Terminator-specific Recycling of a B1-Alu Transcription Complex by RNA Polymerase III Is Mediated by the RNA Terminus-binding Protein La*

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Efficient synthesis of many small abundant RNAs is achieved by the proficient recycling of RNA polymerase (pol) III and stable transcription complexes. Cellular Alu and related retroposons represent unusual pol III genes that are normally repressed but are activated by viral infection and other conditions. The core sequences of these elements contain pol III promoters but must rely on fortuitous downstream oligo(dT) tracts for terminator function. We show that a B1-Alu gene differs markedly from a classical pol III gene (RNA*<sub>Met</sub>) in terminator sequence requirements. B1-Alu genes that differ only in terminator sequence context direct differential RNA 3' end formation. These genes are assembled into stable transcription complexes but differ in their ability to be recycled in the presence of the La transcription termination factor. La binds to the nascent RNA 3’ UUUOH end motif that is generated by transcriptional termination within the pol III termination signal, oligo(dT). We found that the recycling efficiency of the B1-Alu genes is correlated with the ability of La to access the 3’ end of the nascent transcript and protect it from 3’-5’ exonucleolytic processing. These results illustrate a relationship between RNA 3’ end formation and transcription termination, and La-mediated reinitiation by pol III.

Eukaryotic RNA polymerase (pol) III synthesizes 5S rRNA, tRNAs, 7SL RNA, and U6 RNA as well as adenovirus VA1 RNAs and cellular Alu retroposon RNAs (1). Sequences encoding tRNA, VA1 RNA, and Alu-like RNAs contain similar internal A box and B box promoter elements characteristic of class 2 genes, while 5S (class 1), and U6 (class 3) genes use different promoter elements (2, 3). The promoters of the class 2 genes engage the multisubunit transcription factor (TF) IIIC which subsequently recruits TFIIIB to direct pol III transcription. Murine B1-Alu and human Alu sequences are homologous retroposons that represent the least characterized of the known class 2 genes. These genes differ from the other class 2 genes in promoter strength, distance between the B box and terminator, and the presence of a ~40-bp poly(A) tract that resides downstream of the B box (see Fig. 4A). Since mobile B1-Alu and Alu elements carry their promoters but not their transcriptional terminator sequences with them upon transposition they must rely on fortuitous oligo(dT) tracts downstream of their insertion sites for subsequent terminator function (4). As such, elements inserted at different loci contain different stretches of DNA between their core Alu sequence and the variably distanced downstream terminator, a structural feature that must be accommodated by the pol III machinery if the new transposon is to be active (4). B1-Alu and Alu retroposons are intriguing because they represent highly regulated pol III genes. Although B1-Alu and Alu elements are present at high copy number (about 10<sup>5</sup>-10<sup>6</sup> copies per haploid genome) they are normally repressed but become highly activated by viral infection, heat shock, and other stresses, while their conventional pol III gene counterparts are expressed constitutively (5, 6). Although the mechanistic basis for B1-Alu and Alu gene induction probably involves multiple levels of control (7), it is reasonable to assume that their unique architecture directs their regulation.

After TFIIIB is recruited to the template by TFIIIC, the resulting preinitiation complex is stable and can be recycled for multiple rounds of transcription by pol III (8–11). While studies indicate that TFIIIB bound to the template is sufficient to direct reinitiation, other data suggest that proper termination may be required for efficient recycling, suggesting that termination and reinitiation may be mechanistically linked (10, 12). This view is consistent with studies of the human La antigen, a protein that recognizes the 3’ terminal RNA motif UUUOH and serves as a termination factor that can regulate the recycling of pol III transcription complexes in vitro (13–16). Evidence that La plays a role in transcription initiation (14) is consistent with the finding that this protein is associated with a pol III holoenzyme that is capable of autonomous initiation (17). Yet although the mechanisms that link termination and reinitiation represent an intriguing aspect of pol III transcription, these remain incompletely characterized (12).

A B1-Alu RNA gene identified as a result of its expression in vivo was used for the present study (18). This gene is transcribed by pol III as a primary transcript that associates with La in vivo prior to 3’ processing to a small stable RNA that subsequently accumulates in the cytoplasm (19–21). Since retrotransposition appears to be limited to unprocessed transcripts, it has been proposed that 3’ processing can affect the transpositional potential of B1-Alu transcripts (21). Thus, 3’ processing may play a role in the “life cycle” of Alu retroposons (4)

We previously described the effects of subtle terminator mutations on B1-Alu RNA 3’ end processing (22). The B1-Alu wild-type terminator (B1-WT) and its terminator mutant de
rivative (B1-Tm) both support accurate termination while the efficiency of transcript release is relatively low from the B1-WT gene (15). Efficiency of transcript release from the B1-WT template is increased by La protein which also facilitates recycling of polymerase and template, stimulating transcription (15). Furthermore, in the absence of La, the B1-WT transcript is processed at its 3' end, apparently by a 3' - 5' exonuclease, while processing is blocked in the presence of La (15, 23). In this system, human La appears to protect the nascent B1-Alu transcript in a manner similar to the ability of yeast La to protect nascent tRNA precursors from 3' - 5' exonuclease processing in vitro (24). Thus, the La protein acts as a trans-acting factor, and the terminator sequence as a cis-acting element, have both independently been shown to affect the expression of this B1-Alu gene (15, 22, 23).

Here, we document that contrary to the positive effect of La on transcription of the B1-WT template, La does not stimulate B1-Tm transcription, even though both templates are assembled into stable transcription complexes that are equally competent for a single round of RNA synthesis. We show that this differential transcription efficiency is terminator-specific and mediated by La’s ability to differentially promote recycling of B1-Alu transcription complexes. The recycling efficiency of these B1-Alu transcription complexes is correlated with the ability of La to stably protect the 3' end of the nascent RNA from processing.

**EXPERIMENTAL PROCEDURES**

B1-Alu and tRNA<sub>Met</sub> Gene Terminator Mutants—All constructs used in this study were confirmed by DNA sequencing. Sau3A1 fragments containing a B1-Alu gene in pGEM-1 with wild-type (AAATTTTTAA) and mutant (GCTTTTGC) terminators were previously described as pGBI<sub>WT</sub> and pGBI<sub>Tm</sub>, respectively (22). In each of these constructs immediately downstream of the terminator is a second potential terminator sequence, GAATTTTTGT. Additional B1-Alu terminator constructs used for Fig. 4B were generated by polymerase chain reaction site-directed mutagenesis from clone pGBI<sub>WT</sub>. Fragments carrying a 5' HindIII site and a 3' EcoRI site just downstream of the first terminator were generated and inserted into pUC18; these constructs do not contain the second potential terminator found in pGBI<sub>WT</sub> and pGBI<sub>Tm</sub>. Construct pGBI<sub>WT</sub> is identical to pGBI<sub>WT</sub> except for a 3-bp substitution in the B-box (GAGTTCCAGGCC → GAGCCTGAGGCC) that abolishes promoter function.

A ~270-bp fragment containing the tRNA<sub>Met</sub> gene 2 (25) was cloned into the EcoRI/HindIII sites of pBS SK<sup>+</sup> and designated pRNA<sub>Met</sub> (26). Modifications to the terminator of this gene were generated by mutagenesis using appropriate primers as described previously (23), introducing a Bg/II site 9 bp downstream of the terminator. Met-WT1 is the wild-type gene while Met-WT2 is wild-type but with the introduced Bg/II site.

**FIG. 1.** B1-WT and B1-Tm templates differ in multiple round but not single round transcription assays. After preincubation of plasmid template and nuclear extract, each reaction was separated into two batches and simultaneously incubated under conditions that allow multiple round transcription (A), or limit transcription to a single round (B). For A, NTPs and [α-<sup>32</sup>P]GTP were then added, while B also contained Sarkosyl to a final concentration of 0.05%; this concentration of Sarkosyl blocks reinitiation by pol III (14, 39) and inhibits RNA processing. Aliquots were removed after the times indicated above the lanes (minutes) and RNA was prepared. The nascent primary transcript, T1, and the processed species, P, are indicated to the left. RNA products were analyzed on 6% polyacrylamide-urea gels. A and B were performed and analyzed simultaneously although B was exposed longer than A; when exposed for the same time, the band intensities seen in panel B were comparable to the faint T1 band seen at 3 min for B1-Tm in panel A (lane 5).

**RESULTS**

**Terminator-specific Transcription by RNA Polymerase III—** While changing the wild-type B1-Alu terminator from AAATTTTTAA (B1-WT) to GCTTTTGC (B1-Tm); chosen because it represents an efficient terminator of 5 S rRNA gene transcription, Ref. 28) does not alter the accuracy of pol III termination, it accelerates 3' processing of the nascent transcript in vitro (24). The cumulative data indicate that while La is a positive determinant of B1-WT RNA expression, they also suggest that the B1-Tm transcript is insensitive to La. Previous studies had also suggested that the B1-WT template is more actively transcribed than B1-Tm, although this had not been subjected to detailed analysis (15, 22).

The experiment shown in Fig. 1A reveals that significantly more B1-Alu RNA accumulates in a B1-WT reaction (lanes 1–4) than in a B1-Tm reaction (lanes 5–8) using plasmid DNA and nuclear extract under standard conditions that allow multiple rounds of pol III transcription. As will be addressed by experiments in a later section, the high molecular weight transcripts that accumulated in the B1-Tm reaction most likely represent nonspecific, promoter-independent RNA synthesis. Promoter-mediated transcription of B1-WT and B1-Tm templates generates primary transcripts (T1) and processed (P) species, respectively, as the major products (15, 22), as seen in Fig. 1. The
amount of P species that accumulates in B1-Tm reactions represents the majority of the output of promoter-mediated B1-Tm transcription.

The above results suggest that B1-Tm transcription complexes are formed less efficiently and/or recycled less efficiently than B1-WT complexes. As one approach to distinguish between these possibilities, aliquots of the pre-assembled transcription complexes that were used above for multiple round transcription (Fig. 1A), were simultaneously assayed under conditions that limit RNA synthesis to a single round (Fig. 1B). Under these conditions, equal amounts of RNA were produced from B1-WT and B1-Tm templates indicating that these templates formed complexes equally well and that they were equally competent for the first round of de novo transcription by pol III.

It remained formally possible that B1-Tm complexes were unstable relative to B1-WT complexes in multiple round assays. We therefore employed assays that monitor the competitive strength and stability of transcription complexes (8, 9). The B1-WT and B1-Tm plasmids were preincubated with nuclear extract and a second plasmid, either one containing a human Alu element that generates a 320-nt nascent transcript, or a negative control plasmid, was then added and transcription was initiated by the addition of nucleotides and [α-32P]GTP. In this assay, limiting factors bound by one template will not be available to the other template and transcription from one, the other, or both will be reduced. Both B1-WT and B1-Tm templates each prevented transcription from the second template (Fig. 2, lanes 10 and 11), while the transcriptionally inactive control templates pGB1-68 and pGEM (lanes 1 and 2), representing empty vector and B1-WT containing a 3-bp substitution in the B box promoter, respectively, did not (lanes 12 and 13). Similar results were observed using the VA1 gene as the second template (not shown). We note that the above experiments do not rule out the possibility that a subcomponent which on its own would not be sufficient to support transcription from the competing template, might dissociate from the B1-Tm transcription complex during these reactions. Nonetheless, by these standard assays that monitor competitive strength (lanes 6 and 7) and transcription complex stability (lanes 10 and 11), B1-WT and B1-Tm templates were indistinguishable. We conclude that B1-WT and B1-Tm templates are each assembled into stable transcription complexes to promote similar levels of first round transcription but differ markedly in assays that allow multi-round transcription.

Terminator-specific Response of a B1-Alu Transcription Complex to La—The B1-WT and B1-Tm genes, which differ only in the sequence context of their terminators, were examined for their response to pol III and La in transcription assays. Streptavidin-agarose linked HaeII linearized templates were preincubated in transcription buffer containing ATP, CTP, GTP, and HeLa extract. The supernatant was removed and the transcription complexes were washed twice with transcription buffer; 0–40 pmol of recombinant La was then added followed by 25 μl of transcription buffer containing NTPs and 3.5 μCi of [α-32P]GTP. Incubation was continued for 45 min and RNA was analyzed on 6% polyacrylamide-urea gels. The mobilities of the RNA products corresponding to processed (P), T1, T2, and run off (RO) transcripts are indicated to the left. A schematic of the template as described in the text is shown below; note that these templates differ only in the sequence of T1 which is AAITTTTAA in B1-WT and GCTTTTGC in B1-Tm.

Fig. 3 reveals three noteworthy differences in the outcome of these B1-WT and B1-Tm transcription reactions: (i) differential RNA 3′ end processing, (ii) differential levels of promoter-mediated transcription, and (iii) differential levels of promoter-dependent terminator-specific response to La. B1-WT (lanes 1–5) and B1-Tm (lanes 6–10) templates were examined for their response to pol III and La in transcription assays. Streptavidin-agarose linked HaeII linearized templates were preincubated in transcription buffer containing ATP, CTP, GTP, and HeLa extract. The supernatant was removed and the transcription complexes were washed twice with transcription buffer; 0–40 pmol of recombinant La was then added followed by 25 μl of transcription buffer containing NTPs and 3.5 μCi of [α-32P]GTP. Incubation was continued for 45 min and RNA was analyzed on 6% polyacrylamide-urea gels. The mobilities of the RNA products corresponding to processed (P), T1, T2, and run off (RO) transcripts are indicated to the left. A schematic of the template as described in the text is shown below; note that these templates differ only in the sequence of T1 which is AAITTTTAA in B1-WT and GCTTTTGC in B1-Tm.

**Fig. 3.** Terminator-specific B1-Alu templates exhibit differential sensitivity to La. B1-WT (lanes 1–5) and B1-Tm (lanes 6–10) templates were examined for their response to pol III and La in transcription assays. Streptavidin-agarose linked HaeII linearized templates were preincubated in transcription buffer containing ATP, CTP, GTP, and HeLa extract. The supernatant was removed and the transcription complexes were washed twice with transcription buffer; 0–40 pmol of recombinant La was then added followed by 25 μl of transcription buffer containing NTPs and 3.5 μCi of [α-32P]GTP. Incubation was continued for 45 min and RNA was analyzed on 6% polyacrylamide-urea gels. The mobilities of the RNA products corresponding to processed (P), T1, T2, and run off (RO) transcripts are indicated to the left. A schematic of the template as described in the text is shown below; note that these templates differ only in the sequence of T1 which is AAITTTTAA in B1-WT and GCTTTTGC in B1-Tm.

**Fig. 2.** B1-WT and B1-Tm plasmids are both assembled into stable transcription complexes. Lanes 1–5 contain the RNA products of transcription reactions showing the major transcripts produced by the indicated single plasmids. Lanes 6–9 show transcripts of reactions containing the combinations of plasmids indicated when both were present at the beginning of the preincubation period. Lanes 10–13 show the transcripts when the second template was added after the end of the preincubation period. DNA was preincubated in transcription buffer in the presence of HeLa nuclear extract and in the absence of NTPs. At the end of the preincubation period, NTPs and [α-32P]GTP were added, with or without the second plasmid as indicated; incubation was continued for 1 h and RNA was prepared and analyzed on 6% polyacrylamide-urea gels. Positions of the major RNA products from each template are shown. pGEM and B1-Alu represent empty vector and B1-WT carrying a 3-bp mutation in the B box promoter, respectively, used as negative controls. hAlu represents a transcriptionally active human Alu element (pGHAFF-AluE2pol) that produces a 325-nt transcript (see text). A, B1-WT; B, B1-Tm; C, hAlu; D, pGEM; E, B1-Mut.
independent, nonspecific transcription. In the absence of La, the B1-WT and B1-Tm templates both produce low levels of transcription and a significant amount of processed (P) transcript relative to T1 transcript (lanes 1 and 6). In this case, the lower amount of T1 transcript in lane 6 relative to lane 1 is compensated in part by a slight increase in the P species in lane 6 relative to lane 1 (note that T2 serves as an internal control for this comparison). However, these transcription complexes exhibit a marked difference in their response to La. While La stimulated the production of the B1-WT transcript and protected it from processing (T1, lanes 1–5) confirming earlier results (15), these effects were not observed for the B1-Tm transcript (lanes 6–10). Time course experiments had demonstrated that the B1-Tm template indeed produces the T1 primary transcript that is subsequently (and efficiently) converted to the processed B1 RNA (22). It is therefore significant that La cannot stabilize the B1-Tm primary transcript even when added at concentrations comparable to or exceeding that in nuclear extract (lanes 6–10 and data not shown). Although La did slightly alter the mobility of the processed B1-Tm RNA as noted previously (see Fig. 6B in Ref. 15 and Fig. 3 in Ref. 23), the mechanism by which it does so remains unknown. However, for the purposes of the present study it is most important to note that La did not block processing of the B1-Tm primary transcript. Insensitivity of the nascent B1-Tm RNA to La as demonstrated here can explain the rapid processing of B1-Tm RNA relative to B1-WT observed in vivo (22).

The B1-WT and B1-Tm templates also differ in their ability to direct promoter-mediated transcription (Fig. 3). While La did increase overall RNA synthesis from the Tm template, a significant amount of this appeared to be in the form of transcripts longer than run off, resulting from promoter-independent nonspecific transcription rather than promoter-mediated transcription (lanes 6–10). We are less certain of the origin of the smear of transcripts whose lengths are shorter than run off, some of which may be due to promoter-mediated transcription. In contrast to B1-Tm, pol III was efficiently directed to the B1-WT initiation complex in the absence of La, with little production of nonspecific transcripts longer than run off (lanes 2–5). Thus, on the basis of the transcripts of length run off and longer it appeared that the B1-Tm transcription complex allowed more nonspecific transcription than did B1-WT. This indicates that the B1-Tm transcription complexes did not compete for pol III as efficiently as the B1-WT complexes. These results provide evidence to suggest that once assembled, B1-Tm transcription complexes are less active than B1-WT complexes for promoter-mediated reinitiation by pol III in response to La. We propose that differences in the La-dependent recycling of these terminator-specific B1-Alu templates accounts for the substantial difference observed when using plasmid DNA and nuclear extract (Fig. 1).

B1-Alu RNA Genes Are Distinctively Sensitive to the Sequence Context of the Terminator—Effects of terminator sequence variability on B1-Alu transcription was examined in Fig. 4B. The B1-WT and B1-Tm templates differ not only in flanking dinucleotide composition, but also in the number of Ts; i.e. AAT5AA versus GCT4GC, in the terminator. In addition, although these terminators are followed immediately by the potential terminator GAATTTTGT, the effect if any of this sequence on the expression of these templates had not previously been examined. This sequence was deleted in the pUC series of B1-Alu terminator mutants that were compared with the B1-WT and B1-Tm templates in Fig. 4B. Therefore, several comparisons, including effects of flanking dinucleotide composition, number of Ts, and presence or absence of the downstream GAATTTTGT, can be made from the data in Fig. 4B. Changing the number of Ts in the GC-rich terminator from five to four modestly altered the ratio of T1 to P transcripts (Fig. 4B, lanes 2 and 3) but did not increase the amount of T1 to the level obtained with the AA-rich terminator (lane 1). Comparison of the AAT5AA terminator in the pUC-based constructs (lane 1) with the original B1-WT (lane 4) indicated that although removal of the downstream GAATTTTGT sequence appeared to decrease termination efficiency as evidenced by more run off transcripts (lane 1 versus lane 4), this did not significantly alter the amount or distribution of transcripts relative to the corresponding GC-rich terminators (lanes 2, 3, and 5). The GCT4GC template (lane 2) produced more processed B1-Alu RNA than the GCT4GC template (lane 3) with a corresponding decrease in the T1 transcript. It is noteworthy that the transcript from the GCT4GC template is not protected by exogenous La, nor is transcription from this template stimulated by La, similar to the original B1-Tm template GCT4GC (not shown). This indicates that the difference in pol III-related activities of the B1-WT and B1-Tm templates cannot be explained by a different number of Ts. Comparison of lanes 3 and 9 indicated that GC dinucleotides flanking both sides of the T tract promote processing (below).

Certain permutations of dinucleotides flanking the T stretch influenced termination efficiency (lanes 6 and 7) as did reducing the number of Ts from five to four in the context of the AA-rich terminator (not shown). However, even though these templates terminated transcription inefficiently, they were distinguished by the fact that the GCT4GC terminator supported less transcription than its counterpart AAT5GC (lanes 8 and 9). In all cases examined, A-rich dinucleotides supported more transcription than GC-rich dinucleotides adjacent to the same number of Ts, especially when upstream of the Ts. The presence of a single upstream A in the context NGT4CA (lane 6) conferred more transcriptional stimulation than C in the same context (lane 7). We note that the transcriptional stimulation conferred by A (lane 6) as compared with C (lane 7) occurred in this context in the absence of differential RNA processing. Thus although these NGT4CA terminator-mediated differences in transcriptional efficiency may not be as great as with the B1-WT and B1-Tm terminators, they nonetheless demonstrate that transcriptional stimulation and RNA processing can be uncoupled. Since the terminators AGT4CA and CGT4CA, both allowed substantial readthrough but promoted different transcription levels, the results also suggest that the termination and recycling activities of the B1-Alu terminator may be separable. Both of these terminators allowed significantly more readthrough than AAT5AA (not shown).

We also examined the effects of terminator sequence context on the transcription of two other class 2 genes, VA1 and tRNA. The appropriate terminator mutations in the VA1 gene had no significant effect on transcription rate, transcript release, or transcript size. However, since VA1 RNA does not normally undergo processing, it remained possible that the effects of terminator context and/or La might be limited to templates that produced transcripts that undergo 3′ processing. We therefore examined the effects of terminator context on the human tRNA\textsuperscript{Met} gene because its nascent transcript is processed \textit{in vitro} (25, 26). Fig. 4C shows data obtained using human tRNA\textsuperscript{Met} gene mutants carrying terminator sequences including ones corresponding to the B1-WT(AAT5AA) and B1-Tm (GCT4GC) terminators as well as variations of these. The transcription system used here supports some processing of the nascent tRNA precursor in the absence of La (Fig. 4C) but none in the presence of La (not

\textsuperscript{2} R. Maraia and N. Sasaki-Tozawa, unpublished observation.
FIG. 4. The B1-Alu RNA gene is distinguishably sensitive to the sequence context of its terminator. A, models of type 2 genes as represented by the human tRNA^Met gene, the adenovirus associated (VA1) RNA gene and the B1-Alu RNA gene studied here. Cross-hatched boxes represent the A and B box promoter and terminator (T) elements. The vertical hatched rectangle represents the (dA)_m tract in the non-template strand of the B1-Alu RNA gene. Distances from transcription initiation (arrow) to terminator are indicated above the terminators in base pairs (bp). B, transcription from B1-Alu gene constructs carrying various terminator sequences, as indicated above the lanes, using nuclear extract under multiple round transcription conditions. Terminator sequences of the pUC series of terminator mutants is shown above the lanes and compared with the B1-WT and B1-Tm templates which also differ in that they contain a potential downstream terminator (see text). Below the lanes are the raw data in radioactive PhosphorImager counts (×10^9) for each band, T1, run off, and P, C, transcription from human tRNA^Met gene constructs carrying various terminator sequences using multiple round transcription conditions. Plasmid DNA was transcribed under standard conditions and RNA was prepared. RNA products were analyzed on 6% polyacrylamide gels. The terminator sequence of each construct is shown above the lanes; lane 2 contains the wild-type terminator CTT_4CC.

shown, also see Ref. 26), making it suitable for our purpose. Decreasing the oligo(dT) tract to fewer than 4 Ts caused inefficient termination (lane 1). When placed 2 nucleotides upstream of a 4 T terminator an A residue also decreased termination efficiency in the tRNA construct although not nearly as much as the CTT_4CC construct or the equivalent B1-Alu construct (not shown). More importantly, unlike the B1-Alu gene, neither transcription efficiency nor processing was altered in the analogous terminator mutants, AAT_5AA, GCT_3GC, and GCT_4GC (Fig. 4C, lanes 3–5). Most significantly, CTT_4CA, which functioned very poorly as a terminator of B1-Alu (transcription (Fig. 4C, lane 7), functioned well as a tRNA^Met gene terminator (Fig. 4C, lane 6). These terminator sequences exerted little if any influence over tRNA^Met transcription (Fig. 4C), even in the presence of excess La (not shown), clearly different from the B1-Alu genes. We conclude that the B1-Alu RNA gene is distinctly sensitive to the sequence context of its terminator, both with regard to termination efficiency and influence on 3′ end formation.

DISCUSSION

The major conclusion that can be drawn from this work is that two B1-Alu transcription complexes that differ only in a few base pairs surrounding their oligo(dT) terminator exhibit differential recycling by pol III. Although a potential caveat of this study is the possibility that low B1-Tm transcription reflects instability of the nascent B1-Tm transcript, our analyses as listed below, indicate that this is not the case. Multiple experiments like those shown in Fig. 1A as well as pulse-chase experiments do not reveal significant loss of B1-Tm transcript. Also, when pre-synthesized ^32P-labeled RNA representing the B1-Tm primary transcript is added to transcription reactions, processing occurs and the P species is recoverable without major losses (not shown). In addition, only minimal loss of signal, consistent with RNA shortening, occurs when B1-Alu RNA processing is allowed to proceed uninhibited (see Fig. 3 in Ref. 23). Indeed, pulse-chase analysis had demonstrated efficient accumulation of the P species in B1-Tm reactions (22). These data indicate that less RNA accumulates in B1-Tm transcription reactions relative to B1-WT because less B1-Tm RNA is synthesized. The fact that B1-Alu terminator-mediated differences in transcriptional efficiency can be uncoupled from RNA processing as seen by comparing lanes 6 and 7 in Fig. 4B further supports this interpretation.

We showed that the differential recycling efficiency of these genes reflects a differential response to the La transcription termination factor. La is an RNA-binding protein whose affinity for newly terminated pol III transcripts is mediated by recognition of the RNA 3′ end motif, UUUOH, the RNA counterpart of the pol III termination signal (30). We further showed that while La can access, recognize, and protect the 3′ end of the transcript produced from the B1-WT transcription complex, the transcript produced by the B1-Tm complex is refractory to La. Since these genes were previously shown to direct differential 3′ end processing in vivo, it is reasonable to suspect that the same differential sensitivity to La occurs in cells (22). We propose that the B1-WT and B1-Tm sequences represent La-sensitive and La-insensitive genes, both in terms of RNA 3′ processing and transcription complex recycling. In vivo, 3′ processing of tRNA precursors can occur by La-dependent or La-independent pathways although yeast cells genetically depleted of La exhibit no apparent transcriptional defect in vitro (24). However, ectopic expression of La does lead to nascent pre-tRNA levels that are substantially elevated over wild-type (31), suggesting that La may stimulate tRNA synthesis in the fission yeast Schizosaccharomyces pombe. In any case, the correlation between La-mediated recycling and RNA 3′ end binding by La

\(^3\) J. L. Goodier and R. J. Marsaia, unpublished observation.
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...described here supports the contention that La plays a role in linking termination and reinitiation by pol III.

Another conclusion that can be drawn from this work is that the sequence requirements for terminator function differ for B1-Alu and other pol III genes, namely the tRNA<sup>Met</sup> gene and the VA1 RNA gene (Fig. 4, B and C). We wish to emphasize that terminator sequence context not only influences the accuracy of termination but also can affect transcriptional efficiency and post-transcriptional 3' end processing. Although tRNA, VA1 RNA, and Alu genes have been classified as type 2 on the basis of their promoter structure, they differ significantly in other architectural features, most notably downstream of the B box, in their terminator-proximal regions (Fig. 4A). It is unknown whether terminator function is dictated by the sequence of the promoter elements, which differ in tRNA, VA1, and B1-Alu genes, and which may assemble different arrays of transcription factors, or whether the general differences in architecture are more directly responsible. In any case, the sequence requirements for pol III termination appear to be gene specific, suggesting that factors other than pol III itself can modulate terminator function. This analysis of this B1-Alu gene indicates that the composition of the dinucleotides flanking the T residues of the terminator is a major cis-acting determinant of differential B1-Alu gene expression. The present work shows that these sequences exert their effects, at least in part, through the La protein.

B1-Alu and Alu sequences are transcriptionally repressed but are induced to high activity under some conditions (Introduction). Although induction involves changes in chromatin (7), the factors involved in this process are largely unknown. The results presented here suggest that terminator-related processes are involved in the induction of Alu genes. B1-Alu and Alu sequences are mobile genetic elements that have undergone significant evolution within mammalian genomes. These genes are regulated differently from other class 2 genes presumably to control the effects of their transcripts on host cell physiology (32) and should be expected to continue to provide insight into the cellular machinery with which they interact.

Others have reported effects of a pol III terminator on RNA expression (33). Mutagenesis of a terminator-proximal potential RNA hairpin decreased transcription while compensatory mutations restored it (34). In that case, La stimulated expression of the hairpin-lacking RNA. The authors noted that "higher order structure rather than merely a highly localized sequence environment is probably responsible." It should be noted that our mutations were limited to the dinucleotides immediately flanking the T stretch and that these B1-Alu sequences do not exhibit a terminator-proximal hairpin (not shown). It is also noteworthy that the previous study did not distinguish between transcription complex assembly and recycling (33, 34).

The present work can also be distinguished from prior studies that indicated that the 3'-flanking sequence including the terminator, is an important determinant of pol III transcription (11, 35–38). Those studies examined truncated templates in assays that monitor the assembly of transcription complexes and demonstrated that without downstream regions, class 2 genes could not efficiently engage transcription factors. Our study compared the effects of substitution mutations in the terminator, without gene truncation. We documented that terminator sequence context does not affect assembly of B1-WT and B1-Tm transcription complexes. Rather, by using pre-formed, isolated transcription complexes, we showed that the differential transcription is due to differences in recycling.

The data presented here strongly suggest that terminator sequence context can be an important determinant of the recycling efficiency of transcription complexes, at least for B1-Alu sequences. The relationship between terminator sequence, nascent RNA 3' end metabolism, transcription complex recycling, and response to La may provide insight into mechanisms of pol III termination. Correlation between recycling and association of the B1-WT transcript with La suggests that the affinity of La for the nascent transcript may be a direct determinant of recycling efficiency. Although this interpretation may be attractive it is not supported by RNA binding data and RNA 3' protection data that demonstrate that La exhibits similar affinities for, and protection of, nascent B1-WT and nascent B1-Tm RNAs, when each are synthesized with either 3 or 4 terminal uridylates by T7 RNA polymerase. These results are in agreement with data that showed that B1-WT terminator-mediated inhibition of processing is specific to pol III-synthesized RNA (22). Thus, when synthesized by pol III in vivo or in vitro, the 3' end of the B1-Tm transcript is not protected by La. An interpretation that is consistent with all of the data is that the ability of La to associate with and protect the B1-WT but not the B1-Tm nascent transcript may reflect a pol III-dependent differential accessibility of the 3' ends of the nascent transcripts to La. This could be due to different conformational states of the 3' ends of the nascent RNAs in their respective transcription termination complexes, as directed by the sequence context of the terminator.

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REFERENCES

1. Willis, I. M. (1993) Eur. J. Biochem. 212, 1–11
2. Geiduschek, E. P., and Tocchini-Valentini, G. P. (1988) Annu. Rev. Biochem. 57, 873–914
3. Kasaveti, G. A., Bardeleben, C., Bartholomew, B., Braun, B. R., Joaozinho, C. A. P., Pisano, M., and Geiduschek, E. P. (1994) in Transcription: Mechanisms and Regulation (Conaway, R. C., and Conaway, J. W., eds.) pp. 107–126, Raven Press, Ltd., New York.
4. Schmid, C., and Marra, R. (1992) Curr. Opin. Genet. Dev. 2, 874–882
5. Fan, H., Salsich, A. L., Goodier, J. L., Zhang, X., Qin, J., and Marra, R. J. (1997) Cell 88, 707–715
6. Marra, R. J. (1996) Proc. Natl. Acad. Sci. U.S.A. 93, 3383–3387
7. Russanova, V. R., Driscoll, C. T., and Howard, B. H. (1995) Mol. Cell. Biol. 15, 4282–4290
8. Lomovs, A. B., Martin, P. L., and Roeder, R. G. (1983) Science 222, 740–748
9. Bogenhagen, D. F., Workington, W. M., and Brown, D. D. (1982) Cell 28, 413–421
10. Kasaveti, G. A., Braun, B. R., Nguyen, L. H., and Geiduschek, E. P. (1990) Cell 60, 235–245
11. Wang, Z., and Roeder, R. G. (1996) Mol. Cell. Biol. 16, 6681–6680
12. Dinti, G., and Gentesco, A. (1996) Cell 84, 345–352
13. Fan, H., Salsich, A. L., Goodier, J. L., Zhang, X., Qin, J., and Marra, R. J. (1997) Cell 88, 707–715
14. Marra, R. J. (1996) Proc. Natl. Acad. Sci. U.S.A. 93, 3383–3387
15. Marra, R. J., Kenan, D. J., and Reene, J. D. (1994) Mol. Cell. Biol. 14, 2147–2158
16. Gottlieb, E., and Steitz, J. A. (1989) EMBO J. 83, 851–861
17. Wang, Z., Luo, T., and Roeder, R. G. (1997) Genes Dev. 11, 2371–2382
18. Young, R. P., Scott, R. W., Hamer, D. H., and Higman, S. M. (1982) Nucleic Acids Res. 10, 3099–3116
19. Adeniyi-Jones, S., and Zaslouf, M. (1985) Nature 317, 81–84
20. Marra, R., Zaslouf, M., Plotz, P., and Adeniyi-Jones, S. (1988) Mol. Cell. Biol. 8, 4433–4440
21. Marra, R. (1991) Nucleic Acids Res. 19, 5695–5704
22. Marra, R., Chong, D.-Y., Wolfe, A. P., Verce, R. L., and Hsu, K. (1992) Mol. Cell. Biol. 12, 1500–1506
23. Goodier, J. L., Fan, H., and Marra, R. J. (1997) Mol. Cell. Biol. 17, 5823–5832
24. Yoo, C. J., and Wolin, S. L. (1997) Cell 89, 393–402
25. Zaslouf, M., Santos, T., Romeo, P., and Rosenberg, M. B. (1982) J. Biol. Chem. 257, 7857–7863
26. Fan, H., Goodier, J. L., Chamberlain, J., Engelke, D. R., and Marra, R. J. (1996) Mol. Cell. Biol. 13, 3201–3211
27. Arias, J. A., and Dynan, W. S. (1989) J. Biol. Chem. 264, 3223–3229
28. Bogenhagen, D. F., and Brown, D. D. (1981) Cell 24, 261–270
29. Studitsky, V. M., Kasaveti, G. A., Geiduschek, E. P., and Felsenfeld, G. (1997) Science 278, 1960–1963
30. Stefano, J. E. (1984) Cell 34, 145–154
31. Van Horn, D. J., Yoo, C. J., Xue, D., Shi, H., and Wolin, S. L. (1997) RNA 3, 1434–1443
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32. Chu, W. M., Ballard, R., Carpick, B. W., Williams, B. R., and Schmid, C. W. (1998) *Mol. Cell. Biol.* 18, 58–68
33. Chu, W. M., Liu, W. M., and Schmid, C. W. (1995) *Nucleic Acids Res.* 23, 1750–1757
34. Chu, W. M., Ballard, R. E., and Schmid, C. W. (1997) *Nucleic Acids Res.* 25, 2077–2082
35. Allison, D. S., and Hall, B. D. (1985) *EMBO J.* 4, 2657–2664
36. Young, L. S., Rivier, D. H., and Sprague, K. U. (1991) *Mol. Cell. Biol.* 11, 1382–1392
37. Wilson, E. T., Larson, D., Young, L. S., and Sprague, K. U. (1985) *J. Mol. Biol.* 183, 153–163
38. Wilson, E. T., Condliffe, D. P., and Sprague, K. U. (1988) *Mol. Cell. Biol.* 8, 624–631
39. Kovelman, R., and Roeder, R. G. (1990) *Genes Dev.* 4, 646–658