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A Carboxy-Terminal Basic Region Controls RNA Polymerase III Transcription Factor Activity of Human La Protein

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Human La protein has been shown to serve as a transcription factor for RNA polymerase III (pol III) by facilitating transcription termination and recycling of transcription complexes. In addition, La binds to the 3′ oligo(U) ends common to all nascent pol III transcripts, and in the case of B1-*Alu* RNA, protects it from 3′-end processing (R. J. Maraia, D. J. Kenan, and J. D. Keene, Mol. Cell. Biol. 14:2147–2158, 1994). Others have previously dissected the La protein into an N-terminal domain that binds RNA and a C-terminal domain that does not. Here, deletion and substitution mutants of La were examined for general RNA binding, RNA 3′-end protection, and transcription factor activity. Although some La mutants altered in a C-terminal basic region bind RNA in mobility shift assays, they are defective in RNA 3′-end protection and do not support transcription, while one C-terminal substitution mutant is defective only in transcription. Moreover, a C-terminal fragment lacking RNA binding activity appears able to support low levels of transcription by pol III. While efficient multiround transcription is supported only by mutants that bind RNA and contain a C-terminal basic region. These analyses indicate that RNA binding contributes to but is not sufficient for La transcription factor activity and that the C-terminal domain plays a role in transcription that is distinguishable from simple RNA binding. The transcription factor activity of La can be reversibly inhibited by RNA, suggesting the potential for feedback inhibition of pol III transcription.

Eukaryotic RNA polymerase III (pol III) is responsible for synthesizing the abundant transcripts of tRNA, 5S rRNA, 7SL RNA, and U6 RNA genes as well as other small RNA genes (reviewed in reference 44). To produce a sufficient quantity of RNAs of the correct structure, transcription initiation and termination must be accurate and reinitiation must be efficient. pol III alone cannot accomplish this and must rely on transcription factors (TFs) that bind to control elements and direct RNA synthesis. The adenovirus-associated (VA1) RNA gene and cellular tRNA and *Alu* genes have internal promoters and 3′ terminators that direct transcription by pol III. Mammalian TFIIIC1 and TFIIIC2 bind to the control elements of these genes (12, 43). After TFIIIB joins, the resulting preinitiation complex is stable and can be recycled for multiple rounds of transcription by pol III (5, 21, 27, 43).

Mammalian in vitro transcription assays have used reconstituted systems that contain at least one partially purified fraction. For example, mammalian TFIIIC2, TFIIIB, and pol III have been highly purified, while TFIIIC1 and TFIIIC1′ represent relatively crude yet essential fractions (42, 43). In the *Saccharomyces cerevisiae* system, elegant studies have shown that recombinant or highly purified TFIIIB and TFIIIC are sufficient for multiround transcription (21), although it has also been reported that transcription can be stimulated by TFIIIE, a partially characterized fraction (13, 37). Thus, in both yeast and mammalian pol III systems, while the central factors required for in vitro transcription have been identified and continue to be characterized, evidence also suggests that ancillary, less well characterized factors may stimulate transcription. While remarkable progress has been made in understanding assembly of the preinitiation complex, relatively less effort has been directed toward understanding of the mechanisms that mediate transcription termination and the recycling of stable transcription complexes and pol III (13, 24, 31, 33, 42, 43).

Accumulating evidence suggests that the *cis*-acting oligo(dT) termination signal functions not only in RNA 3′-end formation but also in TFIIIC-mediated assembly and utilization of transcription complexes (2, 14, 43, 46). Although TFIIIC fractions in yeast and humans appear to differ significantly, a component of this multisubunit factor appears to bind to the terminators of certain genes in both systems (reviewed in reference 43; see reference 21). Terminator-containing, but not terminator-lacking, templates can efficiently recycle a fraction of pol III to the preinitiation complex by an unknown mechanism (14). Mammalian TFIIIC1 binds at the termination signal as well as near the transcription start site and mediates cooperative assembly of the transcription complex (43, 46). Thus, in yeast and mammalian systems, proper termination appears to facilitate efficient transcription, although the mechanisms that determine recycling efficiency are not yet sufficiently clear (14, 43). Controls at the levels of recycling of stable transcription complexes and pol III represent potential mechanisms for regulating RNA synthesis. Human La protein, a transcription termination factor for pol III, has been shown to facilitate recycling of both stable transcription complexes and pol III (18, 19, 33). Indeed, La can regulate the recycling potential of mammalian pol III transcription complexes (15, 31).

Human La is an abundant nuclear protein that associates with nascent pol III-synthesized RNAs in vivo (20, 28, 36). La binds specifically to the RNA copy of the pol III termination signal, the 3′ oligo(U) terminus found on all nascent transcripts synthesized by pol III (41). However, unlike other pol III TFs which remain bound to the template during multiple rounds of transcription, La associates with each nascent transcript produced, a feature that presumably accounts for high levels of La in the nucleus (20).

The effects of La on transcription have thus far been appar-

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ent only in mammalian cell extracts from which La has been depleted (18, 19, 31, 33). Assays performed on either salt-washed or heparin-and-Sarkosyl-stripped, immobilized transcription complexes showed that utilization of complexes by pol III is increased by La (31, 33). Native complexes isolated by gel filtration are also stimulated by La (17). Assays with salt-washed or gel filtration-isolated complexes produce significant background, presumably due to detectable endogenous La (not shown), which can complicate analysis of mutant La proteins, especially since this protein can homodimerize (10). The present study overcomes this limitation by employing a heparin-plus-Sarkosyl-stripped, immobilized transcription complex assay that is dependent on added La (31). This assay was used to show that phosphorylated and unphosphorylated La proteins exhibit differential transcription factor activities (15). It was unclear how phosphorylation at a single site, serine 366, in the C terminus of La could regulate transcription factor activity, since the only known transcription-related activity of La, i.e., nascent-transcript binding, had been localized to the N-terminal domain (7). The results presented here help clarify this issue (see Discussion).

Bacterial extracts containing N- and C-terminal deletion mutants of La have been used previously for immunoprecipitation and electrophoretic mobility shift assays (EMSAs) to show that RNA binding is mediated by the N-terminal domain of La (9, 23, 35). Guided by the information obtained from those studies, we constructed and purified a panel of His-tagged mutant La proteins and used them in assays of (i) general RNA binding determined by EMSA, (ii) nascent-RNA 3′-end protection, and (iii) La-dependent pol III transcription. Although RNA binding contributes to the transcription factor activity of La, we show that the role of La is not simply to protect nascent transcripts from degradation in this system. RNA binding activity is not sufficient for the transcription factor activity of La, since a discrete basic region near the C terminus of La is also required.

MATERIALS AND METHODS

Generation of mutant La proteins. A human La cDNA clone (gift of D. Kenan, Duke University Medical Center) containing an *Nco*I restriction site at the N-terminal methionine was subcloned into the vector pET28a (Novagen). Deletion constructs of this clone were generated by oligonucleotide-mediated mutagenesis as described previously (25). All constructs, including La 1-408 (the full-length protein), contained a six-His tag at the carboxyl terminus. C-terminal deletions were made by using oligodeoxynucleotides that introduced a common six-His tag followed by a diagnostic *Spe*I site containing the termination codon TAG. Amino-terminal deletions were made from wild-type La that contained a C-terminal six-His tag. N-terminal deletions were constructed by using oligodeoxynucleotides that introduced an *Nco*I site followed by a sequence coding for Met and Ala residues. Upstream DNA was then excised as an *Nco*I-*Nco*I fragment, and the vector was religated. The following substitution mutants were constructed by using antisense oligodeoxynucleotides to introduce the indicated substitutions: for La GXK>DE, amino acids 328 to 344 within the basic glycine-and-lysine-rich (GK) region were replaced with alternating aspartate (D) and glutamate (E) residues; for La K>DE, the K residues at positions 339, 341, and 344 were replaced with D, E, and D, respectively; and for LaΔDXE>GK, residues 337 to 367 were deleted and residues 368 to 376 within the acidic region were replaced with alternating glycines and lysines and juxtaposed to residue 336.

Recombinant plasmids were expressed in *Escherichia coli* BL21(DE3) cells (Novagen) and purified with Ni^{2+}-chelating agarose (Qiagen) under nondenaturing conditions and eluted with imidazole according to standard procedures. All the mutant proteins were verified by Western blot analysis with anti-La serum (data not shown). A few of the proteins appeared to have undergone degradation, as evidenced by the presence of smaller polypeptides. This was most evident for La 26-408 and La 46-408. The smaller products were retained by the Ni^{2+} affinity column and were recognized by anti-La antibodies and were therefore degraded from their N termini.

Purified recombinant La protein that contains no tag (designated rLa) was a gift of D. Kenan (Duke University Medical Center) and was used as a control in some experiments. The concentration of rLa protein was determined by optical density using the extinction coefficient 0.81 as described elsewhere (23). The concentrations of His-tagged mutant La proteins were determined by comparing their intensity following sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and Coomassie blue staining with that of rLa by densitometry using a Molecular Dynamics scanner and ImageQuant software. Nucleic acid content was monitored by determining the optical density at 260 or 280 nm of the preparations. In control experiments, La proteins were further purified by S-Sepharose chromatography which decreased the amount of contaminating nucleic acid; however, this was found not to alter the relative transcriptional activities of the La proteins (not shown).

^{32}P-RNA EMSA analysis. DNA containing a promoter for T7 RNA polymerase was generated by PCR amplification of a plasmid containing the human hY4 RNA gene (34) as described previously (29). The primers used in the PCR were 5′-TAATACGACTCACTATAGGCTGGTCCGATGGTA-3′ and 5′-AAAAG CCAGTCAAATTTAGCAGTGGGGGGTT-3′. Transcription reaction mixtures contained 1 μg of gel-purified PCR product in 40 mM Tris-HCl (pH 8.1)–6 mM $MgCl_2$–1 mM spermidine–10 mM dithiothreitol–0.01% Triton X-100–56 μg of bovine serum albumin per ml–1 U of RNasin (Promega) per μl–4 U of T7 RNA polymerase (Promega) per μl–780 μM ATP, CTP, and UTP–100 μCi of [α-^{32}P] GTP (3,000 Ci/mmol)–77 μM GTP. The ^{32}P-RNA products were excised after electrophoresis in 10% polyacrylamide–8 M urea and eluted in 0.1% SDS–0.5 M ammonium acetate–10 mM magnesium acetate.

For K_d determination, 1,500 cpm of ^{32}P-hY4 RNA was incubated for 30 min on ice with increasing concentrations of La protein in 20-μl reaction mixtures containing 25 mM Tris (pH 8.0), 3 mM $MgCl_2$, 0.1 mM EDTA, 0.5% Nonidet P-40; 100 mM NaCl, 0.5 mM dithiothreitol, and 250 μg of poly(rC) per ml (23). Under these conditions, 1,500 cpm of hY4 ^{32}P-RNA in a 20-μl reaction volume was calculated to be 0.6 nM. For subsequent assays testing the binding affinity of La at the estimated K_d value, 1,500 cpm of labeled hY4 RNA was included in the reaction mixtures (see Fig. 2A). The EMSA experiment with higher La concentrations (see Fig. 2B) utilized 2,250 cpm of hY4 RNA. The products were analyzed by electrophoresis on 8% native polyacrylamide gels followed by autoradiography. All radioactive quantitation was done with a PhosphorImager using ImageQuant software (Molecular Dynamics).

Nuclear extract. Nuclear extract was prepared from murine erythroleukemia (MEL) or HeLa cells as previously described (32). pol III was purified from a 0.29 to 0.7 M KCl elution of a phosphocellulose column fraction of HeLa whole-cell S100 extract by a modification of a procedure described previously (39). The eluate was applied to DEAE-cellulose (DE52), and the material containing pol III activity eluted between 0.09 and 0.13 M $(NH_4)_2SO_4$ was used. By use of a poly(dA-dT) template, pol III activity was determined as the amount of [^{3}H]dUTP incorporation that was sensitive to 1 mg of α-amanatin per ml but resistant to 0.5 μg/ml. One unit of activity incorporates 1 pmol of [^{3}H]dUMP into RNA in 20 min at 37°C. The activity of the pol III preparation used here was 17 U/μg (20 U/μl).

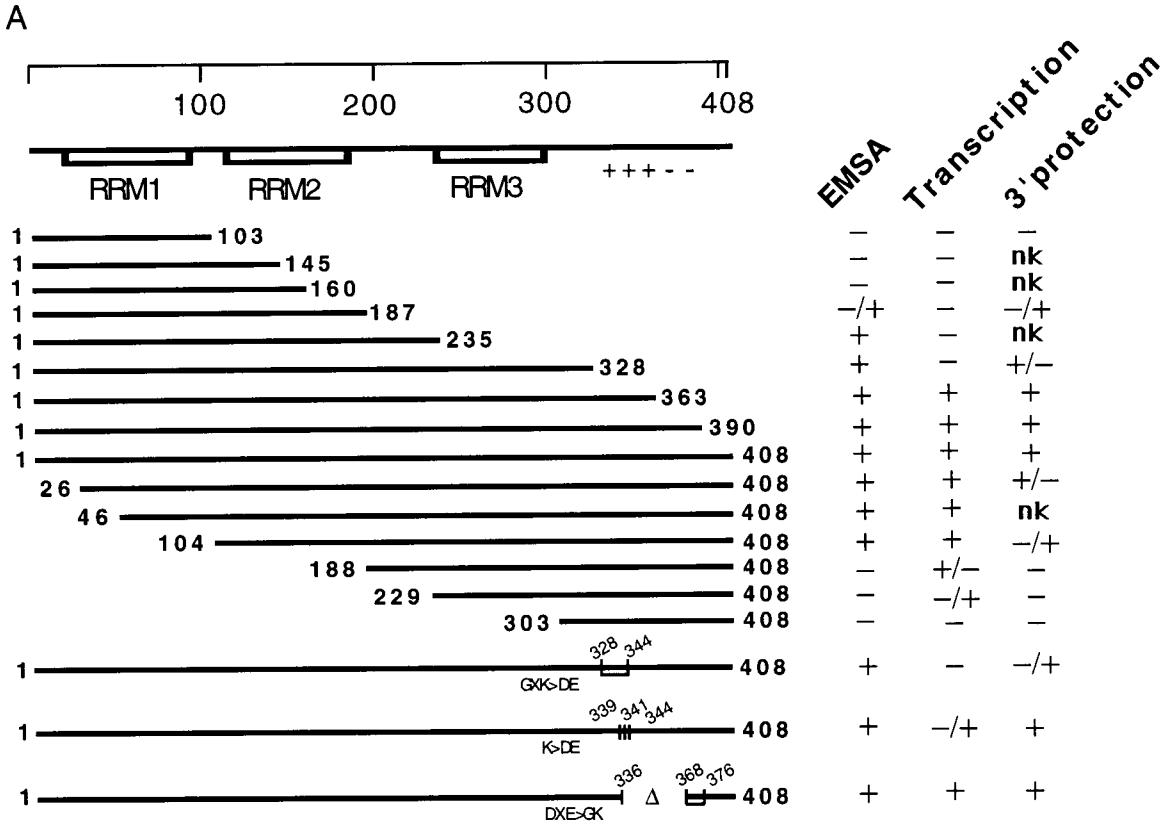
pol III transcription assay. Immobilized transcription complexes containing the VA1 RNA gene were assembled in MEL nuclear extract, washed, and stripped of pol III activity as described previously (31). The complexes were equilibrated, and transcription was reinitiated upon addition of pol III (20 U), La (20 pmol), and α-^{32}P-labeled nucleoside triphosphates (NTPs) in a 25-μl reaction volume as described elsewhere (31). Following a 30-min incubation, the reactions were stopped and the products were purified by phenol-chloroform extraction and ethanol precipitation and analyzed by electrophoresis on a 6% polyacrylamide–8 M urea gel and autoradiography. For some experiments, pol III-synthesized VA1 RNA was gel purified prior to addition to reaction mixtures.

Single-round transcription. pol III-depleted complexes, prepared as described above, were incubated with 20 pmol of La protein, pol III (20 U), 0.5 mM ATP and CTP, 0.03 mM GTP, and [α-^{32}P]GTP for 10 min at 30°C to arrest pol III at the first U residue (position 7 of VA1 RNA). Sarkosyl (final concentration, 0.05%) and UTP (final concentration, 0.6 mM) were then added, and incubation continued for various times as indicated below. The reactions were stopped and the ^{32}P-RNA products were analyzed as described above.

Nascent B1-Alu RNA 3′-end protection. 3′-end protection reactions were carried out by preincubating immobilized, *Eco*RI-linearized pGB1e DNA with HeLa nuclear extract (20 μg of total protein per reaction mixture), ATP, CTP, and GTP for 2 h; the supernatant was then removed, and the immobilized complexes were washed twice with transcription buffer lacking NTPs (33). pol III (20 U), La (20 pmol), ATP, CTP, UTP, and [α-^{32}P]GTP (see Fig. 3A) or T7 RNA polymerase-synthesized, gel-purified nascent B1-*Alu* ^{32}P-RNA (see Fig. 3B) were added, and the reaction mixtures were allowed to incubate for an additional 60 min. The supernatant was recovered, and RNA was purified and analyzed by denaturing PAGE and autoradiography.

RESULTS

A current model of the human La protein is shown at the top of Fig. 1A (3, 23), below which is shown a representation of the La proteins used in this study. The deletion mutant La proteins were purified and examined by SDS-PAGE and Coomassie blue staining (Fig. 1B). These proteins exhibited the expected relative mobilities in SDS-PAGE. A summary of the activities



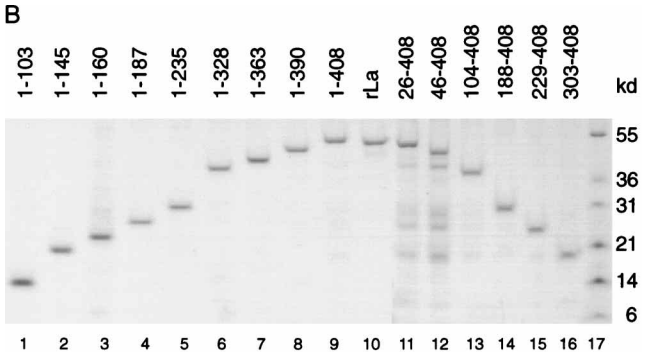


FIG. 1. La proteins used in this study. (A) Domain structure of human La protein (modified from reference 23). Amino acid numbering is shown along the top line. The positions of predicted RRMs and the adjacent basic (+++[GK]) and acidic (−−[DE]) regions as previously described (23) are indicated. A schematic representation of the La deletion and substitution mutants is shown. Results from each of three assays, pol III transcription, RNA 3′-end protection, and EMSA, are summarized beside the respective mutants. −, negative result; +, positive result; −/+, weakly positive result; +/−, moderately positive result; nk, not known. (B) A 0.5-μg sample of each purified La protein was separated on a 4 to 20% gradient SDS-polyacrylamide gel and stained with Coomassie blue. Non-His-tagged rLa was provided by D. Kenan. The sizes of molecular mass standards (lane 17) are shown on the right.

of these La proteins in three assays (below) is presented in Fig. 1A.

RNA binding by mutant La proteins. We examined the ability of purified La proteins to bind hY4 ^{32}P-RNA, a 3′-oligo(U)-containing, 94-nucleotide (94-nt), natural pol III product that remains stably associated with La in vivo (20). The affinity of wild-type La for hY4 RNA was measured in an EMSA by titrating a fixed amount of ^{32}P-RNA with increasing amounts of purified La protein and a constant amount of the nonspecific competitor poly(C) (9). Under these conditions, the apparent K_d of the La-hY4 RNA interaction was determined to be 5 nM (data not shown). In the presence of poly(C), twofold-less hY4 RNA was bound by La at 5 nM than in its absence (not shown). Our results are similar to the K_d of 6 nM obtained for La and hY1 RNA by a similar approach (9).

Our mutant proteins were then tested at 5 nM for their ability to bind hY4 RNA (Fig. 2A). La proteins containing 187 or fewer N-terminal residues did not bind significant amounts of RNA under these conditions (Fig. 2A, lanes 2 to 5). By contrast, La proteins containing more than 187 N-terminal residues bound the RNA efficiently (Fig. 2A, lanes 6 to 10). Constructs lacking 25 or more residues of the N terminus (e.g., La 26-408) failed to bind hY4 RNA (Fig. 2A, lanes 12 to 17). Since La 1-187 terminates at the boundary of RRM-2 and binds RNA inefficiently, we suspect that additional residues might stabilize the structure and increase RNA binding. Therefore, with the possible exception of La 1-187, these results agree with those reported previously (9, 23, 35, 40). Efficient binding of La to hY4 RNA requires amino acids near the N terminus, including ones at the start of RRM-1 as well as RRM-2.

Because our transcription reactions (below) required concentrations of La that exceed its apparent K_d for hY4 RNA, we also examined binding at a higher concentration of La (30 nM; Fig. 2B). RRM-1 alone, as represented by La 1-103, La 1-145, and La 1-160, did not interact efficiently with hY4 RNA (Fig. 2B, lanes 1 to 3). At 30 nM, La 1-187 bound hY4 RNA, although relatively weakly, since it reproducibly appeared to dissociate during electrophoresis (Fig. 2B, lane 4). As expected, La proteins extending beyond amino acid 235 bound

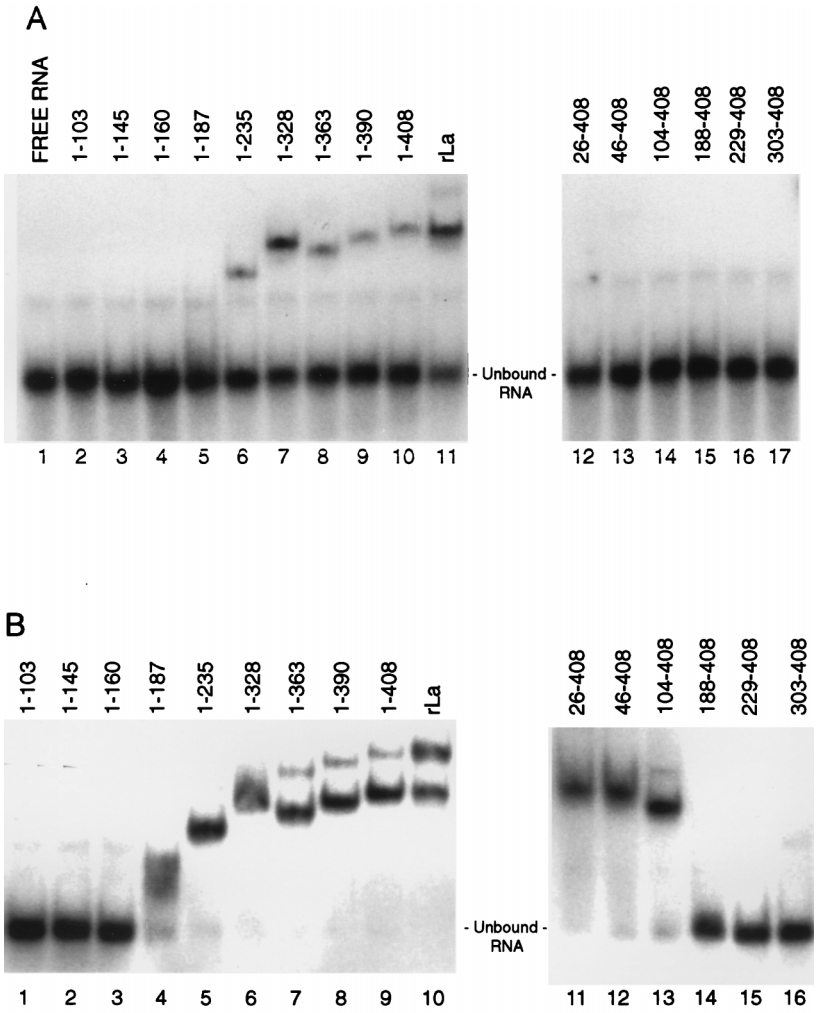


FIG. 2. RNA binding activity of mutant La proteins. Radiolabeled hY4 RNA was incubated with 5 (A) and 30 (B) nM La. Ribonucleoprotein complexes were separated from free unbound RNA and visualized by autoradiography after electrophoresis in nondenaturing 8% polyacrylamide gels. The La mutants, designated according to Fig. 1, used in each reaction are indicated above the lanes.

the RNA efficiently (Fig. 2B, lanes 5 to 10). The doublet bands of La-RNA complexes seen with high concentrations of La proteins extending to amino acid 363 and beyond (Fig. 2B, lanes 7 to 10) may be due to dimerization of La (10), although we have not examined this further. In contrast to the finding of no detectable binding at 5 nM La, mutants La 26-408, 46-408, and 104-408, each lacking part or all of RRM-1, reproducibly bound hY4 RNA at 30 nM (Fig. 2B, lanes 11 to 13). Mutants La 188-408, 229-408, and 303-408, which contain RRM-3 (3) but not RRM-1 or RRM-2, did not bind hY4 RNA under these conditions (Fig. 2B, lanes 14 to 16). Thus, as monitored by EMSA, RRM-1 appears to increase the affinity of La for hY4 RNA but is not necessary for hY4 RNA binding at high concentrations. As will be suggested by data shown below, a role for RRM-1 may be to contribute to high-affinity 3′-oligo(U)-specific binding (23). The EMSA results are summarized in Fig. 1A.

Nascent-RNA 3′-end protection is mediated by the N- and C-terminal domains of La. Previous data indicated that La protected the 3′ end of nascent B1-*Alu* RNA from nuclease attack (33). Employing conditions that allow efficient posttranscriptional processing, we examined the ability of mutant La proteins to protect nascent B1-*Alu* RNA from 3′-end processing in coupled pol III transcription-processing assays (Fig. 3A) and in isolated 3′-end processing assays using presynthesized, gel-purified nascent B1-*Alu* RNA (Fig. 3B). The results are summarized in Fig. 1A.

Stalled transcription elongation complexes were formed during a preincubation period, and excess nuclear extract (containing La) was then washed away; upon incubation with NTPs (and pol III), a primary transcript of 210 nt that then undergoes 3′-end processing to a 135-nt species is produced (1, 32, 33). An equivalent 135-nt transcript known as scB1 RNA accumulates in vivo (1, 8, 29, 30, 32). In the absence of La (Fig. 3A, lane 12), most of the ^{32}P-RNA produced from the B1-*Alu* template in these reactions was processed, although a small amount of primary transcript remained (Fig. 3A, bands P and 1°). Reaction mixtures that contained La 1-408, 1-390, and 1-363 produced mostly the primary transcript, with little if any processed RNA (Fig. 3A, lanes 1 to 3). In contrast, reaction mixtures that contained mutants La 1-145, 1-103, 303-408, and 188-408 exhibited no 3′-end protection activity (Fig. 3A, lanes 6 to 9). Reaction mixtures containing mutants La 104-408 and 26-408 yielded a relatively small amount of primary transcript and some processed RNA, indicating inefficient protection activity (Fig. 3A, lanes 10 and 11). La 104-408 reproduc-

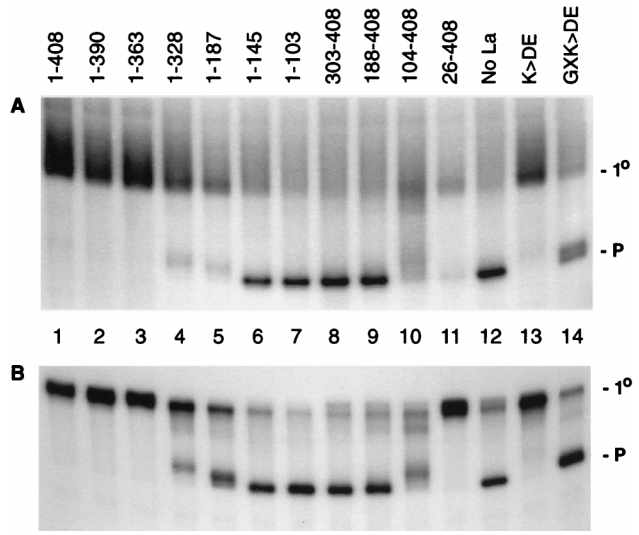


FIG. 3. N- and C-terminal domains of La protect nascent RNA from 3′-end processing. (A) After preincubation of immobilized B1-*Alu* DNA, nuclear extract, ATP, CTP, and GTP, transcription complexes were washed and supplemented with pol III, NTPs including [α-^{32}P]GTP, and the mutant La proteins indicated above the lanes. After an additional 60-min incubation, soluble ^{32}P-RNA was recovered, purified, and analyzed by denaturing PAGE and autoradiography. The positions of the B1-*Alu* primary transcript (1°) and 3′-end-processed RNA (P) are indicated on the right. (B) Same as for panel A except that presynthesized, gel-purified B1-*Alu* ^{32}P-RNA containing a 3′ oligo(U) tract was added instead of [α-^{32}P]GTP.

ibly yielded a smear of transcripts that migrated between the primary and processed RNAs, suggesting that an exonuclease was responsible (Fig. 3A, lane 10).

Reaction mixtures containing mutants La 1-328 and 1-187 produced RNA species whose mobilities were slightly slower than that of the fully processed RNA (Fig. 3A, lanes 4 and 5). This surprising result suggested that residues 329 to 363 play a role in nascent-RNA 3′-end protection. This was supported by results with La GXK>DE. This protein did not protect the primary transcript and yielded a doublet of processed RNA (Fig. 3A, lane 14), the upper band of which exhibited mobility similar to those of the products produced with La 1-328 and La 1-187. Although the discriminating features of the different forms of the processed RNA are unknown, the effect was clear: disruption of the C-terminal basic region (residues 328 to 344), as in La GXK>DE, abrogates protection of B1-*Alu* RNA from 3′-end processing. Since La GXK>DE binds RNA in EMSAs (see Fig. 5B), these results suggest that its inability to protect nascent RNA from 3′-end processing is due to a specific defect which is not observed by monitoring general RNA binding by EMSA. However, alteration of just three residues in the basic region did not compromise the RNA 3′-end protection activity of La (La K>DE; Fig. 3A, lane 13).

A similar pattern was obtained by using presynthesized, gel-purified B1-*Alu* ^{32}P-RNA in isolated 3′-end processing reactions (Fig. 3B).

It is noteworthy that while deletion (La 104-408) or disruption (La 26-408) of RRM-1 decreases 3′-end protection more or less to the same degree as in the C-terminally deleted La 1-187 and La 1-328, these RRM-1 mutants do not abrogate protection to the degree of mutants that also lack RRM-2 (La 1-145, 1-103, 303-408, and 188-408). The cumulative results suggest that RRM-1 and the C-terminal domain may each contribute to the 3′-end protection activity of La.

Only La proteins that bind RNA and contain a C-terminal basic region support efficient transcription by pol III. Having established the pattern of RNA binding exhibited by the mutant La proteins, we next examined them for transcription factor activity. The results are shown in Fig. 4A and summarized in Fig. 1A. La 1-328 and all the more extensively C-terminally deleted proteins did not support transcription (Fig. 4A, lanes 1 to 6), while La 1-363, 1-390, and 1-408 supported efficient transcription (lanes 7 to 9). This indicates that the region containing residues 328 to 363 is required for transcription activity, while the last 44 amino acids are dispensable.

La 26-408, 46-408, and 104-408 were highly active, indicating that the N-terminal 103 amino acids are not required for efficient transcription in this system (Fig. 4A, lanes 11 to 13). La 188-408 reproducibly yielded a faint signal (lane 14) equal to 10 to 15% of the signal produced by La 1-408 (La 188-408 will be examined below). Although not visible in Fig. 4A, a low level of VA1 RNA synthesis was reproducibly detected in reaction mixtures containing La 229-408.

The experiment whose results are shown in Fig. 4B provides direct evidence that La promotes synthesis of nascent RNA as opposed to stabilization of the newly synthesized transcript. After formation, stripping, and washing steps, transcription

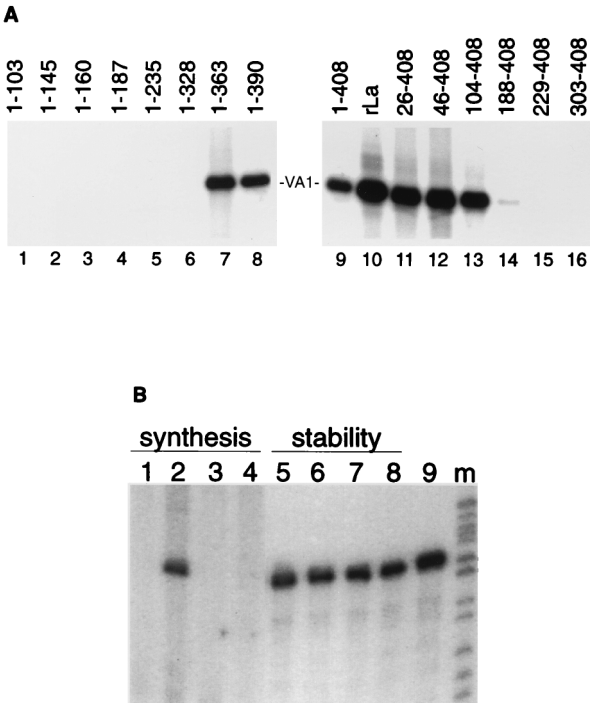


FIG. 4. pol III transcription factor activity of La N- and C-terminal deletion proteins. (A) After VA1 transcription complexes were assembled, depleted of pol III activity, and washed, equal amounts were aliquoted and the La protein (20 pmol) indicated above each lane was added. Purified pol III (20 U) and ^{32}P-NTPs were then added, and the reaction mixtures were incubated for 40 min. VA1 ^{32}P-RNA was visualized on 6% polyacrylamide–8 M urea gels. (B) After VA1 transcription complexes were assembled, depleted of pol III activity, and washed, they were repleted with pol III (20 U) and NTPs with or without added La. [α-^{32}P]GTP was added to monitor de novo RNA synthesis (lanes 1 to 4), or VA1 ^{32}P-RNA was added to reaction mixtures to monitor RNA stability (lanes 5 to 9). The reaction mixtures were then incubated for 40 min, and the products were purified and visualized on 6% polyacrylamide–8 M urea gels. The following La mutants were used: lanes 1 and 6, La 1-328; lanes 2 and 5, La 1-363; lanes 3 and 7, La GXK>DE; and lanes 4 and 8, La 188-408. Lane 9, the input VA1 ^{32}P-RNA loaded directly without exposure to transcription reactions; lane m, molecular mass markers. A faint band was visible in lane 4 upon overexposure (not shown).

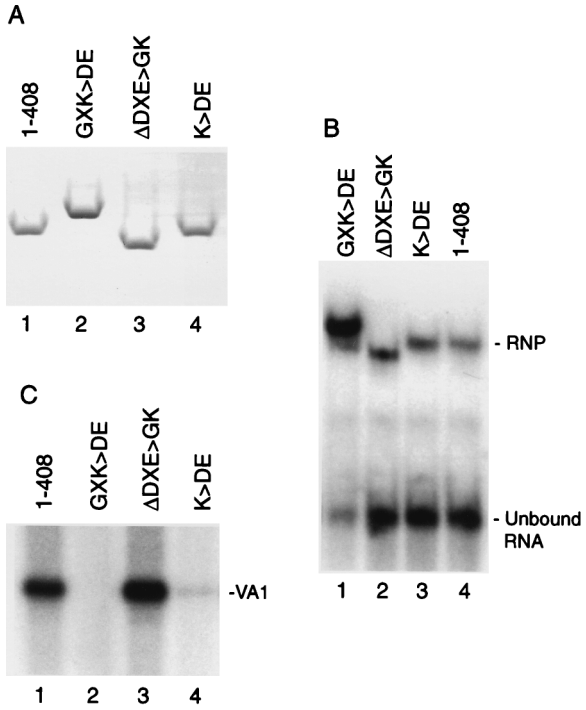


FIG. 5. Characterization of La substitution proteins. (A) SDS-PAGE and Coomassie blue staining. (B) EMSA using hY4 ^{32}P-RNA and the mutant La proteins indicated, as described for Fig. 2B. RNP, ribonucleoprotein. (C) Transcription factor activities of the mutant La proteins assayed as described for Fig. 3. The La mutant used for each reaction is indicated above the lane, designated according to Fig. 1A.

complexes were aliquoted to multiple tubes. The transcription reaction mixtures were set up containing either [α-^{32}P]GTP to monitor de novo RNA synthesis (Fig. 4B, lanes 1 to 4) or gel-purified VA1 ^{32}P-RNA to monitor RNA stability (lanes 5 to 9). After the standard incubation period, RNA was purified as usual and examined. It was clear that these transcription reaction mixtures containing La proteins that were either active (Fig. 4B, lane 2) or inactive (lanes 1, 3, and 4) for de novo RNA synthesis exhibited no RNA degradation activity (lanes 5 to 8). As expected, VA1 ^{32}P-RNA remained intact in these reactions in the absence of added La (not shown) or in the presence of various La proteins (Fig. 4B, lanes 5 to 8), regardless of the activity of these proteins in the transcription assay. Thus, in this transcription assay, La is required for de novo RNA synthesis.

The C-terminal basic region of La is required for transcription factor activity. La residues 329 to 363 are highly enriched in glycine (G) and lysine (K) (23). One approach to further examine the importance of this region in transcription factor activity was to replace amino acids 328 to 344 with alternating aspartate (D) and glutamate (E) residues (designated La GXK>DE). In another construct, we replaced only three residues, lysines at positions 339, 341, and 344, with D, E, and D, respectively (designated La K>DE). In a separate construct, we deleted residues 337 to 367 and in addition replaced residues 368 to 376 with alternating GK (LaΔDXE>GK). The net effect of the LaΔDXE>GK mutation was to replace the C-terminal end of the native GK region with a uniform set of GK residues, producing a shorter protein. These proteins are represented in Fig. 1A and are compared to La 1-408 in the Coomassie blue-stained SDS gel in Fig. 5A. Although La GXK>DE migrated more slowly than expected, DNA sequencing verified this construct and other constructs (not shown).

Each of the three internally modified La proteins bound hY4 RNA well, as expected (Fig. 5B). However, La GXK>DE did not support transcription (Fig. 5C, lane 2). Replacement of three lysines at positions 339, 341, and 344 with acidic amino acids reduced transcription dramatically (La K>DE [Fig. 5C, lane 4]). In contrast to these mutants, LaΔDXE>GK supported efficient transcription (Fig. 5C, lane 3), suggesting that the basic nature of the GK region rather than its exact sequence is required for transcription activity. The possibility that La proteins were differentially degraded during the transcription assay was excluded by examination by SDS-PAGE (not shown).

C-terminal fragment La 188-408 supports low-level transcription but is defective for efficient multiround transcription by pol III. One explanation to account for the reproducibly low but detectable level of transcription observed in the presence of La 188-408 (Fig. 4A, lane 14) might be that initiation by pol III is inefficient in each of the multiple rounds of transcription that occur in these reactions (31). Alternatively, the first initiation by pol III might be efficient but recycling might be inefficient. We investigated these possibilities (Fig. 6A) by comparing the La mutants simultaneously in single-round (lanes 1 to 11) and multiple-round (lanes 12 to 19) transcription assays. Preassembled transcription complexes were prepared and incubated with La protein, pol III, CTP, ATP, and GTP. Under these conditions, pol III initiates transcription but cannot extend the RNA chain due to the lack of UTP (31). Upon addition of UTP, pol III completes a single cycle of RNA synthesis but is unable to reinitiate in the presence of 0.05% Sarkosyl (24, 31). A parallel control reaction demonstrating that pol III was indeed limited to a single cycle in the presence of full-length La is presented in Fig. 6, lanes 20 to 22, which shows that the RNA products do not increase after 5, 25, and 45 min, respectively. Quantitation of the VA1 ^{32}P-RNA is indicated in radioactive counts below the lanes.

In the 5-min single-round assay, La 188-408 reproducibly supported transcription (Fig. 6A, lane 10) to greater than 50% of that of La 1-408 (Fig. 6A, lane 7). In contrast to single-round assays, parallel multiple-round assays with La 188-408 revealed that VA1 RNA synthesis reproducibly occurred at significantly lower levels than in reactions using La 1-408 (Fig. 6A, lanes 18 and 16, respectively). The amount of RNA synthesis in the multiple-round reaction using La 188-408 was reproducibly and remarkably similar to the amount in the single-round reaction using full-length La (Fig. 6A, compare lanes 18 and 7). As a way to examine the capacity for multiple- versus single-round transcription that corrects for the intrinsic activity of each protein, we compared the ratios of multiple- to single-round results obtained with various La proteins. This ratio ranged from 5.9 to 6.7 for La 1-408, 1-363, 1-390, and 26-408 but was only 1.7 for La 188-408 (Fig. 6A). La 188-408 reproducibly yielded a relatively low index of reinitiation (not shown). By this analysis, La 188-408 was significantly less active at recycling pol III transcription complexes than were the other proteins.

Although pol III could complete a single round of transcription within 5 min in the presence of La 1-408, as expected (Fig. 6A, lanes 20 to 22), the results did not rule out the possibility that this was an insufficient amount of time in the presence of La 188-408. Therefore, we also examined the transcription activities of La 188-408 and La 1-408 at different times under single-round conditions. On repeated attempts, we found that La 188-408 was always only moderately impaired in single-round assays. For example, an experiment that examined sin-

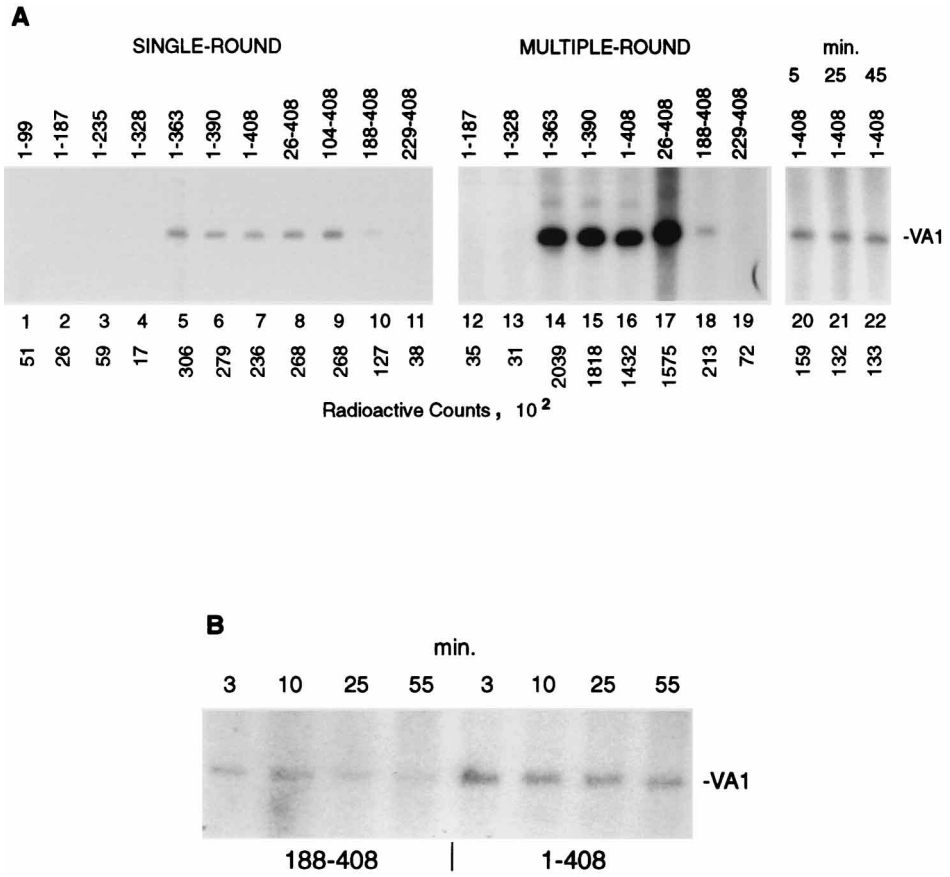


FIG. 6. Single- versus multiple-round transcription assay of La 188-408. (A) After VA1 complexes were assembled, depleted of pol III activity, washed, and aliquoted, transcription was limited to a single initiation (lanes 1 to 11 and 20 to 22) or allowed to continue under conditions that allow multiple-round transcription (lanes 12 to 19). Incubation was for 5 and 40 min for single- and multiple-round assays, respectively. A control for the single-round assay in the presence of wild-type La (see text) is also shown (lanes 20 to 22). All reactions were performed simultaneously, and all products were coelectrophoresed. The La mutant used in each reaction is indicated above the lane, designated according to Fig. 1A. The radioactive counts for each VA1 RNA band as determined by PhosphorImager analysis are shown below the lanes. (B) Single-round assays in the presence of either La 1-408 or La 188-408 performed as described for panel A. Sample aliquots were removed at the times indicated.

gle-round assays at various times showed that La 188-408 exhibited about 50% of the activity of La 1-408 (Fig. 6B).

Although we do not know the mechanistic basis for the apparent difference in single- versus multiple-round transcription exhibited by La 188-408, we can nonetheless conclude that this protein which lacks RNA binding can promote limited transcription by pol III and is most defective in supporting multiple-round transcription.

La transcription factor activity is inhibited by RNA. The data this far suggested that La's ability to bind RNA contributes to but is not sufficient for efficient transcription. We therefore examined whether exogenous RNA would interfere with La's transcription factor activity in this system. Figure 7 shows the effect on transcription of preincubating La with exogenous RNA. Unlabeled hY4 RNA was preincubated with La 1-408 and added to isolated transcription complexes with pol III and

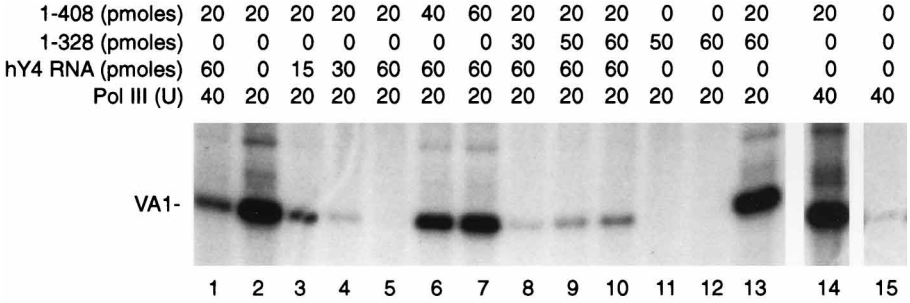


FIG. 7. RNA inhibits La transcription factor activity. Unlabeled hY4 RNA was preincubated with La 1-408 and/or La 1-328 in the amounts shown above the lanes. Together with pol III and ^{32}P-NTPs, La and hY4 RNA were then added to isolated transcription complexes, and VA1 ^{32}P-RNA synthesis was allowed to continue under standard multiple-round conditions.

^{32}P-NTPs, and VA1 ^{32}P-RNA synthesis was monitored in multiple-round assays. hY4 RNA produced a concentration-dependent decrease in transcription (Fig. 7, lanes 2 to 5). Significantly, we could recover VA1 RNA synthesis by increasing the amount of La in the reaction mixture (Fig. 7, lanes 6 and 7).

Maximum inhibition occurred with 60 pmol of hY4 RNA (Fig. 7, lanes 2 to 5). Adding a twofold excess of pol III to hY4 RNA-inhibited reaction mixtures stimulated some transcription (Fig. 7, compare lanes 1 and 5). However, increasing the amount of La twofold led to recovery of substantially more transcription (Fig. 7, compare lanes 5 and 6) than did pol III (compare lanes 1 and 5), and most transcription was recovered by higher concentrations of La (lane 7). The stimulation by excess pol III was not unexpected, since our pol III preparation contains a small amount of La (data not shown) and in excess can promote low-level transcription in the absence of exogenous La (Fig. 7, lane 15). Thus, the increased transcription in response to excess pol III may be due at least in part to the unavoidable addition of La. In any case, although we cannot rule out the possibility that a small amount of hY4 RNA's inhibitory effect may be mediated through pol III directly in these reactions, the results indicate that most of the inhibition is mediated through La.

In contrast to La 1-408, La 1-328 could recover only a fraction of the hY4 RNA-mediated inhibition (Fig. 7, lanes 8 to 10). This protein could not promote transcription on its own (Fig. 7, lanes 11 and 12). The ability of La 1-328 to relieve inhibition did not appear to be due to stimulation of wild-type La, since mixing La 1-328 and La 1-408 led to a slight decrease in transcription in the absence of exogenous RNA (Fig. 7, compare lanes 13 and 2). The results suggest that the RNA-binding activity of La 1-328 was responsible for relieving hY4-mediated transcription inhibition, although it was not as efficacious as was full-length La at reversing inhibition. This is probably because La 1-328 does not interact with nascent RNA as well as full-length La, as reflected by the RNA 3′-end protection results (Fig. 3). Nonetheless, the cumulative data in Fig. 7 allow the conclusion that nascent hY4 RNA can inhibit transcription in these reactions and that this effect is mediated by full-length active La protein.

DISCUSSION

The major conclusion that can be drawn from this work is that RNA binding contributes to but is not sufficient for the pol III transcription factor activity of La. This conclusion is supported by results with deletion mutants La 1-235 and La 1-328 as well as substitution mutants La GXK>DE and La K>DE, which bind RNA efficiently but do not support transcription. In addition, we identified the C-terminal basic region of La, including residues 329 to 363, as a motif that is required for pol III transcription activity in this system. Results obtained with the heparin- and Sarkosyl-prepared complexes used here were confirmed by using native transcription complexes isolated by gel filtration. In that case, the general pattern of differential activity obtained for the La mutants tested was the same as reported here (17).

RNA binding and the transcription factor activity of La. An unexpected finding was that the C-terminal basic region of La appears to be required for nascent RNA 3′-end protection. The presence of an intermediate band in the presence of certain C-terminally altered proteins (e.g., Fig. 3, lanes 4, 5, 10, and 13) suggests the possibility that La recognizes via discrete bipartite signals in the nascent transcript, mediated by N- and C-terminal domains of La, and that nascent-transcript processing may occur in distinct steps. In any case, the data indicate that the C-terminal domain of La is clearly involved in both transcription and the kind of RNA binding that is specific to La, namely, 3′-end recognition. Some of the C-terminally altered proteins that are defective in transcription (La 1-187, 1-328, and GXK>DE) are also defective in RNA 3′-end protection. However, some proteins defective in 3′-end protection, such as La 104-408 and La 26-408, are competent for transcription, whereas the protein La K>DE, which is defective for transcription, is competent for general RNA binding and RNA 3′-end protection. Thus, with regard to efficient transcription factor activity, a deficiency in 3′-end protection is tolerable as long as the ability to interact with RNA is maintained and the C-terminal basic region is intact.

Although RRM-1 increases the affinity of La for hY4 RNA, RRM-1 is not required for RNA binding at high concentrations (Fig. 2) or for efficient transcription (Fig. 4). However, RRM-1 appears to confer some nascent-RNA 3′-end protection, since its deletion or disruption leads to the appearance of processed RNA (Fig. 3, lane 10). Disruption or deletion of RRM-1 does not abrogate protection entirely, suggesting that RRM-2 and/or downstream residues may contribute to oligo(U) binding and 3′-end protection.

The results reported here are consistent with a model in which La interacts with the 3′ oligo(U) tract of nascent RNA to facilitate transcription termination and recycling of transcription complexes. However, as noted above, not all proteins that lead to diminution of 3′-end protection are defective in transcription. For example, La 104-408 provides sufficient RNA binding activity to fulfill La's requirement for RNA binding during multiround transcription, even though this protein exhibits a deficiency in RNA 3′-end protection. An explanation for this may be that while efficient protection from RNA 3′-end processing presumably requires sustained tight binding between La and the oligo(U) terminus throughout the course of the nuclease protection assay, association between La and the oligo(U) tract for a brief time at termination may be sufficient to confer transcription factor activity as assayed here.

Although evidence of involvement of RRM-3 in any of the biochemical activities of La reported here is lacking, we note that this putative RRM (3) has not been systematically examined. While this motif is absent in yeast La, it is conserved in the La proteins from insects to humans, suggesting an important role in RNA biogenesis (23, 45). Additional mutants in which the three RRMs can be internally mutated and/or swapped should help clarify the roles of the RRMs in general RNA binding, 3′ oligo(U) binding, and transcriptional activity.

The C-terminal domain of human La as a regulatory motif. These results reveal a role for the basic region of La in transcription. Transcription factor activity of La is inhibited by phosphorylation of serine 366, the single site found phosphorylated in vivo (15). However, until now, a potential target for phosphoserine 366-mediated transcription inhibition was unknown. Phosphoserine 366 resides just downstream of the basic region that was identified here as being functionally important for transcription. We can now propose that the basic region is required for and controls the transcription factor activity of La and that it can be negatively regulated by phosphorylation at the nearby site, serine 366. The unexpected finding that deletion or disruption of the C-terminal basic region of La leads to loss of RNA 3′-end protection suggests that a role for this region in transcription is to recognize the nascent-RNA 3′ end. An issue for future studies is whether additional, i.e., extragenic, targets in the transcription apparatus that interact with La can be identified.

An important question is why a role for La in pol III transcription in the yeast *S. cerevisiae* has not been observed. In

general, pol III in *S. cerevisiae* and higher eukaryotes is remarkably conserved at the level of transcription initiation (16). However, differences have been noted with regard to the lack of sequence homology of TFIIIC (but not TFIIIB) subunits, pathways of transcription complex assembly, and perhaps function of TFIIIC (11, 26, 38, 43, 46). A more fundamental and noteworthy difference in this regard may be that yeast class III genes require significantly longer termination signals than do vertebrates (i.e., seven T's are optimal in *S. cerevisiae* as opposed to four T's in *Xenopus* [2, 4]). Thus, it may be significant that the tRNA and 5S rRNA genes studied in the yeast system carry the termination signals T_7CT_7 and T_{20}, respectively, and these may facilitate termination and recycling of pol III better than the T_4 terminators of VA1 and other vertebrate class III genes, providing a possible explanation for why La homologous protein is not required in the yeast system (22, 45). These species-specific idiosyncrasies of pol III termination may reflect subtle yet intriguing differences in the pathways of termination and reinitiation in the two phyla.

RNA-mediated inhibition of transcription. La-dependent transcription can be inhibited by exogenous RNA, and, importantly, this inhibition can be reversed by excess La (Fig. 7). In addition to supporting the notion that a free RNA binding site is required for efficient transcription factor activity of La, the results demonstrate a potential for feedback inhibition of RNA synthesis. Two studies have examined the effects of exogenous RNA on pol III transcription. By chelating La with RNA, Gottlieb and Steitz (18) produced transcription complexes in which pol III appeared to be stalled at the terminator, although the amount of transcription was not shown to be decreased by exogenous RNA. Brow and Geiduschek (6) demonstrated that exogenous 5S rRNA inhibited 5S rRNA gene transcription. This effect was presumably due to sequestration of TFIIIA, a transcription complex assembly factor that also binds 5S rRNA. The present study is to be distinguished from the previous ones. By using preassembled, isolated transcription complexes, our analysis focused on the utilization of transcription complexes by pol III, not their assembly.

A model of La action. Efficient termination as has been reported for human La facilitates efficient recycling of preassembled transcription complexes and pol III (33). Facilitated recycling by yeast pol III onto the same template is dependent on the presence of downstream sequences that include the pol III terminator (14). Likewise, downstream sequences are required for efficient assembly and utilization of yeast, insect, and mammalian transcription complexes (2, 43, 47). The requirement for downstream sequences including a functional pol III terminator in facilitated recycling suggests that proper termination plays a role in restoring reinitiation competence to the exiting polymerase and/or the transcription complex, although other explanations are possible and this has not been rigorously examined. We envisage that La promotes efficient transcription by facilitating conversion of a transcription termination complex to a reinitiation-competent complex.

The results presented here and elsewhere support a model in which La interacts with the nascent RNA at termination and then must free itself of the RNA product before it can support another cycle of transcription by pol III. This implies a mechanism by which negative feedback can control RNA synthesis and suggests that dissociation of La and nascent RNA is an important step in the regeneration of a free La protein that will be active for a subsequent round of transcription.

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REFERENCES

1. **Adeniyi-Jones, S., and M. Zasloff.** 1985. Transcription, processing and nuclear transport of a B1 *Alu* RNA species complementary to an intron of the murine α-fetoprotein gene. Nature **317:**81–84.
2. **Allison, D. S., and B. D. Hall.** 1985. Effects of alterations in the 3′ flanking sequences on *in vivo* and *in vitro* expression of the yeast *SUP4*-o $tRNA^{Tyr}$ gene. EMBO J. **4:**2657–2664.
3. **Birney, E., S. Kumar, and A. R. Krainer.** 1993. Analysis of the RNA-recognition motif and RS and RGG domains: conservation in metazoan pre-mRNA splicing factors. Nucleic Acids Res **21:**5803–5816.
4. **Bogenhagen, D. F., and D. D. Brown.** 1981. Nucleotide sequences in Xenopus 5S DNA required for transcription termination. Cell **24:**261–270.
5. **Bogenhagen, D. F., W. M. Wormington, and D. D. Brown.** 1982. Stable transcription complexes of *Xenopus* 5S RNA genes: a means to maintain the differentiated state. Cell **28:**413–421.
6. **Brow, D. A., and E. P. Geiduschek.** 1987. Modulation of yeast 5S rRNA synthesis *in vitro* by yeast ribosomal protein YL3. J. Biol. Chem. **262:**13953–13958.
7. **Chan, E. K. L., and E. M. Tan.** 1987. The small ribonucleoprotein SS-B/La binds RNA with a conserved protease-resistant domain of 28 kilodaltons. Mol. Cell. Biol. **7:**2588–2591.
8. **Chang, D.-Y., and R. J. Maraia.** 1993. A cellular protein binds B1 and Alu small cytoplasmic RNAs *in vitro*. J. Biol. Chem. **268:**6423–6428.
9. **Chang, Y.-N., D. J. Kenan, J. D. Keene, A. Gatignol, and K.-T. Jeang.** 1994. Direct interactions between autoantigen La and human immunodeficiency virus leader RNA. J. Virol. **68:**7008–7020.
10. **Craig, A. W., Y. V. Svitkin, H. S. Lee, G. J. Belsham, and N. Sonenberg.** 1997. The La autoantigen contains a dimerization domain that is essential for enhancing translation. Mol. Cell. Biol. **17:**163–169.
11. **Dean, N., and A. J. Berk.** 1987. Separation of TFIIIC into two functional components by sequence specific DNA affinity chromatography. Nucleic Acids Res. **15:**9895–9907.
12. **Dean, N., and A. J. Berk.** 1988. Ordering promoter binding of class III transcription factors TFIIIC1 and TFIIIC2. Mol. Cell. Biol. **8:**3017–3025.
13. **Dieci, G., L. Duimio, F. Coda-Zabetta, K. U. Sprague, and S. Ottonello.** 1993. A novel RNA polymerase III transcription factor fraction that is not required for template commitment. J. Biol. Chem. **268:**11199–11207.
14. **Dieci, G., and A. Sentenac.** 1996. Facilitated recycling by RNA polymerase III. Cell **84:**245–252.
15. **Fan, H., A. L. Sakulich, J. L. Goodier, X. Zhang, J. Qin, and R. J. Maraia.** 1997. Phosphorylation of the human La antigen on serine 366 can regulate recycling of RNA polymerase III transcription complexes. Cell **88:**707–715.
16. **Geiduschek, E. P., and G. A. Kassavetis.** 1992. RNA polymerase III transcription complexes. Transcr. Reg. **1:**247–280.
17. **Goodier, J. L., H. Fan, and R. J. Maraia.** 1997. Unpublished observations.
18. **Gottlieb, E., and J. A. Steitz.** 1989. Function of the mammalian La protein: evidence for its action in transcription termination by RNA polymerase III. EMBO J. **8:**851–861.
19. **Gottlieb, E., and J. A. Steitz.** 1989. The RNA binding protein La influences both the accuracy and the efficiency of RNA polymerase III transcription *in vitro*. EMBO J. **8:**841–850.
20. **Hendrick, J. P., S. L. Wolin, J. Rinke, M. R. Lerner, and J. A. Steitz.** 1981. Ro small cytoplasmic ribonucleoproteins are a subclass of La ribonucleoproteins: further characterization of the Ro and La small ribonucleoproteins from uninfected mammalian cells. Mol. Cell. Biol. **1:**1138–1149.
21. **Kassavetis, G. A., B. R. Braun, L. H. Nguyen, and E. P. Geiduschek.** 1990. *S. cerevisiae* TFIIIB is the transcription initiation factor proper of RNA polymerase III, while TFIIIA and TFIIIC are assembly factors. Cell **60:**235–245.
22. **Kassavetis, G. A., S. T. Nguyen, R. Kobayashi, A. Kumar, E. P. Geiduschek, and M. Pisano.** 1995. Cloning, expression, and function of *TFC5*, the gene encoding the B″ component of the *Saccharomyces cerevisiae* RNA polymerase III transcription factor TFIIIB. Proc. Natl. Acad. Sci. USA **92:**9786–9790.
23. **Kenan, D. J.** 1995. RNA recognition by the human La protein and its relevance to transcription, translation and viral infectivity. Ph.D. thesis. Duke University, Durham, N.C.
24. **Kovelman, R., and R. G. Roeder.** 1990. Sarkosyl defines three intermediate steps in transcription initiation by RNA polymerase III: application to stimulation of transcription by E1A. Genes Dev. **4:**646–658.
25. **Kunkel, T. A., J. D. Roberts, and R. A. Zakour.** 1987. Rapid and efficient site-specific mutagenesis without phenotypic selection. Methods Enzymol. **154:**367.
26. **Lagna, G., R. Kovelman, J. Sukegawa, and R. G. Roeder.** 1994. Cloning and characterization of an evolutionarily divergent DNA-binding subunit of

mammalian TFIIIC. Mol. Cell. Biol. **14:**3053–3064.
27. **Lassar, A. B., P. L. Martin, and R. G. Roeder.** 1983. Transcription of class III genes: formation of preinitiation complexes. Science **222:**740–748.
28. **Lerner, M. R., J. A. Boyle, J. A. Hardin, and J. A. Steitz.** 1981. Two novel classes of small ribonucleoproteins detected by antibodies associated with lupus erythematosus. Science **211:**400–402.
29. **Maraia, R.** 1991. The subset of mouse B1 (*Alu*-equivalent) sequences expressed as small processed cytoplasmic transcripts. Nucleic Acids Res. **19:** 5695–5702.
30. **Maraia, R., M. Zasloff, P. Plotz, and S. Adeniyi-Jones.** 1988. Pathway of B1-*Alu* expression in microinjected oocytes: *Xenopus laevis* proteins associated with nuclear precursor and processed cytoplasmic RNAs. Mol. Cell. Biol. **8:**4433–4440.
31. **Maraia, R. J.** 1996. Transcription termination factor La is also a reinitiation factor for RNA polymerase III. Proc. Natl. Acad. Sci. USA **93:**3383–3387.
32. **Maraia, R. J., D.-Y. Chang, A. P. Wolffe, R. L. Vorce, and K. Hsu.** 1992. The RNA polymerase III terminator used by a B1-Alu element can modulate 3′ processing of the intermediate RNA product. Mol. Cell. Biol. **12:**1500–1506.
33. **Maraia, R. J., D. J. Kenan, and J. D. Keene.** 1994. Eukaryotic transcription termination factor La mediates transcript release and facilitates reinitiation by RNA polymerase III. Mol. Cell. Biol. **14:**2147–2158.
34. **Maraia, R. J., N. Sasaki-Tozawa, C. T. Driscoll, E. D. Green, and G. J. Darlington.** 1994. The human Y4 small cytoplasmic RNA gene is controlled by upstream elements and resides on chromosome 7 with all other hY scRNA genes. Nucleic Acids Res. **22:**3045–3052.
35. **Pruijn, G. J., R. L. Slobbe, and W. J. van Venrooij.** 1991. Analysis of protein-RNA interactions within Ro ribonucleoprotein complexes. Nucleic Acids Res. **19:**5173–5180.
36. **Rinke, J., and J. A. Steitz.** 1982. Precursor molecules of both human 5S ribosomal RNA and transfer RNAs are bound by a cellular protein reactive with anti-La lupus antibodies. Cell **29:**149–159.
37. **Ruth, J., C. Conesa, G. Dieci, O. Lefebvre, A. Dusterhoft, S. Ottonello, and A. Sentenac.** 1996. A suppressor of mutations in the class III transcription system encodes a component of yeast TFIIIB. EMBO J. **15:**1941–1949.
38. **Sinn, E., Z. Wang, R. Kovelman, and R. G. Roeder.** 1995. Cloning and characterization of a TFIIIC2 subunit (TFIIICβ) whose presence correlates with activation of RNA polymerase III-mediated transcription by adenovirus E1A expression and serum factors. Genes Dev. **9:**675–685.
39. **Sklar, V. E. F., and R. G. Roeder.** 1976. Purification and subunit structure of deoxyribonucleic acid-dependent ribonucleic acid polymerase III from the mouse plasmacytoma, MOPC 315. J. Biol. Chem. **251:**1064–1073.
40. **Slobbe, R. L., W. Pluk, W. J. van Venrooij, and G. J. M. Pruijn.** 1992. Ro ribonucleoprotein assembly *in vitro*. Identification of RNA-protein and protein-protein interactions. J. Mol. Biol. **227:**361–366.
41. **Stefano, J. E.** 1984. Purified lupus antigen La recognizes an oligouridylate stretch common to the 3′ termini of RNA polymerase III transcripts. Cell **36:**145–154.
42. **Wang, Z., and R. G. Roeder.** 1995. Structure and function of a human transcription factor TFIIIB subunit that is evolutionarily conserved and contains both TFIIB- and high-mobility-group protein 2-related domains. Proc. Natl. Acad. Sci. USA **92:**7026–7030.
43. **Wang, Z., and R. G. Roeder.** 1996. TFIIIC1 acts through a downstream region to stabilize TFIIIC2 binding to RNA polymerase III promoters. Mol. Cell. Biol. **16:**6841–6850.
44. **Willis, I. M.** 1993. RNA polymerase III genes, factors and transcriptional specificity. Eur. J. Biochem. **212:**1–11.
45. **Yoo, C. J., and S. L. Wolin.** 1994. La proteins from *Drosophila melanogaster* and *Saccharomyces cerevisiae*: a yeast homolog of the La autoantigen is dispensable for growth. Mol. Cell. Biol. **14:**5412–5424.
46. **Yoshinaga, S. K., P. A. Boulanger, and A. J. Berk.** 1987. Resolution of human transcription factor TFIIIC into two functional components. Proc. Natl. Acad. Sci. USA **84:**3585–3589.
47. **Young, L. S., D. H. Rivier, and K. U. Sprague.** 1991. Sequences far downstream from the classical tRNA promoter elements bind RNA polymerase III transcription factors. Mol. Cell. Biol. **11:**1382–1392.