoff transcripts was reduced when ELL was added to transcription reactions before initiation, the kinetics of appearance of those full-length runoff transcripts that were synthesized under these conditions was similar to the kinetics of appearance of full-length runoff transcripts synthesized when ELL was added to transcription reactions after initiation. Taken together, these results suggested that ELL might inhibit transcription by reducing the total number of initiation events, rather than by inhibiting elongation.

To address this possibility directly, an abortive initiation assay was used to determine whether ELL is capable of inhibiting synthesis of the first phosphodiester bond of transcripts initiated at the AdML promoter. RNA polymerase II will utilize dinucleotides to prime synthesis of promoter-specific transcripts from a dinucleotide-primed abortive initiation assay has been widely used in studies investigating the requirements for synthesis of the first phosphodiester bond of nascent transcripts by both prokaryotic and eukaryotic RNA polymerases (39–42).

As shown previously, and as predicted from the sequence of the AdML promoter (Fig. 2A), RNA polymerase II and the general initiation factors will synthesize the trinucleotide product, which is underlined. B, addition of ELL to transcription reactions after initiation, during the chase phase of the reaction, led to a significant increase in the rate of elongation by RNA polymerase II (Fig. 1, compare lanes 1–5 and lanes 6–10). Furthermore, even though the yield of full-length runoff transcripts was reduced when ELL was added to transcription reactions before initiation, the kinetics of appearance of those full-length runoff transcripts that were synthesized under these conditions was similar to the kinetics of appearance of full-length runoff transcripts synthesized when ELL was added to transcription reactions after initiation. Taken together, these results suggested that ELL might inhibit transcription by reducing the total number of initiation events, rather than by inhibiting elongation.

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As shown previously, and as predicted from the sequence of the AdML promoter (Fig. 2A), RNA polymerase II and the general initiation factors will synthesize the trinucleotide CpApC at the AdML promoter when provided with the dinucleotide primer CpA and [α-32P]CTP. The non-template strand sequence corresponding to the CpApC product is underlined. B, addition of ELL to transcription reactions after initiation, during the chase phase of the reaction, led to a significant increase in the rate of elongation by RNA polymerase II (Fig. 1, compare lanes 6–10). Furthermore, even though the yield of full-length runoff transcripts was reduced when ELL was added to transcription reactions before initiation, the kinetics of appearance of those full-length runoff transcripts that were synthesized under these conditions was similar to the kinetics of appearance of full-length runoff transcripts synthesized when ELL was added to transcription reactions after initiation. Taken together, these results suggested that ELL might inhibit transcription by reducing the total number of initiation events, rather than by inhibiting elongation.

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transcription was initiated by addition of 50 μM ATP, 50 μM UTP, 10 μM CTP, 10 μM GTP to the AdML promoter for 20 min. ELL and the remaining transcription proteins were then added to reaction mixtures, which were incubated for an additional 30 min at 28 °C. Transcripts were analyzed by electrophoresis through 6% polyacrylamide (29:1 acrylamide/bis-acrylamide) gels containing 7.0 M urea and 1 × TBE.

To determine which step or steps is inhibited by ELL during assembly of the preinitiation complex, order of addition experiments were performed using a heparin challenge protocol (29, 44). Elongation of nascent transcripts longer than 4–9 nucleotides is resistant to heparin, whereas assembly of the preinitiation complex and transcription initiation are not. Thus, if heparin is added to promoter-specific transcription reactions shortly after addition of ribonucleoside triphosphates, full-length runoff transcripts will be synthesized only from those promoters at which initiation has already occurred. Furthermore, heparin inhibits ELL activation of elongation by RNA polymerase II (data not shown); consequently, the heparin challenge assay should detect the effect of ELL on assembly of the preinitiation complex or synthesis of the first few phosphodiester bonds of promoter-specific transcripts, but not on further elongation of transcripts.

In the experiments of Fig. 3, various combinations of RNA polymerase II and initiation factors were first preincubated with the AdML promoter for 20 min. ELL and the remaining transcription proteins were then added to reactions. After 20 min, transcription was initiated by addition of ATP, UTP, and CTP to allow synthesis of heparin-resistant elongation complexes containing transcripts of not more than 16 nucleotides. After 3 min, heparin was added to transcription reactions to prevent further initiations, and GTP was added to allow synthesis of full-length runoff transcripts. Consistent with the results of abortive initiation assays, addition of ELL to transcription reactions after assembly of the preinitiation complex resulted in substantial synthesis of full-length runoff transcripts initiated at the AdML promoter (Fig. 3, lanes 6, 8, and 11), whereas addition of ELL to heparin challenge transcription reactions after assembly of the preinitiation complex strongly inhibited synthesis of full-length runoff transcripts (Fig. 3, lanes 1, 7, and 10). Synthesis of full-length runoff transcripts was also strongly inhibited if RNA polymerase II, TBP, or TFIIH was not preincubated with the AdML promoter prior to addition of ELL (Fig. 3, lanes 3, 9, and 12); in contrast, significant synthesis of full-length runoff transcripts was observed if TBP, TFIIH, and RNA polymerase II, but not TFIIH and TFIIH, were preincubated with the AdML promoter prior to addition of ELL (Fig. 3, lane 4). Finally, addition of ELL to transcription reactions before addition of AdML template DNA substantially reduced synthesis of full-length runoff transcripts (data not shown). Taken together, these results suggest that ELL is capable of interfering with selective binding of RNA polymerase II and TFIIH to the TATA factor at the promoter. We note that increased levels of synthesis of full-length runoff transcripts were observed when TFIIH, TFIIF, and TFIIH were included in preincubations along with RNA polymerase II, TFIIH, and TBP prior to addition of ELL. ELL may, therefore, have some effect on TFIIH, TFIIF, and TFIIH func-
tion; alternatively, the increased transcription levels observed when these initiation factors were included in preincubations prior to addition of ELL may be a consequence of their abilities to stabilize binding of RNA polymerase II and TFIIIB to TBP at the promoter.

If ELL inhibits assembly of the preinitiation complex by interacting stably with and sequestering RNA polymerase II or one of the initiation factors, it should be possible to overcome ELL inhibition by adding an excess of one or more of these components of the basal transcriptional machinery. As shown in Fig. 4, addition of excess RNA polymerase II to transcription reactions containing ELL restored synthesis of full-length run-off transcripts to levels approaching those synthesized in the absence of ELL, even though additional RNA polymerase II did not substantially increase transcription in the absence of ELL. In contrast, addition of excess TBP, TFIIIB, TFIIIE, TFIIIF, and TFIIH (Fig. 4) or excess AdML template DNA (data not shown) did not relieve ELL inhibition. These results suggest that ELL is capable of interacting stably with RNA polymerase II and preventing entry of polymerase into the functional preinitiation complex.

Overlapping ELL Functional Domains Are Responsible for Inhibition of Initiation and Activation of Elongation—As part of our effort to understand how ELL inhibits transcription initiation, we sought to establish the relationship between the ELL elongation stimulatory and initiation inhibitory activities. It was possible that inhibition of initiation by ELL might be simply a by-product of ELL-RNA polymerase II interactions involved in stimulating elongation; alternatively, it was possible that the ELL initiation inhibitory activity might be a discrete function of ELL, unrelated to its elongation stimulatory activity.

To address these possibilities, a series of ELL deletion mutants (summarized in Figs. 5A and 6A) were constructed, expressed in E. coli, purified, and assayed for their abilities (i) to inhibit initiation by RNA polymerase II from the AdML promoter in a reconstituted basal transcription system composed of TBP and the general initiation factors TFIIB, TFIIIE, TFIIF, and TFIIH and (ii) to stimulate the rate of elongation by RNA polymerase II on the oligo(dC)-tailed pGR220S/P/X template (30). If inhibition of initiation by ELL were simply a consequence of ELL-RNA polymerase II interactions that are crucial for stimulation of elongation, any ELL mutations that affect its ability to inhibit initiation should also affect its ability to stimulate elongation. As shown below, however, we identified some ELL mutants that do not inhibit initiation, but are fully active in elongation, suggesting that inhibition of initiation by ELL is at least in part a consequence of ELL-RNA polymerase II interactions that are dispensable for stimulation of elongation.

As shown in Fig. 5, B and C, the ELL(51–621) mutant, which lacks the first 50 N-terminal amino acids, was as active as wild type ELL in stimulation of elongation, but did not inhibit promoter-specific initiation at concentrations that were sufficient for almost complete inhibition of initiation by wild type ELL. Within this region, we were unable to identify any small sequence motifs responsible for inhibition of initiation; each of a series of additional N-terminal and small internal deletion mutants were active in elongation, but failed to inhibit initiation (Fig. 5, D and E).

We were also unable to identify ELL mutants that inhibit promoter-specific initiation, but fail to stimulate elongation; thus, there is significant overlap between the regions responsible for these two functions. As shown in Fig. 6, the ELL(1–373) mutant, which lacks 248 amino acids from the C terminus of ELL, stimulated elongation and inhibited initiation as effectively as wild type ELL. The ELL(1–249) mutant, which lacks an additional 124 amino acids from the C terminus, as well as the internal deletion mutants ELL(Δ50–100), ELL(Δ100–150), and ELL(Δ150–200), lacked both activities. Like the ELL(51–620) mutant, the ELL(Δ200–250) and ELL(Δ250–300) mutants stimulated elongation by RNA polymerase II, but did not significantly inhibit initiation. Taken together, the results of our structure-function analysis indicate that ELL sequences required for stimulation of elongation are a subset of those needed for inhibition of initiation. Whereas stimulation of elongation by RNA polymerase II depends
strongly on two ELL regions located between amino acids 60 and 200 and 300 and 373, inhibition of promoter-specific transcription depends on a larger ELL region falling between amino acids 1 and 373. Because the results presented in Figs. 3 and 4 suggest that ELL inhibits initiation through an interaction with RNA polymerase II, we asked whether the ability to inhibit initiation correlates with stable binding to polymerase. To measure binding of ELL to polymerase, we tested the ability of histidine-tagged ELL and ELL mutants to retain polymerase on nickel-agarose. RNA polymerase II was preincubated in the presence and absence of histidine-tagged ELL or ELL mutants and then batch adsorbed to nickel-agarose. Following brief centrifugation, the unbound protein, which remained in the supernatant was removed. After extensive washing (3 washes with 10 volumes of buffer) of the nickel resin, bound protein was eluted with a buffer containing imidazole, and both unbound and eluted fractions were assayed for the presence of RNA polymerase II by Western blotting using the monoclonal antibody 8WG16, which is specific for the C-terminal domain of the largest polymerase subunit (38). As shown in Fig. 7A, similar amounts of RNA polymerase II bound to immobilized wild type ELL and the ELL(51–620) mutant, whereas no significant binding of RNA polymerase II to the ELL(D50–100) mutant was detected. Thus, the results of both types of binding assays argue that stable binding of ELL to RNA polymerase II is not sufficient for inhibition of promoter-specific initiation.

**DISCUSSION**

The human ELL gene on chromosome 19p13.1 was originally identified as a gene that undergoes frequent translocations with the trithorax-like MLL gene on chromosome 11q23 in acute myeloid leukemia (16, 17). The ELL gene encodes an 620-amino acid nuclear protein (45) that we recently demonstrated is capable of regulating the activity of the RNA polymerase II elongation complex (12). Mechanistic studies indicate that ELL activates the overall rate of elongation by RNA polymerase II by suppressing transient pausing by polymerase at many sites along the DNA.

In this report, we have investigated the structure and func-
tion of the ELL protein. These studies led to the discovery that, in addition to its ability to activate elongation, ELL is capable of inhibiting promoter-specific transcription initiation by RNA polymerase II in vitro. Several lines of evidence argue that ELL inhibits initiation by binding directly to polymerase and disrupting proper assembly of the enzyme with TBP and TFIIB at the promoter during assembly of the preinitiation complex. First, ELL binds stably to RNA polymerase II in vitro. Second, results of the order of addition experiments indicate that pre-assembly of RNA polymerase II with TBP and TFIIB at the promoter is sufficient to render transcription initiation substantially resistant to inhibition by ELL. Finally, addition to transcription reactions of excess RNA polymerase II, but not TBP, TFIIB, TFIIE, TFIIF, or TFIIH, relieves inhibition by ELL.

Our identification of ELL mutants such as ELL(51–621), which activate elongation, but do not inhibit initiation, suggests that inhibition of initiation is not simply a by-product of ELL-RNA polymerase II interactions necessary for stimulation of elongation. Furthermore, our observation that wild type ELL and the ELL(51–621) mutant appear to bind RNA polymerase II similarly argues that failure of the ELL(51–621) mutant to inhibit initiation is not due to its inability to bind polymerase.

Taken together, our data suggests (i) that binding of ELL to RNA polymerase II is not sufficient for inhibition of initiation and (ii) that the ability of ELL to inhibit initiation is a function distinct from its ability to activate elongation.

Because many transcriptional regulatory proteins are composed of separable domains that carry out distinct functions such as DNA binding and transcriptional activation or repression (46), we sought to identify separable ELL modules that could independently activate elongation and inhibit initiation. Analysis of a large number of ELL mutants, however, revealed substantial overlap between ELL regions responsible for these two activities. Our findings indicated (i) that both activities are carried out by N-terminal ELL sequences between amino acids 1 and 373 and (ii) that ELL sequences required for activation of elongation are a subset of those required for inhibition of initiation and reside in a bipartite region between amino acids 60–200 and 300–373.

Exactly how ELL prevents entry of RNA polymerase II into the preinitiation complex is not clear. It is possible that ELL inhibits initiation sterically, by physically blocking interaction of RNA polymerase II with TBP, TFIIB, or promoter DNA. Alternatively, it is possible that ELL inhibits initiation by an allosteric mechanism, by binding to and inducing in polymer-
is possible that the ELL elongation activation domain tethers ELL to polymerase, and a distinct ELL region, not directly involved in controlling elongation, is responsible for inhibiting initiation. In light of this possibility, it is noteworthy that, according to a recently proposed model for the structure of the TBP-TFIIB-RNA polymerase II-promoter complex, the polymerase catalytic site for nucleotide addition is located ~100 Å from TBP and TFIIB in the preinitiation complex (47). Thus, if ELL exerts its effects on initiation and elongation through direct, physical interactions with the polymerase catalytic and TBP/TFIIB-binding sites, it must be capable of interacting with widely separated sites on polymerase.

Previous studies have identified a variety of transcriptional repressors that inhibit transcription initiation by RNA polymerase II by blocking assembly of the preinitiation complex. The majority of these repressors inhibit initiation by antagonizing TFIID function and fall into three classes. First, repressors such as LBP-1 (48), the bovine papilloma virus E2 protein (49), and the *Drosophila P* element transposase (50) are sequence-specific DNA-binding proteins that bind to promoters and prevent binding of TFIID. Second, repressors such as Mot1 inhibit assembly of the preinitiation complex by promoting ATP-dependent dissociation of TBP from the promoter (51). Finally, repressors such as NC2/Drl/DRAPI inhibit assembly of the preinitiation complex by binding TFIID and interfering with interactions between TFIID and TFIIB (52–54).

ELL appears to be the first example of a transcriptional inhibitory protein that blocks entry of RNA polymerase II into the preinitiation complex through a direct interaction with polymerase. Besides the human cytomegalovirus immediate early protein 2 (also known as IE86 or UL122) (55), ELL is the only protein known to inhibit transcription initiation by preventing entry of polymerase into the preinitiation complex. The immediate early 2 protein, however, appears to prevent entry of RNA polymerase II into the preinitiation complex, not through a direct interaction with polymerase, but, rather, through a sequence-specific interaction with a promoter element just downstream of the TATA box in the cytomegalovirus major immediate early promoter (55, 56).

Finally, what is the physiological significance of the ELL transcriptional inhibitory activity? It is possible that ELL can function as an inhibitor of transcription initiation in cells. Alternatively, it is possible that ELL-mediated inhibition of initiation *in vitro* results from an ELL-RNA polymerase II interaction that has a different role in cells; it is becoming increasingly clear, for example, that RNA polymerase II interacts in cells with proteins involved in a diverse collection of processes including those of the DNA repair, splicing, and polyadenylation pathways (57, 58).

Our previous demonstration that ELL has the ability to stimulate elongation by RNA polymerase II suggested some possible models for mechanisms by which the t(11;19)(q23;p13.1) translocation might induce leukemogenesis (12, 45). For example, fusion of the N-terminal half of MLL to ELL could disrupt the ability of ELL to serve as an elongation factor for target genes whose expression is particularly sensitive to changes in elongation rate. Alternatively, fusion of the MLL-A-T hook domains to ELL could lead to overexpression of certain genes by inappropriately targeting ELL elongation activity to A-T rich regions of DNA. Our findings, which identify an ELL-RNA polymerase II interaction that (i) results in inhibition of promoter-specific initiation *in vitro* and (ii) is specifically disrupted by mutations in the small N-terminal region of ELL lost in the t(11;19)(q23;p13.1) translocation, now require us to consider additional models in which fusion of MLL to ELL alters the expression of critical target genes by altering ELL-
RNA polymerase II interactions that are not involved in regulation of elongation.

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