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Eukaryotic Transcription Termination Factor La Mediates Transcript Release and Facilitates Reinitiation by RNA Polymerase III

RICHARD J. MARAIA,[1]* DANIEL J. KENAN,[2] AND JACK D. KEENE[2]

Laboratory of Molecular Growth Regulation, National Institute of Child Health and Human Development, Bethesda, Maryland 20892,[1] and Department of Microbiology, Duke University Medical Center, Durham, North Carolina 27710[2]

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Ample evidence indicates that *Alu* family interspersed elements retrotranspose via primary transcripts synthesized by RNA polymerase III (pol III) and that this transposition sometimes results in genetic disorders in humans. However, *Alu* primary transcripts can be processed posttranscriptionally, diverting them away from the transposition pathway. The pol III termination signal of a well-characterized murine B1 (*Alu*-equivalent) element inhibits RNA 3′ processing, thereby stabilizing the putative transposition intermediary. We used an immobilized template-based assay to examine transcription termination by VA1, 7SL, and *Alu* class III templates and the role of transcript release in the pol III terminator-dependent inhibition of processing of B1-*Alu* transcripts. We found that the RNA-binding protein La confers this terminator-dependent 3′ processing inhibition on transcripts released from the B1-*Alu* template. Using pure recombinant La protein and affinity-purified transcription complexes, we also demonstrate that La facilitates multiple rounds of transcription reinitiation by pol III. These results illustrate an important role for La in RNA production by demonstrating its ability to clear the termination sites of class III templates, thereby promoting efficient use of transcription complexes by pol III. The role of La as a potential regulatory factor in transcript maturation and how this might apply to *Alu* interspersed elements is discussed.

Transcripts involved in a diversity of biochemical activities are synthesized from RNA polymerase III (pol III)-dependent genes (reviewed in reference 69). In addition to these templates, the genomes of most higher eukaryotes contain short interspersed elements of high copy number which contain promoters for pol III (27, 31). The best characterized of these are the human *Alu* repeats and their rodent counterparts, B1 (B1-*Alu*). A significant biological consequence of *Alu* expression is that their transposition within the human genome sometimes causes heritable disorders (19, 47, 67). Ample structural evidence at the DNA and RNA levels indicate that Alu short interspersed elements were retrotransposed through poly(A)-containing intermediaries synthesized by pol III (reviewed in reference 57). One model suggests that pol III termination may be partly responsible for *Alu* transposition by creating oligo(U) 3′ termini which can base pair intramolecularly with the internal poly(A) tract of *Alu* transcripts to prime reverse transcriptase (28, 57, 66). However, *Alu* and B1-*Alu* transcripts undergo a 3′ processing event which inactivates potential transposition intermediaries by conversion to the poly(A)-minus RNAs found in vivo (1, 39, 41, 42). Intriguingly, and perhaps relevant to transposition, it was noted that the sequence flanking the termination signal of a well-studied pol III-dependent B1-*Alu* has a profound effect on the posttranscriptional fate of the nascent transcripts it produces in vitro and in vivo (1, 35, 39, 40, 41, 58, 71). Inhibition of 3′ processing of this B1-*Alu* transcript is coupled to its synthesis by pol III, suggesting that a terminator-specific factor protects nascent transcripts from 3′ processing (41). In addition to affording insight into the role of 3′ flanking sequences in *Alu* expression and transposition (41, 57), this system provides an opportunity to examine mechanisms of pol III transcription termination.

cis-acting signals of four or more dT residues confer pol III termination in most cases (reviewed in reference 18). However, the base composition flanking the dT_4 signal affects the efficiency of transcriptional termination (5, 45). Biochemical and genetic studies indicate that pol III itself is involved in utilizing dT_4 termination signals (8, 13, 30, 68). Evidence that the RNA-binding protein La is involved in pol III termination has also been obtained (21, 22). La is a 50-kDa phosphoprotein first identified as a human autoimmune antigen and later found to be transiently associated with all primary transcripts synthesized by pol III (53; reviewed in reference 22). La binds to the 3′-terminal oligo(U) tracts on these transcripts, which correspond to the RNA copy of the oligo(dT) pol III termination signal (60). It was demonstrated that certain pol III-dependent transcripts synthesized in extracts depleted of La were slightly shorter at their 3′ ends than those synthesized when La was present. Various findings suggested that these RNAs were contained in transcription complexes arrested at the termination signal (21). However, while depletion of La from nuclear extract drastically reduced the efficiency of pol III-mediated RNA synthesis, subsequent repletion with biochemically purified La antigen restored only a small fraction of the activity (22). While the results were consistent with a role for La in stimulating transcriptional reinitiation, this remained undetermined. Nonetheless, the results directly implicated La in pol III transcription termination and RNA 3′ end formation and prompted a model in which La is required for the synthesis of the terminal U residues of nascent pol III transcripts (21, 22; see Discussion).

Previous findings indicated that inhibition of B1-*Alu* RNA 3′ processing was coupled to the pol III terminator (41) and that the B1-*Alu* nascent transcript was associated with La (40).

* Corresponding author. Mailing address: Laboratory of Molecular Growth Regulation, Building 6, Room 416, National Institute of Child Health and Human Development, Bethesda, MD 20892. Tel: 301-402-3567. Fax: 301-480-9354. Electronic mail address: maraia@ncbi.nlm.nih.gov.

These observations strongly suggested that La was involved in modulating 3′ processing of B1-*Alu* RNA and thus stabilizing potential transposition intermediaries. Therefore, we wanted to examine a role for La in B1-*Alu* RNA expression. The molecular cloning of La from human (9, 10), bovine (11), *Xenopus laevis* (56), and mouse (64) cDNAs and its overexpression in recombinant bacteria provides new opportunities to study its effects on pol III transcription.

In this study, we used streptavidin-agarose and biotin-immobilized pol III-dependent templates encoding B1-*Alu*, *Alu*, VA1, and 7SL RNAs to examine transcription termination, transcript release, and B1-*Alu* posttranscriptional processing. We found that RNAs transcribed by nuclear extract from these class III templates accumulate at their naturally occurring dT_4 termination signals but are inefficiently released. Thus, this immobilized transcription system which is inherently limited in transcription termination has allowed us to examine the effects of pure recombinant La protein on B1-*Alu* and other class III gene expression. Exogenously added La exhibits three effects: (i) it facilitates transcript release from B1-*Alu*, *Alu*, VA1, and 7SL RNA gene terminators, (ii) it protects nascent B1-*Alu* RNA from posttranscriptional 3′ processing, and (iii) it stimulates multiple rounds of RNA synthesis when added to isolated preassembled pol III transcription complexes. La can clear pol III termination signals of arrested transcripts and allow efficient use of stable transcription complexes by pol III. Because the termination signal in conjunction with La can modulate B1-*Alu* nascent transcript turnover, we suggest that La may also modulate posttranscriptional regulation of other pol III transcripts and that transcription and RNA turnover may be coupled.

MATERIALS AND METHODS

AATTTTTAA (AA) and GCTTTTGC (GC) terminator-containing B1-*Alu* templates were previously described as $pGB1e_{(WT)}$ and $pGB1_{Tm}$, respectively (41). *Eco*RI-linearized templates were filled in by Klenow enzyme (Bethesda Research Laboratories) with biotin-7-dATP (Bethesda Research Laboratories) as described by Arias and Dynan (2). The material was spun through Sephadex G-50 to remove unincorporated biotin-7-dATP prior to incubation with streptavidin-agarose (immunopure; Pierce). After a 16-h incubation at 4°C, the supernatant was removed and the agarose beads were washed three times with 1 M KCl and twice with Tris-EDTA; each wash equal to at least 10 times the bead volume. To attain similar coupling efficiencies for various templates, an excess of biotinylated DNA was used; after coupling, the amount of DNA retained was approximated by ethidium bromide staining. The material was then stored in Tris-EDTA at 4°C as a 50% slurry and equilibrated by two washes with transcription buffer (TB) just prior to use.

The 7SL RNA gene 7L30.1 (65) subcloned into the *Hin*dIII-*Eco*RI sites of pUC19 was a gift from K. Sakamoto and B. Howard (55). The VA1 RNA gene-containing plasmid pAd2VA1 was a gift of W. Doerfler (32); it was subcloned into pT7/T3 α18 (Promega) to introduce an *Eco*RI site 30 bp downstream of its CCTTTTGG termination signal. The *Alu* element which recently transposed into the neurofibromatosis I gene (NF1-Alu_{-U4}) and its transcripts were previously described (42, 67).

In vitro transcription reactions using nuclear extract prepared from murine erythroleukemia cells were performed as previously described (41). Reaction mixtures typically contained 15 μl of DNA-agarose beads (~200 ng of DNA) in TB (20 mM *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid [HEPES; pH 7.9], 10 mM $MgCl_2$, 0.2 mM EDTA, 0.06 M KCl) containing 500 μM each ATP, CTP, and UTP and 30 μM GTP plus [α-^{32}P]GTP. Typically, a ratio of 3 μl of nuclear extract (protein concentration, 3.35 mg/ml; 20% glycerol, 20 mM HEPES, 0.1 M KCl, 12.5 mM $MgCl_2$, 0.2 mM EDTA, 0.2 mM EGTA, 2 mM dithiothreitol) was used per 15 μl of agarose beads. Incubations were at 30°C for the times indicated in the figure legends. All transcription products were phenol-chloroform extracted in the presence of 2% sodium dodecyl sulfate (SDS), 10 mM EDTA, and carrier tRNA and ethanol precipitated prior to electrophoretic separation by 6% polyacrylamide–8 M urea-containing gels.

For separation of released material from template-associated material, Spin-X microcentrifuge spin filtration tubes (0.2-μm pore size, cellulose acetate; Costar) were used. The agarose bead slurry was transferred into the filter cup and spun for 1 min; the agarose beads were retained in the filter and could be washed with TB, and the filtrates were combined with stop buffer (SB; 2% SDS, 10 mM EDTA, 50 μg of tRNA carrier per ml), prior to purification. The filter cup containing the retained templates was then placed into a clean microcentrifuge tube, SB was added, and the filtrate was collected. The SB extraction was then repeated, and filtrates were combined prior to purification. (The great majority of retained transcripts are resistant to release for at least 20 min and multiple washes with TB (55a). More than 95% of the remaining retained counts per minute can then be recovered by extraction with the SDS-containing stop buffer.)

For the experiments in which preformed complexes were used, immobilized templates were preincubated in TB plus extract containing ATP, GTP, and CTP but no UTP (41). After preincubation, the beads were washed three times, each wash equal to 10 bead volumes, with TB or TB containing 0.5 M KCl or as indicated in the figure legends or text, followed by equilibration by two washes with TB. All four nucleoside triphosphates (NTPs) were then added along with [α-^{32}P]GTP and bovine serum albumin (BSA) carrier (RNase and DNase free; Pharmacia), and the reaction was allowed to proceed as indicated. BSA carrier was included in all reactions in which preformed transcription complexes were used. Because of an ~8-fold decrease in activity associated with preincubated washed complexes, the number of complexes in Fig. 3 was adjusted for direct comparison. For single-round transcriptions [α-^{32}P]GTP at 20 μM was included in the preincubation step along with CTP and ATP. After preincubation, the beads were washed three times with TB–0.5 M KCl as described above and then subjected to two equilibration washes with TB (removing unincorporated ^{32}P), and all four NTPs (nonradiolabeled) were added with a large excess of unlabeled GTP (final concentration, 1.5 mM), along with heparin to 300 μg/ml (15, 33). The reactions were stopped after 3 min, and total RNA was purified. Results identical to those shown in Fig. 8C were obtained when heparin was omitted (not shown). Tagetitoxin (Tagetin; Epicentre Technologies), a pol III inhibitor, was used at 30 μM, a concentration known to specifically inhibit pol III-transcribed genes (23, 61), including the B1 gene used in this study, under plasmid-mediated standard transcription conditions (data not shown), as does α-amanitin at moderate but not low doses (1, 39a).

Recombinant La protein was expressed from human La cDNA (9, 10) in *Escherichia coli* and purified by ion-exchange and affinity chromatography to >99% homogeneity (33a). Purified TFIIIA was a gift of Dan Lee and Alan Wolffe (37). Purified recombinant U1 small nuclear RNA (snRNA)-associated A protein was prepared by Charles Query (26). Recom-

binant TATA-binding protein was obtained from Promega, which marketed it as TFIID.

Anti-La and other autoimmune sera were obtained as reference sera from the Arthritis Foundation at the Centers for Disease Control and Prevention (Atlanta, Ga.). The anti-La serum immunoprecipitated small cellular RNAs characteristic of anti-La and detected a single major polypeptide of ~50 kDa by Western blot (immunoblot) analysis (12a). Immunoprecipitations were performed with immunoglobulin G (IgG) preadsorbed onto protein A-Sepharose (Pharmacia) and washed four times with NET-2 (62). After formation of preformed complexes, nuclear extract was removed and the immobilized complexes were washed with 2 bead volumes of TB to minimize retention of excess material. This low-volume washing removes excess factors but does not strip complexes, as judged by several criteria (not shown). Synthesis then occurred in the presence of [α-^{32}P]GTP for 45 min. The released fractions were collected and incubated with protein A-Sepharose-IgG for 60 min at 4°C. The protein A-Sepharose-IgG was sedimented, and the supernatants were collected for purification and analysis. The immunoprecipitates were washed four times with NET-2, extracted with phenol-chloroform in the presence of 2% SDS, 10 mM EDTA, and tRNA carrier, and ethanol precipitated.

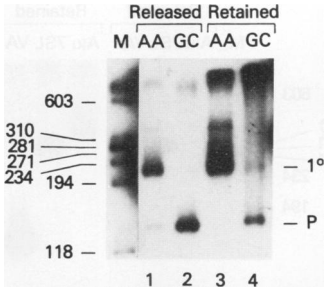

FIG. 1. Separation of released from template-associated B1-*Alu* RNA reveals that transcript release is not the only determinant of 3′ processing. Products of standard nuclear extract-mediated in vitro transcription of biotinylated templates coupled to streptavidin-agarose beads in the presence of [α-^{32}P]GTP are shown. Following a 90-min transcription reaction, a portion of the RNAs is readily released from the template while some are stably retained with the template after multiple washes. RNA products from linearized B1-*Alu* templates carrying either the AA or the GC terminator, as indicated above the lanes were analyzed. The primary transcript (1°) and processed species (P) as previously characterized (1, 39, 41) are indicated. *Hae*III-digested, ^{32}P-labeled size markers were coelectrophoresed in lanes M; sizes in nucleotides are indicated to the left.

RESULTS

Pol III transcription complexes remain stably associated with class III templates, including B1-*Alu*, through multiple rounds of RNA synthesis by pol III (4, 6, 36, 41, 59). Furthermore, a B1-*Alu* template carrying either its natural termination signal AA or the predictably more efficient site-mutated sequence GC derived from the 5S gene (5) produce primary transcripts of 210 nucleotides (nt) which differ in stability in vitro and in vivo (41). The pol III-generated GC transcript is rapidly and efficiently processed at the 3′ end to produce a 135-nt small cytoplasmic B1-*Alu* RNA, while the AA primary transcript is significantly more stable (41). Those studies indicate that inhibition of B1-*Alu* RNA 3′ processing is AA terminator dependent and coupled to synthesis by pol III (41).

Transcript release is correlated with but not sufficient for B1-*Alu* RNA 3′ processing. Plasmids containing either the B1-*Alu* AA or GC terminator sequence (41) were linearized with *Eco*RI, filled in with biotin-7-dATP, and bound to streptavidin-agarose beads (2). RNA products from these templates were analyzed after nuclear extract-mediated in vitro transcription in the presence of [α-^{32}P]GTP. Pol III-initiated RNAs synthesized from these templates will be terminated at either the 210-nt pol III termination signal or a downstream CATTT TCG signal to generate a 275-nt transcript or extended to the end of the template to generate a transcript of 350 nt. In practice, most transcripts that read through the 210-nt signal also read through the 275-nt signal to the end of the template (see below and Discussion). RNAs released by immobilized complexes were fractionated from those which remained associated with the template by centrifugation microfiltration and were then purified. The template-associated material was washed twice with transcription buffer (TB), and the retained RNA was then extracted with SDS, purified, and analyzed by denaturing polyacrylamide gel electrophoresis (Fig. 1). Although the AA terminator released some 210-nt transcript (primary [1°]) and less of the processed form (P, lane 1), typically about 70% of the total RNA (210 nt plus 135 nt) accumulated at the 210-nt termination signal and was retained with the template (lane 3). Readthrough transcripts of 350 nt were preferentially but not completely retained with the template in addition to much larger RNAs which presumably represent non-promoter-mediated transcription. Transcript release from the AATTTTTAA terminator was only 25 to 30% efficient (compare 1° in lanes 1 and 3). In contrast, the GC template produced processed RNA that accumulated in the released fraction (lane 2), with relatively less 210-nt species remaining associated with the template (lane 4). We conclude that the AA terminator retained more of the 210-nt RNA species in a template-bound complex than did the GC terminator (compare 1° in lanes 3 and 4).

Unexpectedly, we found that the GC-retained fraction reproducibly contained processed RNA (P, lane 4) in addition to primary transcript, supporting previous results which indicated that the GC 210-nt primary transcript is rapidly and efficiently processed (41). This result suggests that a B1-*Alu* RNA 3′ processing activity may also remain associated with washed B1-*Alu* transcription complexes. However, further studies will be needed to confirm the specificity of this association.

A small but significant amount of released RNA resulting from AA template transcription was reproducibly found as the processed form, while no processed species remained associated with the AA template (Fig. 1; compare P in lanes 1 and 3). In contrast, most GC RNA was processed (lanes 2 and 4). These data indicate that transcript release and 3′ processing are separable steps in the production of the 135-nt B1-*Alu* RNA species and indicate that release alone is not sufficient for efficient processing. It was shown that AA and GC transcripts were processed at approximately the same efficiency if synthesized by T7 RNA polymerase in the presence of nuclear extract or if added as prepurified RNAs (41). The cumulative data demonstrate that processing is specifically inhibited by the AA pol III termination signal apparently independently of release. We conclude that AA transcripts were released from transcription complexes yet remained refractory to 3′ processing in comparison with GC transcripts.

Naturally occurring pol III termination signals inefficiently release transcripts synthesized from immobilized templates. Although the data showed that the AA terminator retained more of its RNA product than did the GC terminator, to interpret this as a correlation of G+C richness and terminator strength was potentially confounded by the efficient processing

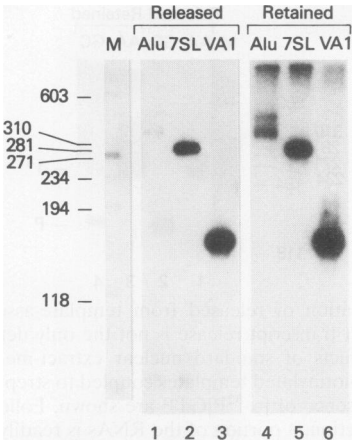

FIG. 2. Natural pol III termination signals inefficiently release transcripts synthesized in vitro from immobilized templates. Class III genes carrying TCTTTTGC (7SL), CCTTTTGG (VA1), and AGTTT TAG (*Alu*) terminators were used for standard in vitro transcription, and the products were separated into released and retained fractions as in Fig. 1. The upper band of the doublet seen in the *Alu* lanes reflects readthrough beyond the 348-nt AGTTTTAG signal. *Hae*III digested, ^{32}P-labeled size markers were coelectrophoresed in lane M; sizes in nucleotides are indicated to the left.

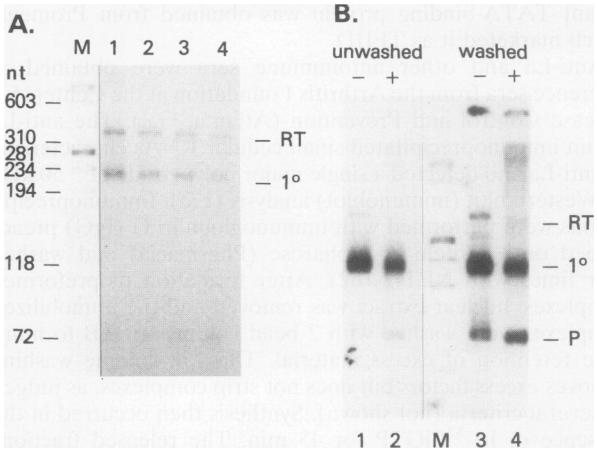

FIG. 3. Evidence that the factor which blocks 3′ processing of a B1-*Alu* RNA is acquired by the pol III transcription complex before termination. B1-*Alu* AA template transcription complexes were formed by incubating immobilized templates in nuclear extract containing ATP, CTP, and GTP but no UTP (41). Non-template-associated factors were removed by washing, and synthesis was then allowed to proceed upon addition of all four NTPs, including [α-^{32}P]GTP, and BSA carrier. (A) Stability of transcription complexes to stringent washes with various concentrations of KCl (lane 1; 0.06 M, lane 2; 0.25 M, lane 3; 0.5 M, lane 4; 1.0 M). After synthesis, total RNA from nonfractionated reactions was analyzed. Only a tiny fraction of total RNA generated from this template is processed (41) (e.g., Fig. 1A), which is why in unfractionated samples such as these, the processed RNA is usually not detectable. The positions of primary transcript (1°) and 350-nt readthrough transcript (RT) are indicated on the right. (B) Examination of processability of released B1-*Alu* RNA. After a scaled-up preincubation reaction as described above, complexes were either washed three times with transcription buffer (0.06 M KCl; each wash equal to 10 bead volumes) or left in nuclear extract unwashed. Following a subsequent 40-min synthesis in the presence of [α-^{32}P]GTP, the released fractions were collected, subjected to gel filtration to remove unincorporated NTPs, and further incubated in fresh extract (+) or buffer alone (−) for an additional 40 min, and RNA was prepared. The positions of primary transcript (1°), 350-nt readthrough (RT), and processed species (P) are indicated on the right. Size markers (lane M) are as in Fig. 1.

of GC transcripts. In any case, we wanted to know whether inefficient release was a specific characteristic of the B1-*Alu* A-rich terminator. Therefore, we examined whether other class III genes which contain G+C-rich terminators such as the well-characterized VA1 and 7SL templates would demonstrate more efficient release in this system (Fig. 2). Although we found that release from the 7SL and VA1 terminators was somewhat more efficient than release from the A-rich terminators tested, the major conclusion drawn from the results is that all of these pol III-dependent transcripts were distributed between the released (lanes 1 to 3) and retained (lanes 4 to 6) fractions. We conclude that in this system, for each of the templates tested, their pol III promoters initiated more transcription than their terminators were able to release.

Evidence that the factor which blocks 3′ processing of B1-*Alu* RNA is acquired by the pol III transcription complex. To further investigate the B1-*Alu* RNA processing inhibitor, we used immobilized transcription complexes to examine the possibility that the inhibitor can associate with the template early as part of the transcription complex. In addition, it is possible that we can dissociate it from preassembled transcription complexes by washing them prior to termination. For this analysis, we allowed transcription complexes to form by preincubating the immobilized B1-*Alu* AA template with nuclear extract in the presence of ATP, CTP, and GTP but not UTP. It was shown that under these conditions, this B1-*Alu* template is assembled into transcription complexes and that efficient synthesis proceeds upon addition of UTP (41).

We first determined the stability of preformed immobilized B1-*Alu* complexes by subjecting them to extensive washes with various concentrations of KCl (Fig. 3A). Others have shown that pol III-containing transcription complexes remain assembled on class III genes under similar conditions (15, 29, 33). After washing, transcription complexes were equilibrated with TB, and synthesis was monitored after addition of all four NTPs, including [α-^{32}P]GTP, and BSA carrier. RNA was extracted from the total (nonfractionated) reaction mixtures (Fig. 3A). We noted that washing the complexes led to a high proportion of 350-nt readthrough transcripts, indicating that termination efficiency at the 210-nt AA signal was decreased by the wash protocol (data not shown; cf. Fig. 1), but we have not quantitated this effect. Instead, we examined the ability of washed transcription complexes to subsequently produce 3′ processed B1-*Alu* RNA (Fig. 3B). After formation, complexes were either washed with TB or left in the extract-containing reaction mixture. ^{32}P-NTPs were then added to both reactions, and syntheses were allowed to proceed. The released fractions were collected in native form and further incubated either with or without the addition of fresh extract. A substantial amount of processed RNA appeared in the fractions produced from washed transcription complexes (Fig. 3B, lanes 3 and 4), whereas RNA released from unwashed complexes remained relatively unprocessed (lanes 1 and 2). It is noteworthy that readthrough transcripts increased with washing as did the amount of processing (data not shown), suggesting that these effects are related. Adding fresh extract to the released [^{32}P]RNA products led to slightly more processing in both cases (lanes 2 and 4). We interpret the results as evidence that washing removes a processing inhibitory factor from preformed transcription complexes as opposed to removing a soluble inhibitor present in the extract. We have not deter-

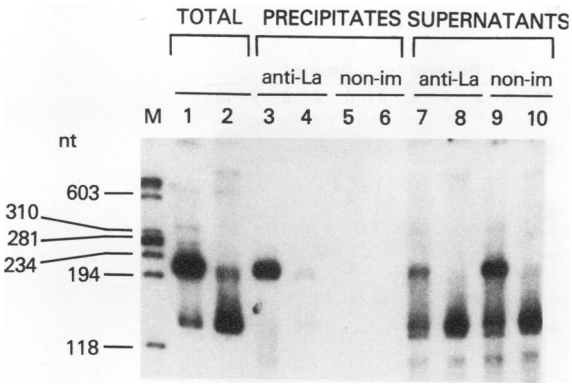

FIG. 4. B1-*Alu* primary transcripts released from transcription complexes are associated with the La protein. Only the released products of in vitro transcriptions were analyzed in this assay. After a 45-min in vitro transcription of preincubated complexes, aliquots of the released materials were purified directly (total; lanes 1 and 2) or subjected to immunoprecipitation with anti-La (lanes 3 and 4) or nonimmune (non-im; lanes 5 and 6) IgG preadsorbed onto protein A-Sepharose. The material remaining nonimmunoprecipitable after incubation with IgG was also analyzed (supernatants; lanes 7 to 10). Odd-numbered lanes, AA terminator template; even-numbered lanes, GC terminator template. Size markers (lane M) are as in Fig. 1.

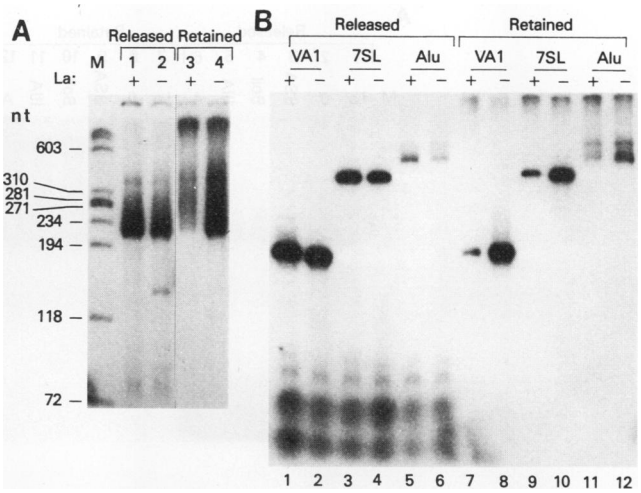

FIG. 5. La facilitates release of transcripts that otherwise remain at the pol III termination signal. Standard in vitro transcriptions of immobilized pol III-dependent templates were carried out in nuclear extract containing endogenous La protein, to which highly purified supplemental recombinant La protein (+) or BSA (−) was added. After a 90-min transcription reaction, released and retained components were separated and analyzed. (A) B1-*Alu* template carrying the AA terminator; (B) VA1, 7SL, and *Alu* templates, as indicated above the lanes.

mined whether the inhibitor binds to transcription complexes per se or directly to DNA. However, it was previously shown that the processing inhibitor was pol III dependent and AA terminator specific (41). Thus, the present data support the notion that this pol III-dependent factor inhibits processing of released B1-*Alu* RNA and that it associates with the transcription complex at the initiation or early elongation phase, prior to termination of RNA synthesis. In the following sections, we present evidence that this factor is La.

B1-*Alu* primary transcripts released from transcription complexes are efficiently associated with the La protein. Previous results indicated that the B1-*Alu* primary transcript synthesized in microinjected *Xenopus* oocytes exists as a small ribonucleoprotein which is associated with La (40). However, those experiments did not address the relative amount of released RNA that was associated with La. Therefore, it is not known whether the released 210-nt primary transcript existed as a La-free RNA. In Fig. 4, we used immunoprecipitation to determine how much of the RNA released from B1-*Alu* templates was associated with La. Immobilized AA and GC templates were transcribed in vitro by using nuclear extract, and the released fractions were analyzed. Aliquots of the total RNAs released from AA and GC templates are shown in lanes 1 and 2, respectively. The remaining released material was subjected to immunoprecipitation. Both the precipitated (lanes 3 to 6) and nonreactive (lanes 7 to 10) supernatant components were analyzed after immunoprecipitation. Most of the 210-nt AA RNA was found to be associated with La (lane 3), while much less remained in the supernatant (lane 7). As expected, the 3′ processed 135-nt RNA produced from the GC template was not immunoprecipitated (lane 4) but remained in the supernatant (lane 8). However, a small but significant amount of unprocessed 210-nt GC primary transcript was immunoprecipitable by anti-La, as can be seen in lane 4. We conclude that nascent transcripts released as pol III-terminated RNAs were preferentially associated with La. Thus, although the AA template released its 210-nt RNA inefficiently (Fig. 1), most of this was associated with La and was resistant to processing. Nonimmune IgG (lanes 5 and 6) as well as other autoimmune IgG such as anti-Ro and anti-Sm did not precipitate this RNA which remained in the supernatants (lanes 9 and 10 and data not shown). The results also demonstrated that the nuclear extract contained functional La protein in specific association with the 3′ end of nascent B1-*Alu* RNA but not with the processed species.

Washing of transcription complexes (e.g., as for Fig. 2) decreased the proportion of B1-*Alu* transcripts that were immunoprecipitable by anti-La (data not shown). These data suggested a relationship between termination efficiency, association with La, and resistance to processing. Therefore, we chose to examine directly the effects of purified recombinant La on B1-*Alu* RNA expression.

Recombinant La mediates transcript release and inhibition of B1-*Alu* RNA 3′ processing. We examined the effects of adding recombinant La to promoter-mediated in vitro transcription of the B1-*Alu* template which accumulates RNA at the AA termination signal. Two effects were reproducibly attributable to the added La protein (Fig. 5A): (i) efficiency of transcript release was increased by La (compare lanes 1 and 3 and lanes 2 and 4), since most of the 210-nt RNA was no longer retained with the immobilized complexes (compare lanes 3 and 4) but instead was recovered in the released fraction (compare lanes 1 and 2), and (ii) no processed RNA species appeared in the released fraction in the presence of recombinant La (lane 1) despite the increased amount of released 210-nt RNA (compare lanes 1 and 2). The data show that La caused a redistribution of much of the 210-nt B1-*Alu* RNA from the template-associated fraction to the released fraction. Furthermore, addition of La to nuclear extract caused a preferential release of the 210-nt RNA from the template, while the trail of larger species was not as affected (Fig. 5A; compare lanes 3 and 4). It is important to note that the addition of La did not increase the amount of RNA produced from these nuclear extract-mediated transcriptions (see Discussion). As shown below, La can stimulate RNA synthesis;

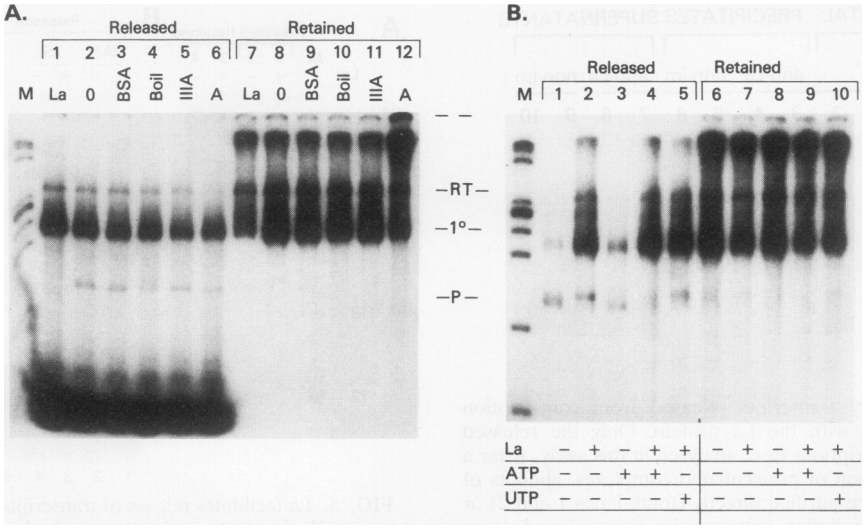

FIG. 6. Transcript release and inhibition of B1-*Alu* RNA 3′ processing are specific activities of La. (A) Extract-mediated in vitro transcription of immobilized B1-*Alu* template was carried out in the presence of various added purified proteins, and the products were prepared from the released (lanes 1 to 6) and retained (lanes 7 to 12) fractions. Lanes: 1 and 7, La; 2 and 8, buffer alone; 3 and 9, BSA; 4 and 10, heat-treated La; 5 and 11, TFIIIA; 6 and 12, U1-A protein. (B) After a 90-min in vitro transcription reaction as described above, the retained termination complexes only were further analyzed (see text). The [^{32}P]RNA-containing arrested complexes were washed twice with buffer lacking NTPs, resuspended in transcription buffer containing additives as indicated in the figure and below, incubated for 5 min, and separated into released (lanes 1 to 5) and retained (lanes 6 to 10) fractions. Lanes: 1 and 6, no additions; 2 and 7, La; 3 and 8, ATP; 4 and 9, La plus ATP; 5 and 10, La plus UTP. The mobility of the processed species was slightly altered in the presence of recombinant La; the reason for this is unknown.

however, this effect was demonstrable only when preassembled washed transcription complexes were used.

We found that La also increased release of 7SL, VA1, and *Alu* RNAs that otherwise accumulated at their pol III termination signals (Fig. 5B). Direct quantitation revealed that La increased the amount of RNA in the released fractions by ~35% for both VA1 and 7SL and more than that for the B1 and *Alu* templates (lanes 1 to 6). Thus, a significant portion of the nascent RNAs were shifted by La from the template to the released fraction. Examination of the template-retained fractions (lanes 7 to 12) revealed that La addition resulted in a dramatic clearance of nascent transcripts that would otherwise remain at the termination signals. In the absence of added La, about half of these transcripts were released. Addition of recombinant La to the reactions led to a higher efficiency of release. We conclude that La efficiently clears these pol III terminators of paused transcripts.

Both the transcript release activity and processing inhibition activity were specific to La (Fig. 6A), since equal amounts of other known RNA-binding proteins such as TFIIIA (lanes 5 and 11) and the snRNA U1-A protein (lanes 6 and 12) demonstrated neither of these activities when compared with the BSA control (lanes 3 and 9) or buffer alone (lanes 2 and 8). Furthermore, these activities of La were heat sensitive, since boiling La prior to addition abrogated transcript release and diminished 3′ processing inhibition (lanes 4 and 10). Thus, although the extract used to synthesize these RNAs contained endogenous La antigen, as demonstrated by immunoprecipitation (Fig. 4), it appears to be limited in transcript release activity. The addition of recombinant La to the extract overcomes this limitation.

We wanted to know whether La could release transcripts when added to complexes after the RNA had accumulated at the termination signal. In Fig. 6B, we examined the effects of adding La to previously arrested transcription complexes which contained [^{32}P]RNA paused at the AA termination signal. After a standard 90-min transcription reaction, the isolated termination complexes were washed with TB lacking NTPs prior to further analysis. To these naturally arrested complexes we added La, with and without NTPs, or BSA as a control. After a 5-min incubation, we separated released from retained fractions and examined their RNAs (Fig. 6B). In the absence of added La, a small amount of RNA was released, presumably as a result of La-independent spontaneous release (lane 1), but most of the RNA remained associated with the template (lane 6). All such spontaneously released RNA appeared within the first 3 min after addition of the incubation buffer and did not increase with longer incubation (64a). Exogenous La caused release of a substantial amount of 210-nt transcripts, indicating that they were not irreversibly arrested at the termination signal (lane 2). Furthermore, the La-released RNA was recovered as predominantly unprocessed species (1°, lane 2). These results demonstrated that La disengaged from complexes the transcripts which were arrested at the pol III terminator by releasing them as intact nascent RNA products. Although some RNAs other than the 210-nt species were also shifted to the released fraction by La, the high-molecular-weight RNA, which presumably represents non-promoter-mediated transcription, was preferentially retained by the template and was not affected by La (compare lanes 2 and 7). Addition of ATP or UTP in the presence or absence of added La (lanes 3 to 5 and 8 to 10 and data not shown) had little effect above background, as was the case for other NTPs (not shown). This finding showed that inefficient release was not primarily due to a limitation of NTPs. We conclude that La releases transcripts which are paused at the dT_4 termination signals of class III genes. Since the purified recombinant La protein used here was >99% homogeneous, as judged by examination of overloaded, overdeveloped, silver-stained polyacrylamide gels (data not shown), the data unequivocably establish La as a pol III terminator-mediated transcript release factor.

B1-*Alu* RNA released in the absence of added La was quite

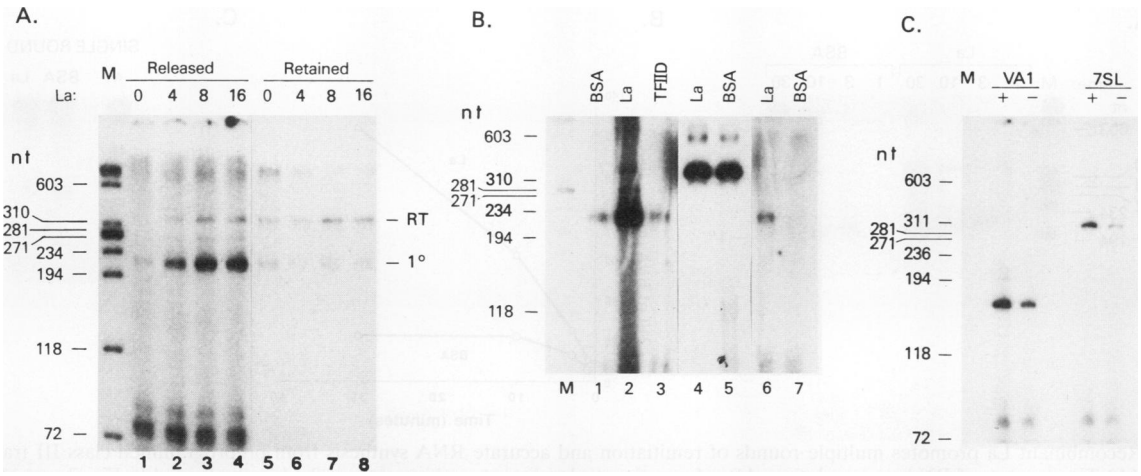

FIG. 7. Recombinant La enhances pol III-mediated RNA synthesis from preprogrammed washed transcription complexes. (A) Preformed complexes were allowed to assemble in the presence of nuclear extract plus ATP, CTP, and GTP but no UTP and were then washed three times with 0.5 M KCl-containing buffer. After equilibration by two washes with TB, equal amounts were aliquoted into reaction mixtures containing all four NTPs, including [α-^{32}P]GTP, and increasing amounts of recombinant La as indicated above the lanes in picomoles. The total added protein content was held constant with BSA. After a 15-min synthesis period, the released (lanes 1 to 4) and retained (lanes 5 to 8) fractions were analyzed. The band at ~72 nt is nontemplate specific and is of unknown identity; it serves as an internal control. (B) Stimulatory effects of La are dependent on pol III activity. Stringently washed preformed complexes were aliquoted and supplemented with NTPs, including [α-^{32}P]GTP, plus various additions as indicated above the lanes and below. Lane 1, BSA; lane 2, La; lane 3, recombinant TATA-binding protein (Promega TFIID). Lanes 4 to 7 contained 30 μM tagetitoxin. Lanes 4 and 5 contained T7 RNA polymerase plus a T7 RNA polymerase-dependent template to produce an *Alu* sequence transcript ending in oligo(U); this template was not preincubated with extract but added later as naked DNA. (C) La stimulates RNA synthesis from preassembled 0.5 M KCl-washed 7SL and VA1 transcription complexes. Templates were preincubated with extract and ATP, CTP, and GTP but no UTP, complexes were stringently washed as described above, and synthesis was allowed to proceed upon addition of NTPs, including [α-^{32}P]GTP, as described above in the presence (+) or absence (−) of La. The concentration of La used in the reactions in C was comparable to the 4 pmol used in the reaction shown in panel A.

susceptible to processing, since about half of it was recovered as the processed species (Fig. 6B, lane 1). La did appear to protect against processing, as can be seen by comparing the ratio of primary to processed transcripts in lanes 1 and 2. Thus, inhibition of processing was conferred by recombinant La and coupled to La-mediated release from the AA pol III signal.

La stimulates multiple rounds of pol III-dependent promoter-mediated transcription when added to preassembled, isolated transcription complexes. If La can mediate specific transcript release, it might also disengage pol III and allow it to reinitiate RNA synthesis from preprogrammed transcription complexes as previously hypothesized (21, 22). Therefore, we examined the effects of La when added to reactions which contained affinity-selected, preassembled, washed transcription complexes as the only source of pol III activity. La stimulated RNA synthesis in a dose-dependent manner (Fig. 7A). Nearly all of the 210-nt RNA produced in the La-containing reactions was released (lanes 1 to 4), while relatively little RNA remained associated with the template (lanes 5 to 8). In control reactions, release of 210-nt RNA was relatively inefficient (Fig. 7A, lanes 1 and 5). In reactions without added La (lanes 1 and 5), about half of the transcripts read through the 210-nt termination signal to the end of the template (also see Fig. 3A). This inefficient use of the 210-nt termination signal relative to standard transcriptions was a result of washing the preformed transcription complexes. The addition of La shifted this ratio toward more efficient use of the 210-nt dT_4 termination signal (Fig. 7A). Thus, recombinant La increased utilization of the pol III termination signal, increased efficiency of transcript release, and increased RNA synthesis from preassembled transcription complexes.

Several other purified DNA- and RNA-binding proteins, including recombinant TATA-binding protein, had no effect over background in this assay (Fig. 7B, lanes 1 to 3, and data not shown). Boiling La prior to addition abrogated its stimulatory effect (not shown). The effects of La were specific to pol III, as T7 RNA polymerase-dependent templates showed no differences in RNA production in control or La-containing reactions (Fig. 7B, lanes 4 and 5). Furthermore, the effects of La on RNA synthesis were inhibited by tagetitoxin (Fig. 7B; compare lanes 2 and 6) at 30 μM, a concentration known to specifically inhibit pol III transcription of this and other templates (23, 61) (data not shown). Finally, a stimulatory effect of La was also seen with VA1 and 7SL preprogrammed transcription complexes, indicating that La plays a general role in pol III-mediated RNA synthesis (Fig. 7C).

Next, we used preassembled stalled B1-*Alu* transcription complexes to investigate the mechanism of La's stimulatory action. In the experiment whose results are shown in Fig. 8, we examined the kinetics of transcript accumulation in the presence of recombinant La. An advantage of immobilized transcription complexes is that they can be isolated and washed free of factors not associated with the template. Also, the number of committed transcription complexes will be fixed by the stringent wash protocol, and subsequent promoter-mediated RNA synthesis should occur only from those complexes which had been previously programmed. Furthermore, after extensive washing with 0.5 M KCl and transfer to clean tubes, the only source of pol III activity is that which associated with the immobilized template during the preincubation period.

After washing of preassembled B1-*Alu* complexes, La or BSA was added along with NTPs, including [α-^{32}P]GTP, and equal aliquots were removed at 1, 3, 10, and 30 min thereafter. RNA yields from the La and control reactions were comparable only at early times, whereas synthesis continued unimpeded in the La reactions (Fig. 8A). The control reactions reproduc-

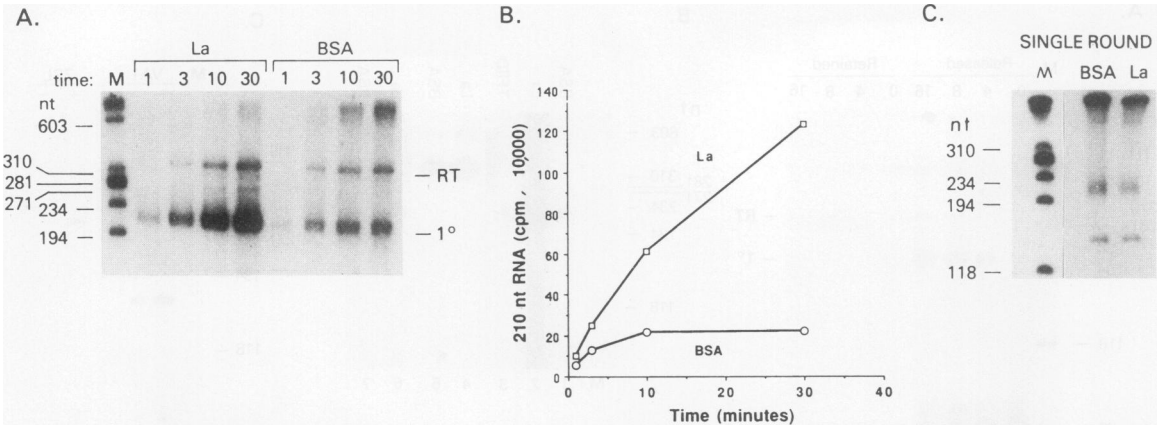

FIG. 8. Recombinant La promotes multiple rounds of reinitiation and accurate RNA synthesis from preprogrammed class III transcription complexes. (A) Time course of RNA accumulation. After formation and stringent washing as described in the legend to Fig. 7, complexes were equilibrated with TB supplemented with La or BSA carrier alone. NTPs, including [α-^{32}P]GTP, were added, equal aliquots were removed at 1, 3, 10, and 30 min thereafter, and RNAs from the total reactions were purified and analyzed by electrophoresis on a 6% polyacrylamide gel. (B) The counts in the 210-nt bands from panel A were quantitated with a Molecular Dynamics phosphor storage imager and were plotted. (C) Products of single-round transcriptions from stringently washed preassembled complexes. In these reactions, [α-^{32}P]GTP was included in the preincubation step to label the stalled transcript. After ternary complex formation, the nonincorporated [α-^{32}P]GTP was washed away, La or BSA carrier was added, and synthesis proceeded in the presence of heparin and all four NTPs, with an excess of unlabeled GTP. Indistinguishable results are obtained when heparin is omitted (not shown; see text).

ibly yielded lower maximum RNA levels at earlier time and indicated that synthesis ceased after only 10 min (Fig. 8B). Other experiments revealed that the stimulatory effect of La extended beyond 30 min (data not shown). These results directly demonstrated that the stimulatory effect of La was time dependent and are consistent with multiple reinitiations by pol III. By labeling the stalled transcript during the preincubation step and then washing away unincorporated ^{32}P-NTP prior to elongation and termination, we could show that La did not increase RNA synthesis over control levels when transcription was limited to a single round (Fig. 8C). Although heparin is included as part of the single-round transcription assay (15, 33), its effects on La's activity were unknown. Therefore, we also performed the single-round transcriptions in the absence of heparin; the results with La and BSA were indistinguishable from those shown in Fig. 8C (data not shown). Control experiments which monitored the kinetics of the appearance of single-round transcripts revealed that RNA synthesis was completed by 3 min. As expected, incubations of up to 30 min did not increase the yield of single-round transcripts by La. These data argue against the possibility that La increased RNA synthesis by increasing efficiency of elongation of stalled transcripts (55a). We conclude that La facilitates multiple reinitiations from stable transcription complexes, thereby promoting efficient use of both template and pol III.

DISCUSSION

Pol III-mediated transcription from immobilized templates can be used to study transcription termination and transcript release and the effects of *trans*-acting factors on these processes. We conclude that human La protein purified from recombinant bacteria can (i) mediate release of B1-*Alu* and other transcripts from pol III termination signals, (ii) stabilize a B1-*Alu* primary transcript, thereby inhibiting its conversion to the poly(A)-minus species, and (iii) convert arrested transcription termination complexes to ones which can be reinitiated by pol III for multiple rounds of RNA synthesis.

Gottlieb and Steitz showed that La was associated with the pol III transcription machinery (20). On the basis of results of La-depletion experiments, it was argued that La facilitated transcription termination and efficient RNA synthesis (21, 22). However, although repletion with biochemically purified La led to recovery of transcripts with the correct termini, the mechanism of the effect on RNA synthesis remained undetermined. Our study was directed toward examining pol III terminator-dependent RNA 3′ processing. The assays developed here used immobilized templates which allow direct partitioning of released and template-associated transcripts as well as facile isolation of transcription termination complexes. We did not immunodeplete La; we added La to a fractionated system which was inherently limited for transcript release. We provided convincing evidence that La stimulates RNA synthesis (Fig. 7 and 8). The use of isolated transcription complexes was crucial, since La exhibited no stimulatory activity when added to nuclear extract-mediated transcription reactions (see below). The results indicate that stimulation of RNA synthesis by La is due to reinitiation by pol III. Thus, La facilitates a productive interaction between pol III and preprogrammed transcription complexes. Without La, these complexes are not used efficiently. These results clearly demonstrate the importance of transcription termination in the efficient production of RNA from pol III-dependent templates and that La plays a decisive role in that process.

The cumulative data suggest that La gains access to the B1-*Alu* transcript in a pol III-dependent manner. It was previously demonstrated that factors specifying termination of pol II-mediated transcription and RNA 3′ end formation were recruited into initiation complexes by snRNA gene promoters (25, 48). Our findings are consistent with a role for La acting through the transcription initiation complex or early elongation complex. The highly efficient use of preprogrammed transcription complexes by pol III in the presence of La (Fig. 8B) is consistent with the possibility that La is involved in directing polymerase to the initiation complex. We must note that our experiments do not rule out a requirement for La in single-round transcription or in initiation complex assembly,

since these complexes were assembled in nuclear extract which contained endogenous La.

A previous study demonstrated that when purified pol III is directed to poly(dC)-tailed templates by a promoter-independent mechanism, extensive RNA-DNA hybrids are generated which are not released from the template (8). Our experiments showed that nuclear extract generates transcription termination complexes which are unable to efficiently release their nascent transcripts. However, the B1-*Alu* transcripts generated by nuclear extract were sensitive to RNase T_1 and resistant to RNase H (data not shown). This finding suggests that template-associated full-length transcripts are tethered at the termination signal. Addition of La to the termination complexes causes transcript release. Although we have not directly assessed whether pol III is also released from arrested termination complexes by La, the cumulative data support a model in which La leads to the disassembly of these complexes by disengaging both pol III and nascent transcript from the termination signal. According to this model, pol III can then reinitiate additional rounds of RNA synthesis from cleared templates.

Our results support a bifunctional role of dT_4 transcription termination signals: transcript pausing and release (8). It was shown that purified pol III can recognize and pause at the 5S RNA gene GC termination signal in the absence of added La protein (8, 13). Using purified pol III, Campbell and Setzer determined that 5S RNA was released from the template and that addition of recombinant La had no effect on termination (8). Thus, the results presented here appear to be in conflict with those of the previous study. Several possibilities may account for this apparent discrepancy. First, we used native mammalian pol III as opposed to pol III purified from *X. laevis*. Second, we demonstrated that La, when added to promoter-mediated transcription reactions, facilitated efficient transcript release from multiple different dT_4 sequences at which pol III paused, whereas the previous study used promoter-independent transcription in the absence of ancillary factors. It was noted that although pol III may be able to release transcript from the 5S termination site, its ability to do so may be obscured in a complete transcription complex and might therefore require La (8). Third, contamination of the pol III preparation with *Xenopus* La could not be rigorously ruled out (8). Finally and perhaps most importantly, the efficient 5S terminator GC (5) used by purified pol III may be less dependent on La for transcript release than other terminators are. Our data suggest that release from the GC terminator, when it is connected to the B1-*Alu* gene, is less dependent on La than is the AA terminator. Unfortunately, however, although this interpretation may reconcile the discrepancy discussed above, it is presently confounded by the rapid processing of B1-*Alu* transcripts produced from the GC terminator. Therefore, this aspect of terminator activity will require study of additional constructs.

While recombinant La exhibits transcript release in nonfractionated extracts, it does not lead to increased RNA synthesis in the same reactions (Fig. 5), yet the latter activity is readily detectable when preassembled washed complexes are used (Fig. 7 and 8). Although we do not know the reason for this difference, the data suggest that the transcript release activity of La and RNA synthesis stimulatory activity may have components which are mechanistically distinct. Results of preliminary experiments using heat-denatured La protein are consistent with this interpretation. Endogenous La antigen exists in multiple phosphorylated forms (17), and although it is abundant in nuclear extracts, it appears to be limiting for release activity. We suspect that recombinant La is hypophosphorylated, since it is produced in *E. coli*. To consider the alternative possibility that La's activities are instead phosphorylation dependent would require that a fast-acting La kinase be associated with washed transcription complexes. Although it is plausible that La's ability to enhance RNA synthesis is inhibited in nonfractionated extracts by modification such as phosphorylation, at the present time we cannot discriminate between this and the alternative possibility that added La squelches pol III-associated factors and or that La's stimulatory activity is inhibited by a component in crude extract which is removed during affinity isolation of transcription complexes. Finally, it remains possible that the difference in conditions used to prepare native versus washed transcription complexes accounts for some of the apparent inhibition of La transcriptional stimulation in unfractionated nuclear extracts.

The report that one preparation of La exhibited nucleic acid-dependent ATPase and helicase activities (3) has prompted us to look for evidence of these activities. We have been unable to demonstrate any helicase activity by purified recombinant La protein despite its activity in a range of biochemical assays reported here and elsewhere (33b, 46). Furthermore, depletion of ATP and or inclusion of nonhydrolyzable ATP analogs had no effect on recombinant La's ability to release transcripts from termination complexes (55a). Thus, ATPase activity is not required for La-mediated transcript release.

Our results indicate that a B1-*Alu* RNA 3′ processing activity appears to be associated with transcription termination complexes and that it may be part of the polymerase-containing complex. A report from Brow (7) suggested association between 5S rRNA 3′ processing and pol III termination and the possibility that a yeast analog of La is involved. More recently, a factor with activities similar to those described here was isolated from yeast cells. This component, designated TFIIIE, was found to stimulate multiple rounds of pol III transcription and to protect nascent tRNA from what appears to be a processing event in a highly purified reconstituted system (15).

The RNA processing activity illustrated in this report may be a 3′ → 5′ exonuclease which is inhibited by secondary structure in the substrate RNA. For example, the 5′ region of B1-*Alu* RNA is a highly structured molecule of 135 nt (39) followed by a 38-nt stretch of single-stranded poly(A) before terminating in downstream residues. In this regard, we address a model of La action which proposed that La was required for the synthesis of the 3′-terminal U residues of pol III transcripts (21, 22). In light of the present results, we must reexamine the data obtained with VA_{200} RNA, the pol III product of a terminator-mutant VA1 template (22). RNase T_1 fingerprint analysis revealed that the VA_{200} RNA produced by La-depleted extracts was specifically decreased in a CUAG oligonucleotide (spot 7 in Fig. 6 of reference 22) which is penultimate to the terminal oligo(U)-containing oligonucleotide. Decreased amounts of this oligonucleotide would not be predicted by the La-dependent oligo(U) synthesis model. Examination of the predicted secondary structure of VA_{200} RNA reveals that the CUAG sequence is part of a 3′-terminal single-strand region of the RNA that would be susceptible to the putative 3′ → 5′ exonuclease (39a). The decreased amount of the penultimate CUAG oligonucleotide would be consistent with posttranscriptional trimming by a transcription complex-associated 3′ exonuclease. Thus, the possibility exists that 3′-shortened VA_{200} RNAs produced in the absence of La are a result of shortening by a transcription complex-associated exonuclease. In any case, a role of La may be to ensure release of correctly terminated transcripts and their delivery away from the putative poly-

merase-associated nuclease. La may be part of a complex regulatory apparatus involved in a general pol III-associated RNA processing activity.

A minor conclusion derived from this study is that B1-*Alu* and *Alu* templates which utilize A-rich terminators inefficiently terminate pol III compared with the G+C-rich terminators found associated with other class III genes. We found that B1-*Alu* and *Alu* transcripts were inefficiently released (<30%) compared with 7SL and VA1 (45 to 55%) in standard reactions (Fig. 1, 2, and 5). In addition, both the B1-*Alu* and *Alu* terminators allowed significant amounts of transcription through the primary termination signal, which is an independent measure of terminator strength, whereas 7SL and VA1 did not (Fig. 1, 2, and 5). Furthermore, when the number of T's in the B1-*Alu* AA terminator was reduced from five to four, termination was severely impaired, as the great majority of transcripts read through this signal to the downstream CATTTTCG, whereas four T's surrounded by GC was an efficient termination signal in the same context (41) (data not shown). These results confirm the observations of Bogenhagen and Brown which demonstrated a correlation between G+C richness of the bases surrounding the dT_4 core termination signal and efficiency of termination (5). However, we note that exceptions to these rules do exist, indicating that the effects of sequence context are complex and in some cases template specific (39a, 45).

Alu elements exhibit a high propensity for transposition into A+T-rich DNA sites (14, 19, 31, 47, 67). This finding in conjunction with the fact that *Alu* repeats lack their own dT_4 termination signals suggests that *Alu*-directed pol III will read into fortuitous downstream A-rich terminators. Although the significance of such *Alu* transcription is unknown, we may speculate on the possible effects. First, some transcriptionally active *Alu* elements appear to be involved in the regulation of certain pol II transcription units (50, 70). In these scenarios, *Alu* transcription blocks passage of pol II and is referred to as transcriptional insulation (49). An arrested pol III-containing *Alu* transcription complex could plausibly provide a more stable block to an oncoming pol II than if the termination complex disassembled. Second, the presence of thousands of interspersed *Alu* promoters followed by inefficient terminators suggests the potential for sequestering pol III if termination becomes limiting. For those *Alu* and B1-*Alu* elements that overcome negative influences (16, 34, 38) to initiate pol III in vivo (12, 39, 42, 43), the inability to release transcript and prepare the template for reinitiation may also help explain the paucity of *Alu* transcripts (12, 42, 43, 51). Thus, the sequence context of the terminators that *Alu*s acquire upon de novo insertion may determine the relative efficiencies with which different Alu elements produce RNA. A transposed *Alu* that inserts near an efficient termination signal may be more active as a result.

Although many pol III transcripts become dissociated from La, some remain associated to various degrees (24, 44, 52, 54). For U6 snRNA, it was suggested that failure to dissociate from La might be inhibitory to the function of the U4/U6 small nuclear ribonucleoprotein in pre-mRNA metabolism (63). These observations suggest that association of some pol III transcripts with La might be dynamic and regulated. Our data indicate that the base composition of the termination signals used by certain class III templates may play a specific role in posttranscriptional regulation of their RNA products. We showed that the posttranscriptional fate of B1-*Alu* RNA released from transcription termination complexes can be modulated by the base composition surrounding the core dT_4 termination signal. B1-*Alu* RNA processing inhibition was previously shown to be dependent on transcription by RNA polymerase III, in contrast to T7 RNA polymerase-synthesized or naked B1-*Alu* RNA, and that the terminator-specific inhibition occurred in vivo (41). In the present study, we found that the B1-*Alu* RNA 3′ processing activity can be specifically inhibited by recombinant La in coupled transcription-processing reactions. These cumulative data, in conjunction with the demonstration that endogenous La associates with B1-*Alu* primary transcripts in vitro (Fig. 4) and in vivo (40), provide compelling evidence that La mediates terminator-specific processing inhibition of B1-*Alu* transcripts. The possible consequences of stabilizing *Alu* and B1-*Alu* primary transcripts which correspond to transposition intermediaries appear to be an increasingly important question for human genetics (19, 47, 57, 67). The role of candidate *cis*-acting sequences and *trans*-acting factors such as La in this process can be examined once an *Alu* source gene and an experimental system to study *Alu* transposition become available.

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