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The RNA Polymerase III Terminator Used by a B1-*Alu* Element Can Modulate 3′ Processing of the Intermediate RNA Product

RICHARD J. MARAIA,[1]* DAU-YIN CHANG,[1] ALAN P. WOLFFE,[2] ROSEANN L. VORCE,[1] AND KARL HSU[1]

Laboratory of Molecular Growth Regulation[1] and Laboratory of Molecular Embryology,[2] National Institute of Child Health and Human Development, Bethesda, Maryland 20892

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The dispersion of short interspersed elements (SINEs) probably occurred through an RNA intermediate. B1 is a murine homolog of the human SINE *Alu*; these elements are composed of 5′ G+C-rich regions juxtaposed to A-rich tracts and are flanked by direct repeats. Internal promoters direct RNA polymerase III to transcribe B1 and *Alu* elements and proceed into the 3′ flanking DNA until it reaches a $(dT)_4$ termination signal. The resulting transcripts contain 3′-terminal oligo(U) tracts which can presumably base pair with the A-rich tract to form self-primed templates for reverse transcriptase and retrotransposition. Nuclear extracts from mouse tissue culture cells contain an RNA processing activity that removes the A-rich and 3′-terminal regions from purified B1 RNAs (R. Maraia, Nucleic Acids Res. 19:5695–5702, 1991). In this study, we examined transcription and RNA processing in these nuclear extracts. In contrast to results with use of purified RNA, nascent transcripts synthesized in nuclear extract by RNA polymerase III are not processed, suggesting that the transposition-intermediate-like RNA is shielded from processing by a protein(s). Alteration of an AATTTT TAA termination signal to a GCTTTTGC signal activated processing by >100-fold in coupled transcription/processing reactions. A similar difference was found when expression was compared in frog oocytes. No difference in processing was found if the transcripts were made by T7 RNA polymerase in the presence of the nuclear extract, indicating that the different processing effects of the two terminators were dependent on synthesis by polymerase III. The modulation of processing of B1-*Alu* transcripts and the potential for retrotransposition of B1 and *Alu* DNA sequences are discussed.

Short interspersed elements (SINEs) are ubiquitous in the genomes of higher eukaryotes as RNA polymerase III templates of high copy number, yet they remain an enigma (13, 16, 29). The *Alu* family of SINEs consists of structurally related elements found in primates and rodents thought to be derived from 7SL RNA and which themselves encode small cytoplasmic RNA (21, 24). B1 is the murine SINE most homologous to *Alu* and consists of a 5′ region of ~138 bases of 60% G+C content, followed by a poly(A) region of ~40 bases. In vivo, RNA polymerase III-dependent B1 SINEs are actively expressed in germ line tissue (19, 21), early stages of development, and states of proliferation and transformation (7, 35, 36). It was recently shown that a specific subset of B1 sequences is expressed as small processed transcripts in cultured cells, developing mice, and adult germ line-containing tissue (21).

Presumably, *Alu* and B1 elements have responded similarly to a selective process since discrete subfamilies of each were fixed in their respective genomes at multiple times during recent evolution (2, 6, 18, 21, 23, 24, 26, 27, 37). Their similarity extends to the fact that specific subsets of active *Alu* and B1 sequences produce transcripts which appear to undergo processing and cytoplasmic localization (21, 24). Although the function of the small cytoplasmic RNA is not known, the conservation of a structural motif (21) found throughout evolution in the translational control domain of signal recognition particle RNAs (20, 31) suggests a role for this RNA in cytoplasmic regulation of gene expression. Furthermore, this conservation suggests that the B1 and *Alu* families consist of functional genes that have evolved under selective pressure plus a large number of transposed copies of these genes.

Dispersion and amplification of SINEs are thought to have occurred by retrotransposition: SINE transcripts are copied into DNA and inserted into the genome. The model for this process is dependent on RNA polymerase III termination at oligo(dT) tracts 3′ of the SINE insertion site such that the transposition intermediate is an RNA containing a poly(A) stretch (3, 24) and ending in oligo(U). Presumably, these sequence features allow for base pairing of the terminal oligo(U) with internal poly(A) to form self-primed templates for reverse transcriptase and help explain why these promoter-containing elements have been the subjects of extensive amplification (14, 32). Polymorphisms caused by the de novo insertion of *Alu* sequences indicate that this process is presently active and capable of causing genetic variability and damage (28, 33, 34). As yet, few experimental investigations of *Alu* mobility have been described, and the factors involved are unknown.

Unlike other RNA polymerase III-dependent genes, transposed *Alu* elements lack $(dT)_4$ termination signals and therefore rely on 3′ flanking DNA for this motif. As a result, *Alu*s interspersed at different loci generate heterogeneous transcripts whose lengths and 3′ sequences are variable and dependent on the position and efficiency of the termination signals encountered by RNA polymerase III (reviewed in reference 16). In this study, we examined factors that affect transcriptional termination of B1-*Alu* RNA. We used a previously characterized B1 which resides within the first intron of the mouse α-fetoprotein (AFP) gene (1, 38) (hereafter referred to as AFP-B1). This sequence produces B1 RNA upon introduction into a variety of mammalian cells (9, 38) and frog oocyte nuclei (1, 21, 22). Transcripts from this element are synthesized by RNA polymerase III (1). These

* Corresponding author.

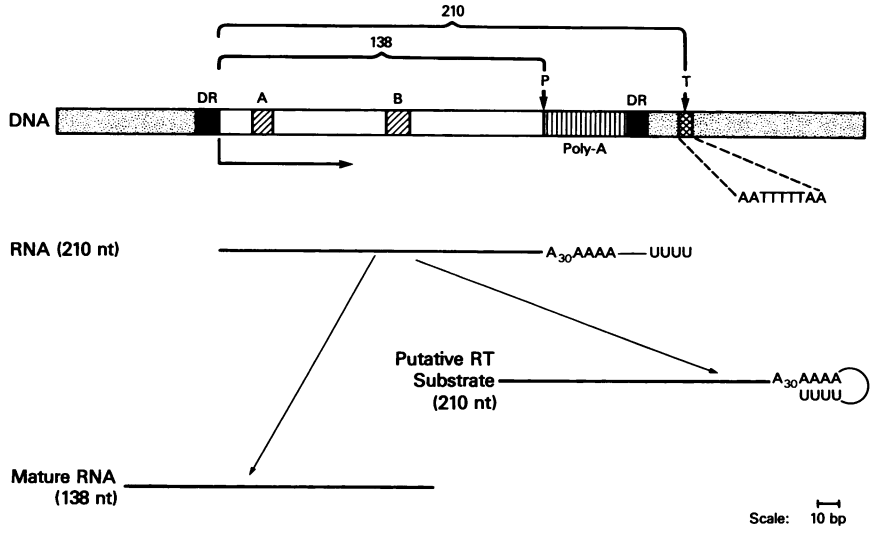

FIG. 1. Schematic diagram of the AFP-B1 gene. Direct repeats (filled boxes) are 5′ of the initiation of transcription (bent arrow) and 3′ of the pure poly(A) (vertical hatched) region. The A and B boxes of the split internal promoter for RNA polymerase III are indicated as diagonal hatched boxes. Stippled areas correspond to nonrepetitive sequences which flank the B1 element and in the 3′ region contain the polymerase III termination signal, AATTTTTAA (vertical arrow T). The 3′ end of the processed transcript (1, 21) is indicated by vertical arrow P. Once produced, the nascent transcript either is converted to the mature cytoplasmic transcript (lower left) by 3′ RNA processing or may become a substrate for retrotransposition (RT; lower right) (14, 32).

transcripts can be processed in microinjected frog oocytes (1, 21, 22) and homologous nuclear extracts (21) by removal of the poly(A) and flanking sequences 3′ of the B1. RNA identical to the processed transcript is found as a small cytoplasmic species in mouse cells (21). The processed transcripts produced would no longer be substrates for reverse transcriptase, as depicted in Fig. 1. Identification of cellular factors involved in modulation of the B1-*Alu* RNA processing activity should yield insight into small cytoplasmic B1 (scB1)-*Alu* RNA metabolism as well as SINE retrotransposition.

We show that conversion of the AATTTTTAA RNA polymerase III termination signal of the AFP-B1 gene to GCTTTTGC dramatically increases the rate of posttranscriptional processing. Differential processing of the wild-type (WT) and termination mutant (Tm) transcripts is dependent upon and coupled to their synthesis in nuclear extracts by RNA polymerase III. The difference between processing of Tm and WT transcripts synthesized by nuclear extracts is due to inhibition of processing of the WT but not the Tm transcript. When both transcripts are first purified and then added to the nuclear extract, they lose their differential inhibition and are processed with nearly equal efficiency as they are if synthesized by T7 RNA polymerase in the presence of nuclear extract. These results suggest that one or more protein factors bind to or remain complexed with the WT transcript, as a result of its synthesis by RNA polymerase III, and inhibit RNA processing. Since the unprocessed transcript could be a substrate for reverse transcriptase (Fig. 1), the modulation of processing could be important for transposition.

MATERIALS AND METHODS

Plasmids. The AFP-B1 gene-containing plasmid pGB1e was previously described (21). Site-directed mutagenesis was performed as described previously (17), using appropriate AFP-B1 primers; the mutated plasmid is designated $pGB1_{Tm}$. Sequence analysis of the mutated B1 gene and flanking DNA confirmed that only the four bases indicated above were changed by the mutagenesis protocol.

In vitro transcriptions. In vitro transcriptions were performed by using murine erythroleukemia (MEL) cell nuclear extract described previously (21), according to standard methods (4). MEL extract-mediated transcription of both the WT and Tm B1 genes displayed sensitivity to α-amanatin and tagetitoxin characteristic of RNA polymerase III (data not shown). A 3-bp substitution in the B box of the internal promoter of the WT gene virtually abolished transcription by this extract and in the injected oocyte (data not shown), confirming its dependence on RNA polymerase III, which was previously established for the 210-nucleotide (nt) precursor and 135-nt processed RNA products of this gene by α-amanatin sensitivity in the microinjected oocyte (1). In some experiments, plasmid DNA was preincubated (4) with extract in transcription conditions lacking UTP; a 25-μl reaction mixture contained 1 μg of plasmid DNA, 4 μl of extract (protein concentration, 7.5 mg/ml), 600 μM each CTP and ATP, 50 μM GTP, and 5 μCi of [α-^{32}P]GTP. Synthesis was initiated (at various times thereafter; see Fig. 5A) by addition of UTP to 600 μM. For the pulse-chase experiment, the labeling mixture was as described above except that the total GTP concentration was 4.2 μM (50 μCi of [α-^{32}P]GTP) in a reaction volume of 80 μl; after preincubation at 21°C for 30 min, synthesis was initiated and proceeded for 6 min and was then chased by increasing the unlabeled GTP to 8 mM in a final volume of 280 μl. RNAs were phenol extracted and ethanol precipitated prior to polyacrylamide gel electrophoresis (PAGE) and autoradiography.

T7 promoter-directed transcriptions in the presence of nuclear extract. The conditions used were as described above either with or without nuclear extract (see Fig. 4D). Plasmid DNA was replaced by a WT B1 template containing the T7 promoter generated by polymerase chain reaction (PCR) as described previously (21), and 2 μl of T7 RNA

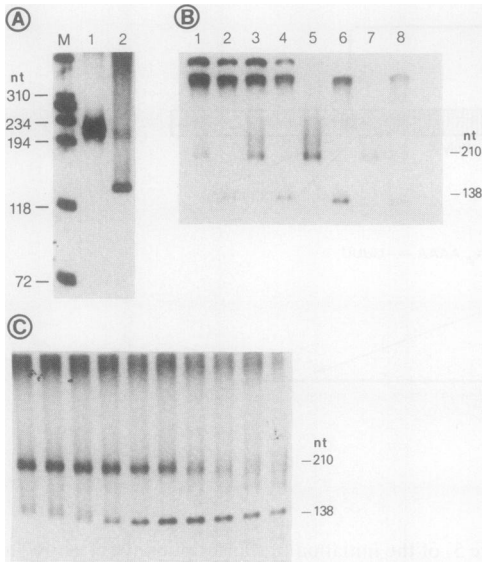

FIG. 2. In vitro transcription using mouse cell nuclear extract. (A) Transcription products of the WT (lane 1) and Tm (lane 2) plasmids after a 60-min incubation with nuclear extracts as described in Materials and Methods. Lane M, denatured *Hae*III-digested øX174 size markers. (B) Reactions (25 μl) using various amounts of extract for WT (odd-numbered lanes) and Tm (even-numbered lanes) transcriptions. Lanes: 1 and 2, 1 μl; 3 and 4, 2 μl; 5 and 6, 4 μl; 7 and 8, 8 μl. (C) Pulse-chase synthesis of Tm RNA. After a 6-min pulse of [α-^{32}P]GTP incorporation, the reaction volume and unlabeled GTP concentration were increased 4-fold and 2,000-fold, respectively, and incubated at 21°C to slow processing. Aliquots were removed at 0, 2, 5, 10, 20, 40, 80, 120, 180, and 240 min thereafter for RNA analysis.

polymerase (Promega) was included where indicated. For PCR generation of the Tm template, the 3′ primer was 5′-AAAAGCCCCATATTTGCA-3′. The transcription/processing reaction was carried out for 30 min.

Processing of exogenously added [^{32}P]RNA. Processing was done as described previously (21) or slightly modified as indicated. The ^{32}P-labeled substrate RNAs were made by T7 RNA polymerase-mediated transcription of PCR-generated templates as described previously (21) with either the WT- or Tm-specific 3′ primer (see above). In some cases, substrate [^{32}P]RNA was synthesized in vitro by nuclear extracts (RNA polymerase III). RNAs were gel purified prior to use (21).

RESULTS

It was previously reported that the nucleotides surrounding the oligo(dT) tract at the 3′ end of a 5S rRNA gene influence efficiency of termination by RNA polymerase III (5). The GpC dinucleotide in these positions appeared to confer the most efficient termination, while ApA appeared to be least efficient. In contrast, termination efficiency of tRNA gene transcription decreased when the 3′ dinucleotide ApA was changed to CpG (25). The primary products of AFP-B1 transcription are the result of a tRNA-like AATTTTTAA termination signal located ~210 nt from polymerase III-directed transcriptional initiation (1) (Fig. 1). This was changed to GCTTTTGC by site-directed mutagenesis (17).

In vitro transcription, using murine-derived nuclear extract previously shown to specifically process AFP-B1 RNA (21), was performed on the WT and Tm AFP-B1 plasmids in the presence of [α-^{32}P]GTP. Figure 2A shows the products of 60-min transcription reactions after phenol-chloroform extraction, denaturing PAGE, and autoradiography. Although the mutation was predicted to affect the efficiency of transcriptional termination at 210 nt, the results indicated an effect on the size rather than on the amount of 210-nt RNA produced. The WT gene produced the expected primary transcript of ~210 nt (lane 1) in addition to the ~138-nt mature RNA as a minor band, while the major RNA from the Tm gene (lane 2) was ~138 nt, with a minor amount of ~210 nt. The ratio of 210- to 138-nt RNA was typically >100-fold higher in WT than in Tm transcriptions. This pattern was also readily produced by *Xenopus* and HeLa nuclear extracts (not shown) and was not influenced by the ratio of extract to DNA used (Fig. 2B). The synthesis of these RNA products was previously demonstrated to be dependent on RNA polymerase III in the microinjected oocyte (1). As expected, the murine cell nuclear extract-mediated synthesis of these RNAs also displayed sensitivity to α-amanatin and tagetitoxin characteristic of RNA polymerase III and was virtually abolished by a 3-bp substitution in the B-box internal promoter (data not shown). Although the Tm transcriptions sometimes yielded a trail of RNA larger than the expected size of 210 nt (Fig. 2A, lane 2), which suggests that transcription extends beyond the termination site, this was not always the case (Fig. 2B). The efficiency of transcriptional termination was not determined, as we chose instead to characterize the basis of the differential size effect.

A previous report of in vitro transcriptional termination within the A-rich tract of an *Alu* sequence (12) suggested that the 138-nt B1 RNA might be due to premature termination as a result of altering the wild-type termination signal. However, this was found not to be the case. Figure 2C shows in vitro transcription products of the Tm gene after a short pulse of [α-^{32}P]GTP incorporation and subsequent chase, using decreased incubation temperature and dilution conditions in order to slow processing and demonstrate the precursor-product relationship of the mutant transcript. This experiment demonstrated that the primary transcript of the Tm gene is the major product at early times and that it is subsequently converted to the ~138-nt RNA. Therefore, the pattern of RNAs produced by the WT and Tm genes was due to differential processing of their nascent RNA products.

RNA polymerase III-dependent B1-*Alu* RNA synthesis and processing occur in the microinjected frog oocyte (1, 21, 22). Recent analyses (21) indicated that this in vivo germ cell expression system faithfully processes the transcripts from a subset of B1 genes, as does mouse nuclear extract, to produce the scB1 RNAs found in mouse cells and germ line tissue (21). We compared the expression of WT and Tm genes after oocyte injection. Plasmids were injected along with [α-^{32}P]GTP into the germinal vesicles of mature oocytes, and the RNA products were purified at various times thereafter (Fig. 3). Our results (Fig. 3, lanes 1 to 4) were in agreement with those of the previous study (1), which showed that the RNA polymerase III-dependent primary transcript from the WT gene was processed 1 to 2 h after its appearance. In contrast, the mutant gene expressed the processed RNA sooner and without a discernible lag time (lanes 5 to 8). These data were obtained from pooled oocytes for each time point; however, analysis of multiple individual oocytes at every time point examined consistently revealed the same pattern (not shown). Thus, the Tm RNA more readily became a substrate for processing than did the WT RNA. It should be noted that although the WT RNA is processed in a time-dependent manner in the injected oocyte

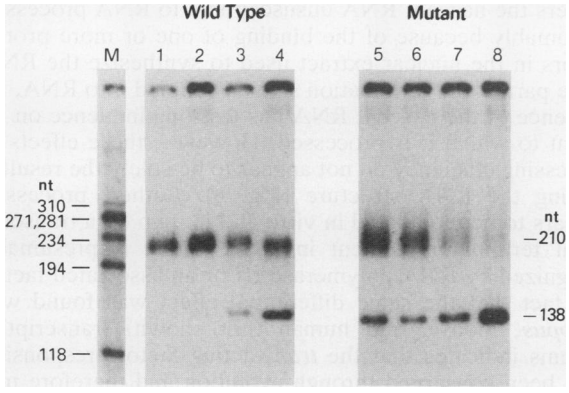

FIG. 3. Expression of WT and Tm plasmids in the injected oocyte as previously described (21). RNA was purified and analyzed at various times after injection. Lanes: 1 and 5, 30 min; 2 and 6, 45 min; 3 and 7, 60 min; 4 and 8, 120 min.

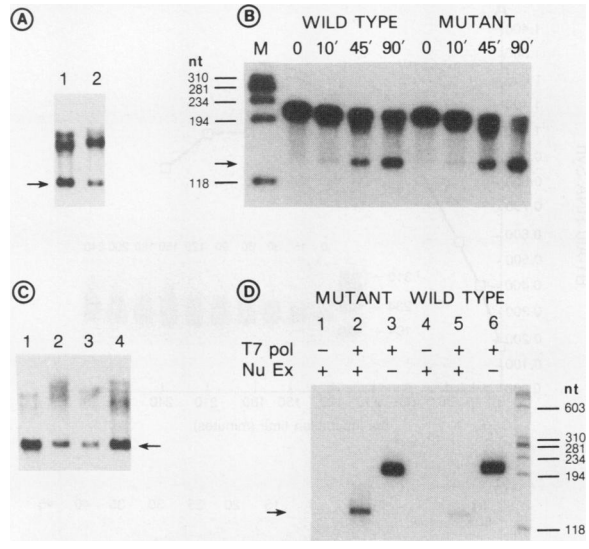

FIG. 4. RNA processing in vitro. (A) Comparison of purified RNAs after incubation with nuclear extract as previously described (21). Lanes: 1, Tm RNA; 2, WT RNA. (B) Processing kinetics of purified WT and Tm RNAs in nuclear extract. Purified RNAs corresponding to either the WT or Tm sequence were added to nuclear extract, and aliquots were removed at the times (minutes) indicated above the lanes, purified, and analyzed (21). Lane M, end-labeled denatured *Hae*III-digested øX174 size markers. (C) Inhibition of WT RNA processing can be uncoupled from RNA polymerase III activity. [^{32}P]RNAs were synthesized in vitro by either T7 RNA polymerase or MEL nuclear extracts (polymerase III), purified, and subsequently added to active transcription reactions. Lanes: 1, Tm 210-nt RNA made by T7; 2, WT RNA made polymerase III; lane 3, WT RNA corresponding to termination at 220 nt (10 bp downstream of AATTTTTAA) made by T7; 4, WT RNA corresponding to 210-nt RNA made by T7. (D) Efficient processing of WT and Tm nascent transcripts synthesized by T7 RNA polymerase in the presence of nuclear extract. T7 promoter-containing templates corresponding to either the WT or Tm sequence were transcribed by T7 RNA polymerase (T7 pol) in the presence or absence of nuclear extract (Nu Ex). Lanes: 1 to 3, Tm template; 4 to 6, WT template. Arrows indicate positions of mature 138-nt RNA.

(1, 22) (Fig. 3), in vitro WT transcription reactions of up to 4 h yielded only a relatively tiny fraction of processed RNA (data not shown). The data suggest that the inhibition of processing of WT RNA (see below) may be overcome in frog oocytes more readily than it is in mammalian cell nuclear extract.

Thus, subtle changes flanking the core termination signal lead to increased processing of the RNA product. To determine whether this effect might be due to a signal in the RNA created by changing its terminal sequence, which could render it a better substrate than the WT transcript for in vitro processing, we compared purified WT and Tm RNAs for the ability to undergo processing. WT and Tm sequence [^{32}P] RNAs were synthesized in vitro by T7 RNA polymerase and purified as described in Materials and Methods. These RNAs were then incubated with the nuclear extract as previously described for AFP-B1 (WT) RNA (21) (Fig. 4A) and in time course processing reactions (Fig. 4B). In contrast to the results shown in Fig. 2A and B, Fig. 4A and B show that the difference in processing efficiency between the purified WT and Tm primary transcripts was much reduced compared with the difference observed in the coupled transcription/processing reactions (Fig. 2A and B). Therefore, the main conclusion from these experiments is that WT RNA was significantly inhibited from processing only when the in vitro transcription and processing reactions were coupled.

To determine whether polymerase III transcription per se would inhibit processing of WT RNA, we added purified [^{32}P]RNAs to transcription reaction mixtures containing unlabeled deoxynucleoside triphosphates and WT plasmid. Figure 4C shows that purified WT RNA synthesized by either RNA polymerase III (lane 2) or T7 RNA polymerase (lanes 3 and 4) was processed as efficiently as was Tm RNA (lane 1). Determination of [α-^{32}P]GTP incorporation into WT B1 RNA from aliquots of these reactions confirmed the presence of polymerase III transcriptional activity (data not shown). The results demonstrated by Fig. 4 coupled with the results of Fig. 2 indicated that exogenous WT RNA was processed efficiently in the nuclear extract transcriptions, while endogenously synthesized nascent WT RNA synthesized by these extracts remained virtually unprocessed.

To determine whether the purification protocol altered the WT RNA and rendered it susceptible to processing, we synthesized WT or Tm transcripts by using T7 RNA polymerase in the presence (Fig. 4D, lanes 2 and 5) or absence (lanes 3 and 6) of nuclear extract. Both WT and Tm transcripts as generated by T7 polymerase were reproducibly and efficiently processed by the extract (lane 6 was relatively underloaded because of inadvertent loss of sample). Therefore, we conclude that the efficient processing of purified WT RNA by the nuclear extract does not appear to be due to a change in its conformation induced by the purification protocol. In addition, the extract does not contain factors that inhibit the processing of WT RNA synthesized by exogenous RNA polymerase. These data demonstrated that the inhibition of WT B1 RNA to undergo processing was tightly coupled to its transcription by RNA polymerase III.

Since nascent WT RNA synthesized by RNA polymerase III in the nuclear extracts was not available for processing, we next examined whether B1 WT RNA was retained in a stalled transcription complex or turned over. These experiments used limiting amounts of extract and a preincubation protocol in which plasmid template is premixed with the extract to overcome the rate-limiting intermediate which forms prior to transcription initiation (4). This leads to a progressive assembly of B1 preinitiation complexes up to a

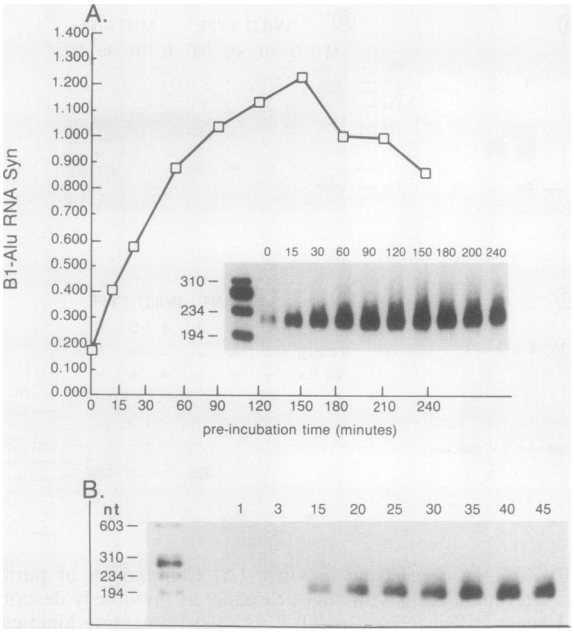

FIG. 5. Evidence that multiple rounds of polymerase III synthesis occur from the WT plasmid. (A) Kinetic profile of AFP (WT)-B1 transcription complex assembly as described previously for 5S transcription (4) and in Materials and Methods. A transcription reaction lacking UTP was incubated at 30°C. At various times thereafter, aliquots were removed, UTP was added, and synthesis was allowed to proceed for 15 min, after which the reaction was stopped and the RNA was purified and analyzed by denaturing PAGE. The resulting autoradiograph is shown in the inset, with the times (minutes) of incubation prior to initiation of synthesis shown above the lanes. An underexposure of this autoradiograph was scanned with a densitometer, and the results are plotted. (B) Kinetic profile of B1 synthesis after maximal transcription complex assembly of WT plasmid and nuclear extract. Synthesis was initiated after a 150-min preincubation period, and aliquots were removed and analyzed at the indicated times (minutes) thereafter.

period of maximal complex formation, which occurs after preincubation for 150 min and then declines (Fig. 5A). This general pattern is reminiscent of 5S rRNA gene transcription complex assembly (4). We next preincubated the template and extract for 150 min to allow maximal complex assembly and then initiated synthesis and sampled aliquots for RNA analysis at various intervals thereafter (Fig. 5B). The appearance of increasing amounts of transcript with increasing time indicated that production of full-length RNA was not limited to a single round of synthesis, as might be expected if the primary transcript did not dissociate from the transcription complex (11). These results are consistent with and support our previous findings for *Xenopus* oocytes, which showed that the nascent B1 RNA product of WT transcription was part of a small ribonucleoprotein complex (of about 8S) (22) released from transcription complexes. We conclude that the inhibition of WT transcript to undergo processing is not due to its retention in a transcription complex.

DISCUSSION

We have shown that the nature of the termination signal used by RNA polymerase III has a profound effect on the fate of the nascent transcripts produced from a B1-*Alu* gene. In particular, use of the AATTTTTAA termination signal renders the nascent RNA unsusceptible to RNA processing presumably because of the binding of one or more protein factors in the nuclear extract used to synthesize the RNA. Since part of the termination signal is copied into RNA, the sequence of the nascent RNA has a strong influence on the extent to which it is processed. However, these effects on processing efficiency do not appear to be solely the result of altering the RNA structure (Fig. 4). Rather, processing appears to be modulated in vitro and in vivo by a transcriptional terminator element in DNA which is presumably recognized by RNA polymerase III or an associated factor. The fact that the same differential effect was found with *Xenopus*, mouse, and human (not shown) transcription systems indicates that the *trans*-acting factors responsible have been conserved through evolution and therefore may play an important role in the regulation of (some) class III genes.

The efficiency of the core termination signal $(dT)_4$ is most positively affected by GpC dinucleotides surrounding it, while ApA yield less efficient termination of *Xenopus* 5S rRNA gene transcription in vitro (5). However, *Xenopus* lysine tRNA transcriptional termination efficiency at $(dT)_4$ is affected by surrounding base composition in a way exactly contrary to the 5S rules (25). In addition, polymerase III appears to use termination signals other than a cluster of dT residues (reviewed in reference 25). Nonetheless, factors that mediate RNA polymerase III termination have been identified. Mutations that affect termination map to a large subunit of yeast RNA polymerase III (15). Likewise, it was reported that purified vertebrate polymerase III could terminate transcription (8), while a factor termed La has been reported to mediate transcript release (10, 11). Therefore, it is reasonable to examine the role of La in the differential processing of the two RNAs discussed in this report, since La is also known to transiently bind nascent transcripts of polymerase III (30). Both the WT and Tm primary transcripts from in vitro transcription reactions were immunoprecipitable by anti-La antibodies (data not shown). However, the primary product of Tm transcription is unstable compared with the WT species, which limits the interpretation of such experiments. Thus, the role of La, if any, in determining the susceptibility to processing of the two primary transcripts might be dependent on the stability of the association between La and the RNA rather than association itself. Preliminary experiments indicate that the primary transcript released from wild-type template is in the form of a ribonucleoprotein which can be made susceptible to nucleolytic attack by treatment with protease. We conclude that identification of the inhibiting activity will be a complex matter and will require substantial additional investigations.

Our study identified an activity that inhibits B1-*Alu* transposition-intermediate-like RNAs from undergoing conversion to the small cytoplasmic species. The processing of WT transcripts in the injected oocyte (1, 21) (Fig. 3) but not in vitro transcription reactions (Fig. 2A and B) suggests that association of the primary transcript with the putative inhibitory activity is more stable in vitro than in vivo. Nonetheless, there was a significant period of time in vivo during which WT nascent RNA was not susceptible to processing while mutant RNA was. A functional role of this inhibitory activity in SINE mobility may be sought by designing experiments that compare the abilities of the WT and Tm B1 genes to retrotranspose in a defined system. Once the inhibitory activity is characterized further, its involvement, if any, in transcription of B1 and other polymerase III-

dependent genes may help us better understand the regulation of polymerase III transcription and the mobility of SINEs within eukaryotic genomes.

The observations made in this and a previous report (21) indicate that only a subset of B1 transcripts synthesized by RNA polymerase III are converted to the small cytoplasmic species by RNA processing. Many interspersed B1 elements examined give rise to polymerase III-generated transcripts which do not undergo processing in vitro or in vivo (21; unpublished observations), while the transcript of the AFP-interspersed B1 is processed in vivo only after a significant lag period (Fig. 3). Although the B1 genes that give rise to scB1 RNA in mouse cells are unknown, they presumably produce primary transcripts which undergo relatively efficient processing. B1-Alu primary transcripts which do not undergo efficient processing would be theoretically more likely to be copied by reverse transcriptase and dispersed by retrotransposition than are transcripts that are efficiently processed (Fig. 1). Thus, one might expect that evolutionary fixation of amplified sets of SINEs might have been through transcription of retrotransposed elements which lost their ability to effectively produce processed transcripts rather than through retrotransposition of elements that efficiently produce small cytoplasmic transcripts. This distinction may be important, since it recognizes the possibility that the source genes for new transpositions may be different from the genes that produce sc*Alu* RNA. We suspect that there are at least two different mechanisms which could lead to inhibition of efficient processing of *Alu*-related primary transcripts: (i) mutation of sequences within the element which are presumably no longer recognized by the processing enzyme and (ii) transposition into loci which negatively influence processing as a result of sequences that flank the element such as the termination signal phenomenon described in this report.

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