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Palindromic sequences preceding the terminator increase polymerase III template activity

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ABSTRACT

Four consecutive T residues in the sense strand are sufficient to terminate transcription by RNA polymerase III (pol III). Previously we observed that compared with this minimally sufficient terminator, five T residues immediately preceded by a palindromic sequence increases transcriptional expression both *in vitro* and *in vivo*, raising the question of whether a palindromic sequence has a role in pol III termination. Here we observe that site-directed mutations which eliminate the dyad symmetry of the palindromic sequence decrease transcriptional expression. Similar effects are observed whether dyad symmetry is eliminated in regions of the palindrome which are proximal or distal with respect to the terminator. Compensatory mutations at either site to restore dyad symmetry rescue transcriptional activity. These observations suggest that a higher order structure, such as a RNA hairpin, immediately preceding the terminator increases pol III transcriptional activity.

INTRODUCTION

In higher eukaryotes, four or more consecutive T residues in the sense strand terminate transcription by RNA polymerase III (pol III) (1). The functional significance of such 'T runs' in termination was immediately apparent upon sequence comparisons of pol III-directed transcription units (2). In those same comparisons, short palindromic sequences immediately preceding terminators for various 5S RNA genes and the adenovirus VA1 RNA gene were also noted (2). Such structures potentially form RNA hairpins, evoking obvious comparison with rho-independent termination in prokaryotes. However, deletion of a palindrome preceding the terminator had no obvious effect on template activity or termination efficiency of the *Xenopus* 5S rRNA gene, leading to the well-established conclusion that dyad sequence symmetry is not required for pol III termination (1,3).

Human Alu elements acquire their individual termination signals from unique 3′ flanking sequences adjacent to their genomic insertion sites (4,5). In contrast, the 7SL RNA gene, which is ancestrally homologous to Alu, has a defined terminator consisting of four T residues immediately preceded by a 5 nt palindrome, specifically 5′-GAGACCCCGTCTCTTTT-3′ (6). By five targeted base substitutions, a minimal endogenous Alu terminator consisting of four T residues was replaced by five T residues immediately preceded by this 5 bp inverted repeat (7). This '7SL-like terminator' stimulates Alu template activity *in vitro* and also increases the steady-state accumulation of Alu RNA *in vivo* (7). The lifetime of the resulting transcripts is not changed by this terminator replacement, leading us to conclude that this efficient 7SL-like terminator increases pol III-directed template activity both *in vivo* and *in vitro*. Either the additional T residue or sequence elements associated with the palindrome or their combination constitute a more effective terminator as monitored by template activity. The goal of this study was to distinguish between these possibilities.

Five T residues are expected to terminate transcription more effectively than four (1). While sequence context surrounding the T residues can also greatly affect termination efficiency, there is no general agreement as to which sequence context is most favorable (1,3,8,9). Effects resulting from terminator context might depend upon the particular gene, the species of origin of pol III and experimental details of the assay. For example, in one case a GC dinucleotide provides the optimal context for termination, but in another case AA is optimal (3,8; see also 9 for discussion).

Sequence context surrounding a terminator can affect template activity by determining the rate at which a template is cleared for subsequent rounds of transcription (10). As assayed *in vitro*, the termination factor La stimulates template clearance in a context-dependent manner (10). La preferentially binds U-terminated RNA, giving it a special affinity for nascent pol III transcripts, by which it also directs post-transcriptional events (9–11). The binding affinity of La is apparently sensitive to the sequence context immediately preceding the terminal U residues, so that bases at these positions determine the rate with which La is released and its subsequent availability (10,12). The critical role of La in regulating pol III activity is further underscored by the recent discovery that it is also required for transcriptional initiation (12). This implies that extremely subtle sequence features surrounding terminators might increase pol III transcriptional activity by promoting more efficient template clearance, more efficient La recycling and, consequently, higher rates of initiation, while simultaneously directing a transcript's fate.

Although context immediately surrounding a terminator is important, there is no evidence that a RNA hairpin structure has any special role in pol III termination and hairpins are certainly not essential (1,3). Nonetheless, as reviewed above, terminators for 5S rRNA, VA1 RNA and 7SL RNA genes are all preceded by palindromic sequences potentially capable of forming RNA hairpins (2,6). As yet other examples, the 7SK RNA gene terminator (GGCAGTCTGCCTTTCTTTT) contains a 5 nt

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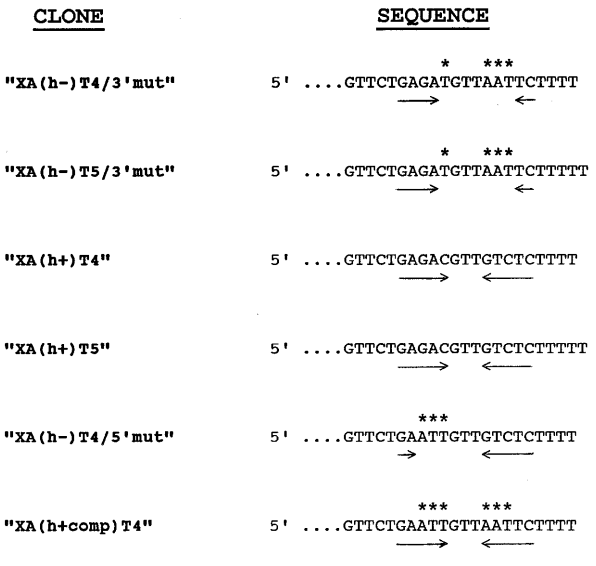

Figure 1. Comparison of termination sequences. Templates employed in this study were generated by a PCR strategy using primers to generate the sequence differences shown above (Materials and Methods). All inserts were cloned having the same orientation with respect to the vector so that immediately 3′ to the termination signal the vector sequence 5′-AAGCCGAA-3′ was common to all constructs. Clones designated h+ had 5 bp palindromes as indicated by underlining arrows. Asterisks indicate substitutions compared with the parent palindromic sequence and truncated underlining arrows are used to highlight residual nucleotides derived from the parent palindromic sequence.

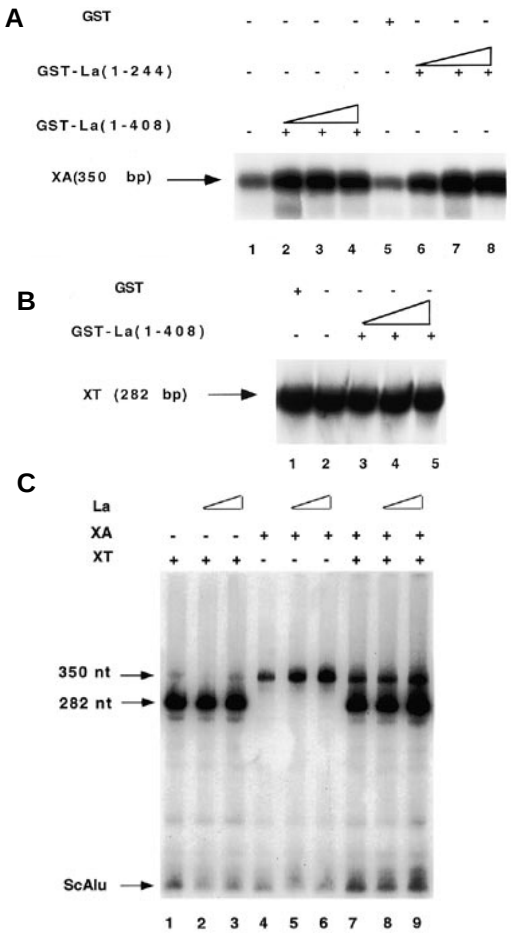

Figure 2. Effects of La protein upon differently terminated templates *in vitro* and *in vivo*. (**A**) The template XA (7) was transcribed *in vitro* with HeLa nuclear extract alone (lane 1) or additionally with GST (500 ng, lane 5), wild-type GST–La(1–408) (25, 50 or 100 ng, lanes 2–4) or mutant GST–La(1–244) (25, 50 or 100 ng, lanes 6–8). (**B**) The template XT (7) was transcribed *in vitro* with HeLa nuclear extract alone (lane 2) or additionally with GST (500 ng, lane 1) or with wild-type GST–La(1–408) (25, 50 or 100 ng, lanes 3–5). (**C**) The templates XT (10 µg, lanes 1–3), XA (10 µg, lanes 4–6) and both XA and XT (10 µg of each, lanes 7–9) were co-transfected into 293 cells with 0 (lanes 1, 4 and 7), 7.5 (lanes 2, 5 and 8) or 15 µg (lanes 3, 6 and 9) La-overexpressing clone and sufficient pUC DNA to provide 40 µg total DNA. The resulting transcripts were analyzed by Northern analysis for Alu transcripts. Positions of scAlu RNA primary XT Alu transcripts (282 nt) and primary XA Alu transcripts (350 nt) are indicated. Phosphorimager analysis was used to determine the intensities of the Alu transcripts (Table 1).

palindrome (13) and short palindromes are present near the termini of some tRNA genes.

In some of these examples the palindrome is probably required for RNA function. In particular, the palindromic sequence represented in the 7SL-like terminator does not form an independent hairpin in either 7SL RNA or in its sequence homolog Alu RNA (14,15). In mature RNA this palindromic sequence is folded into a more extensive secondary structure by a complementing sequence far removed from the transcript terminus. However, this palindrome might transiently form an independent hairpin during RNA synthesis. Also, we previously observed that the 7SL-like terminator acts in a position-independent manner, including constructs in which it is entirely isolated from the body of the Alu element (7). In those constructs this palindrome would be expected to form a stable, independent hairpin.

Based on these comparisons of different terminators, the extraordinary sensitivity of transcription to termination context and previous results (7), the possible significance of palindromes in pol III termination deserves further consideration. Here we have observed that palindromic sequences immediately preceding the terminator increase transcription.

MATERIALS AND METHODS

Construction of chimeric Alu clones

Six constructs were generated by PCR from Alu clones XA or XAT (7) using oligonucleotide 709 as the 5′-primer with respect to the Alu element and the oligonucleotides identified in Figure 1 as 3′-primers (7). PCR products were cloned into TA vector (Invitrogene Inc.), selected for identical orientation of the Alu element with respect to the vector and verified by base sequence.

Cell culture and transfection

HeLa and human 293 cells were cultured as previously described (7). One day prior to transfection, cells were seeded at a density of 5×10^5 on 100 mm plates. Plasmid DNA (consisting of 10 µg of the indicated Alu construct, the indicated quantity of La expression vector and a sufficient quantity of pUC to yield 40 µg total) was transfected by calcium phosphate precipitation (16). After 48 h, cells were harvested for RNA. For RNA lifetime

determinations (data not shown), cells were treated with actinomycin D (5 μg/ml) for the indicated periods of time.

Table 1. Intensities of XA (350 nt) and XT (282 nt) Alu transcripts in 293 cells co-transfected with pCMV-La were determined by phosphorimager analysis of the results shown in Figure 2C

pCMV-La transfected (μg)	XA Alu RNA	XT Alu RNA	XA/XT Alu RNAs
0	133	783	234/7792
7.5	252	630	253/949
15	424	874	302/1247

Transcriptional analysis

In vitro transcription using HeLa nuclear extracts, isolation of cytoplasmic RNA, Northern analysis and primer extension were performed as previously described except for the following minor modifications (7,17). For primer extension assays, the reaction mixture was incubated with 200 U MMLV reverse transcriptase (BRL) for at least 2 h at 37°C. Northern analysis was performed from 6% polyacrylamide gels (40:1 acrylamide:bisacrylamide). Prehybridization and hybridization were both performed overnight at 46°C. Quantitation of primer extension and Northern analysis were performed with a Fuji BAS 1000 phosphorimager. The oligonucleotide PV comp. (ACCGTTTTAGCCGGGATG) was used as the probe for Northern analysis and oligonucleotide Alu 21mer (GCGATCTCGGCTCACTGCAAG) was used for primer extension analysis of Alu RNAs (7). Oliognucleotide 7SL (ATGCCGAACTTAGTGCGG) was used as an internal control to test for the amount of RNA loaded in either primer extension or Northern analysis of endogenous 7SL RNA (7; data not shown).

La constructs

Full-length La cDNA (PBS-La) cloned into pBluescript was a gift of Dr Kuan-teh Jeang (18). The cDNA insert released by restriction with *Not*I and *Apa*I was subcloned into vector PRc/CMV (Invitrogen), resulting in the construct PCMV-La. GST–La fusion constructs were generated from La cDNA by PCR and cloned into pGEX (Pharmacia). One construct provided wild-type fusion protein GST–La(1–408), while another was a deletion construct, GST–La(1–244). These constructs were expressed in *Escherichia coli* DH5a and proteins were purified as previously described (19). Products of the expected size were observed on SDS–PAGE by Coomassie staining and confirmed by staining with anti-La human serum (a gift of Dr Kuan-Teh Jeang).

RESULTS

Terminator-dependent transcriptional stimulation by La

Previously we observed that an Alu clone 'XA' having a minimal terminator consisting of four T residues is less actively transcribed than a corresponding Alu construct 'XT' having a 7SL-like terminator (7; Introduction). Because of the direct involvement of La in terminating pol III transcription, we initially tested the effects of GST–La fusion protein on the *in vitro* template activities of these same constructs (Fig. 2). GST alone had no effect on either construct (Fig. 2). Recombinant La increased the template activity of XA (Fig. 2A), but had no effect on the activity of XT (Fig. 2B). The first 244 residues of recombinant La were sufficient to stimulate transcription (Fig. 2A).

To avoid possible artifacts associated with an *in vitro* assay, we employed a co-transient transfection assay to compare the possible effects of La protein on the expression of XA and XT (Fig. 2C and Table 1). Northern analysis of clone XA revealed two discrete Alu transcripts (Fig. 2C, lanes 4–6). The 350 nt species is the full-length primary Alu transcript corresponding to the expected termination signal; the lower molecular weight species is processed scAlu RNA (7). Processing of primary B1 transcripts into scB1 RNA is modulated by the terminator and is La dependent (9,10). We have not investigated these possibilities for scAlu RNA and restrict this study to the expression of full-length primary Alu transcripts.

The primary transcript from clone XT was 282 nt because of the different position of its terminator (7; Fig. 2C). Previously, we observed that the 7SL-like terminator stimulated template activity ~5-fold compared with a minimal terminator consisting of four T residues (7). In agreement with that observation, we presently observe a 6-fold stimulation (Fig. 2C, lanes 1 and 4 and Table 1). However, while co-transient expression of La increased the steady-state expression of the XA primary transcript 3-fold (lanes 4–6 and Table 1), it has essentially no effect upon the level of XT primary transcript (lanes 1–3 and Table 1).

The steady-state level of RNA was determined both by the rate of transcription and the rate of degradation. Surprisingly, we previously observed that the XA and XT primary transcripts have indistinguishable half-lives (7). This observation was based upon a very sensitive comparison of the relative lifetimes of the two transcripts. Since this palindrome did not increase the lifetime of the resulting transcripts *in vivo* (7), we infer that this sequence increased the rate of transcription, an inference verified by the *in vitro* transcription results (Fig. 2). There is, however, an alternative explanation which we regard as being unlikely: weaker terminators might be leaky, resulting in longer transcripts that terminate at the next available downstream signal. The paucity of such transcripts as assayed by Northern analysis (Fig. 2C and also Fig. 3 as described below) would further require that these hypothetical read-through transcripts were extremely short lived compared with the major product. While we have not rigorously disproven this possibility, differences in the *in vitro* template activity of these two constructs and others described below (7; Fig. 2) support the simpler interpretation, i.e. differences in the steady-state abundance of these transcripts *in vivo* results from differences in template activity. We therefore also interpret *in vivo* results with this simplifying qualification.

Endogenous La is an extremely abundant protein (20,21). Nonetheless, because the two differently terminated constructs respond so differently to exogenous La, we concluded that a comparison of *cis*-acting termination signals, the primary goal of this study, should also simultaneously explore their possible differential response to La.

The insensitivity of the XT construct to additional La implies that its activity both *in vitro* and *in vivo* must be limited by some other factor(s) (Fig. 2 and Table 1). A direct comparison of XA and XT expression in the presence of overexpressed La was performed by co-transient transfection (Fig. 2C, lanes 7–9 and Table 1). The level of expression of XT is 3- to 4-fold higher than that of XA in this co-transient assay, confirming the different

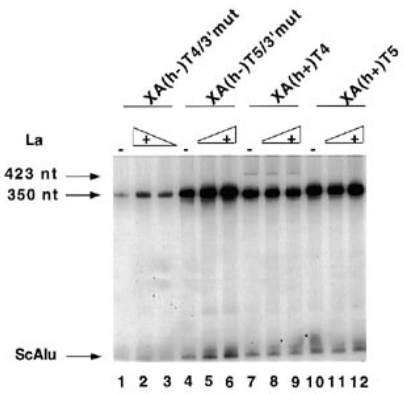

Figure 3. Effects of La protein on terminator-dependent transcription *in vivo*. Alu constructs examined for expression were XA(h–)T4/3′mut (lanes 1–3), XA(h–)T5/3′mut (lanes 4–6), XA(h+)T4 (lanes 7–9) and XA(h+)T5 (lanes 10–12). These Alu templates (10 µg) were co-transiently transfected with pUC alone (lanes 1, 4, 7 and 10) or with pCMV-La (7.5 and 15 µg in lanes 3–2, 5–6, 8–9 and 11–12 respectively) and sufficient pUC to provide 40 µg plasmid DNA in all lanes. Resultant transcripts were assayed for Alu RNA by Northern blot. Positions of flAlu RNA (350 nt), scAlu RNA and a 423 nt transcript are indicated. A 423 nt transcription product is expected for those transcripts which leak through the defined terminator and end at the next downstream terminator. Intensities of the 350 nt bands were determined by phosphorimager analysis and are reported in Table 2. Reprobing of this blot showed that the intensity of 7SL RNA was the same in all lanes (12% maximum deviation comparing lanes 1, 4, 7 and 10).

transcriptional activities of these two constructs (Table 1). However, the presence of co-transfected XT reduces the effect of La overexpression upon the steady-state abundance of the XA transcript, but, conversely, co-transfected XA may slightly sensitize XT to added La (Table 1). These results may both be explained by *trans* effects between the XA and XT constructs. In this co-transient experiment, the factor which normally limits XT expression would now also be limiting for XA expression and, conversely, La, which may be limiting for XA expression, might become limiting for XT expression. Because of this complication, subsequent studies were performed exclusively as parallel comparisons between different constructs.

Dissection of *cis*-acting termination elements

The XA and XT constructs and their resulting primary Alu transcripts differ in a number of features so that these constructs are not suitable for a precise comparison of different terminator sequences. Four constructs were prepared to compare the relative contributions of the number of T residues (either four or five and named accordingly) and sequences immediately located 5′ of termination (Fig. 1). Two of the four constructs (called h+) retained the palindromic sequence derived from the 7SL-like terminator, but in two constructs [called XA(h–)/3′mut] this symmetry was eliminated by four substitutions: a C→T transition at position –9 (numbered 5′ with respect to the T residues), a G→A transition at position –5, a T→A transversion at position –4 and a C→T transition at position –3 (Fig. 1). The dinucleotide sequence context (i.e. TC) at positions –2 and –1 was conserved in all four constructs (Fig. 1). Since all constructs contained identical vector sequences immediately following the T residues, this study does not test the influence of sequences located 3′ of the terminator. Expression of these construct was monitored by transient transfection using Northern analysis; endogenous 7SL RNA controlled for equivalent RNA loading (Fig. 3, Table 2 and data not shown).

Again, Northern analysis revealed discrete Alu transcripts: a 350 nt full-length primary Alu transcript corresponding to the expected termination signal and lower molecular weight processed scAlu RNA (7). Additionally, we detected a very low level of a transcript (423 nt) which would correspond to termination at the next downstream site. As discussed above, the very low level of this product indicates that either termination is not very leaky or that such products must be extremely short lived compared with the 350 nt transcripts. Previously we observed that the 7SL-like terminator stimulated template activity by ~5- to 6-fold compared with a minimal terminator consisting of four T residues (7; Table 1). In agreement with those results, clone XA(h+)/T5, which has an intact palindrome and five T residues, is expressed at a higher level (7-fold) than clone XA(h–)T4/3′mut, which has a minimal terminator consisting of four T residues (Fig. 3, lanes 1 and 10 and Table 2). Thus, these new constructs allowed us to address the central question posed in this study: are the number of T residues, some element of the palindrome or a combination of both responsible for this difference in template activity?

Constructs having terminators defined by five T residues (T5) are expressed at higher levels (3.6- and 1.3-fold) than otherwise identical constructs having terminators defined by four T residues (T4) (compare Fig. 3, lanes 1 and 4 and 7 and 10; Table 2). Constructs with the intact palindromic sequence [XA(h+)] were also expressed at higher levels (5.7- and 2.0-fold) than those with the palindrome disrupted [XA(h–)] (compare lanes 1 and 7 and 4 and 10; Table 2). Thus, both the number of T residues and some feature associated with this palindromic sequence each influence the steady-state accumulation of RNA. As previously discussed, we attribute these effects to differences in the rate of transcription, and *in vitro* transcription assays described below support this interpretation.

In further agreement with the previous results for the XA and XT constructs, co-transient expression of La protein stimulated expression from the two XA(h–)/3′mut templates having the disrupted palindrome (lanes 1–6) but had no effect on the two XA(h+) templates having the intact palindrome (lanes 7–12). Evidently, this difference in La sensitivity accounts for part or all of the difference in template activity observed for clones having intact and disrupted palindromic sequences (Fig. 3).

Table 2. Intensities of primary Alu transcripts in 293 cells co-transiently transfected with pCMV-La and the constructs described in Figure 3 were determined by phosphorimager analysis

pCMV-La transfected (µg)	XA(h–)T4/3′mut	XA(h–)T5/3′mut	XA(h+)T4	XA(h+)T5
0	502	1819	2884	3659
7.5	902	2910	2848	3629
15	1401	4122	2733	3919

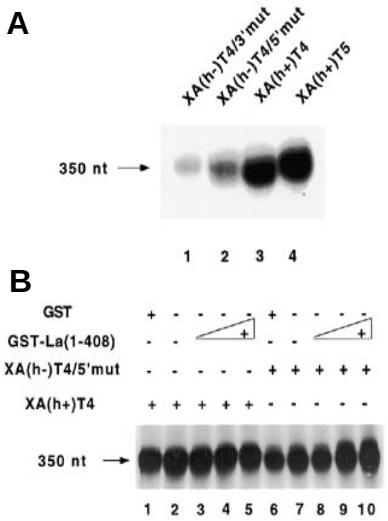

Figure 4. Activity of templates having substitutions distal or proximal to the terminator. (**A**) HeLa nuclear extract was used to transcribe clones XA(h–)T4/3′mut (lane 1), XA(h–)T4/5′mut (lane 2), XA(h+)T4 (lane 3) and XA(h+)T5 (lane 4). The resulting transcripts were analyzed by gel electrophoresis. (**B**) Effects of La and terminator-distal substitutions upon template activity. HeLa nuclear extract was used to transcribe clones XA(h+)T4 (lanes 1–5) and XA(h–)T4/5′mut (lanes 6–10) with GST (500 ng, lanes 1 and 6), with wild-type GST–La (25, 50 or 100 ng, lanes 3–5 and 8–10 respectively) or without additional protein (lanes 2 and 7). Resulting transcripts were analyzed by gel electrophoresis.

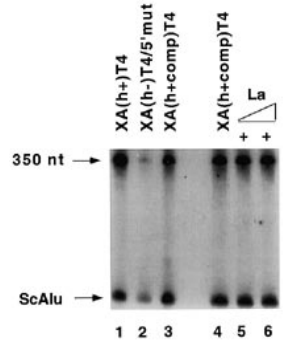

Figure 5. Restoration of dyad symmetry by compensatory substitutions increases transcription. Alu-expressing clones (10 μg) were transfected into 293 cells and the level of expression was assayed by Northern analysis using hybridization probes toward flAlu RNA (350 nt band) as well as scAlu RNA and toward 7SL RNAs, to control for the amount of RNA loaded. These clones were XA(h+)T4 (lane 1), XA(h–)T4/5′mut (lane 2), XA(h+comp)T4 (lane 3) and XA (h+comp)T4 with 0 (lane 4) 7.5 (lane 5) or 15 (lane 6) μg pCMV-La. Sufficient pUC DNA was added to provide 40 μg DNA in all transfections.

A palindrome *per se* increases template activity

Termination is very sensitive to the dinucleotide context immediately surrounding the T residues, suggesting that context might be viewed as a highly localized sequence environment immediately surrounding the terminator (9). Considering the previous results, effects of sequence context on termination must extend upstream from position –3 and the dyad symmetry of the 7SL-like terminator may be an element of that context. To distinguish between these alternatives, the sequence GAC at positions –11 to –9 was replaced by ATT, thereby disrupting the palindrome by about one DNA helix turn, or ~30 Å, upstream from the terminator (Fig. 1). This template, XA(h–)T4/5′mut, was less actively transcribed *in vitro* than XA(h+)T4, which retained the palindromic sequence (Fig. 4A, lanes 2 and 3). Since these substitutions were rather far removed from the actual termination signal as defined by the T residues, we conclude that a higher order structure attributable to the palindromic sequence rather than merely a highly localized sequence environment is probably responsible for these differences in template activity (Discussion).

In vitro transcription was used to test whether mutating the palindrome at positions –11 to –9 sensitizes the template to La (Fig. 4B). As previously observed, the template having the intact 7SL-like terminator [XA(h+)T4] was virtually insensitive to La (Fig. 4B, lanes 1–5). Addition of recombinant La stimulated transcription from the template having the distally mutated palindrome [XA(h–)T4/5′mut], largely overcoming the difference in activity between the two constructs (Fig. 4B, lanes 6–10). Using the co-transient transfection strategy, we also observed that transcription of clone XA(h–)T4/5′mut was increased by La *in vivo* (data not shown). In summary, mutations in either the terminator-distal or terminator-proximal regions of the palindrome decreased template activity and made it dependent upon exogenous La.

Compensatory substitutions creating a new palindrome increase transcription

We wished to test whether compensatory base substitutions that restore palindromic sequence symmetry also restore the template activity of XA(h–)T4/5′mut. The sequence GTC at positions –5 to –3 in this construct was replaced by the sequence AAT, creating a new 5 bp palindrome in clone XA(h+comp.)T4 (Fig. 1). This same construct could be equally well described as a compensatory substitution of AT in place of GA at positions –11 and –10 in XA(h–)T4/3′mut to form a palindrome. The expression of XA(h+comp) was higher than that of XA(h–)T4/5′mut and approximated the expression of XA(h+)T4 (Fig. 5, lanes 1–3).

Transcription from clones having disrupted palindromes was La sensitive (Figs 3 and 4B). Compared with either of its two cognates [i.e. XA(h–)T4/3′mut and XA(h–)T4/5′mut], transcription of XA(h+comp) T4 was also insensitive to La (Fig. 5, lanes 4–6).

DISCUSSION

Previously we observed that a few point mutations converting a minimal terminator into a structure resembling the 7SL RNA gene terminator significantly increased (~6-fold) activity of a template both *in vitro* and *in vivo* (7). One of these substitutions was insertion of an additional T at the termination signal, which potentially might fully account for this increase. Not surprisingly, we observed here that for two pairs of otherwise identical constructs, those terminated with five T residues were more actively transcribed (1.3- to 3.6-fold) than those terminated by four. However, upon comparing two pairs of constructs having identical numbers of T residues, those having the intact palindrome were more actively transcribed (2- to 5-fold) than those having a minimal terminator. Also, the addition of exogenous La stimulated transcription from constructs having minimal terminators both *in vitro* and *in vivo*, but had no effect upon transcription of constructs having the intact palindrome. Thus there are two separate issues to resolve: the effect of *cis*-acting terminator elements, including especially

palindromic sequences, and possible differential effects of La addition upon templates having different terminators.

We observed that mutations which eliminated the dyad symmetry within the 7SL-like terminator decreased template activity, whether these substitutions were positioned distal or proximal to the terminator. In addition, compensatory mutations which restored dyad symmetry increased template activity compared with constructs in which the 7SL-like palindrome was disrupted in either the proximal or distal regions. These data indicate that dyad symmetry preceding the terminator stimulates transcription. Furthermore, positions within the palindrome far from the terminator affect template activity, implying that a higher order structure, such as a RNA hairpin, is probably responsible for these effects.

We have not systematically investigated the effects of other palindromic sequences on template activity. Sequence context, meaning here the identity of DNA bases immediately surrounding the terminator, also influences transcription, thereby complicating any attempt to compare different palindromic sequences. We have, however, observed that a construct having a terminator immediately preceded by an extremely large hairpin (the R17 coat protein binding site) was transcribed at the same levels as a construct having the 7SL-like terminator (unpublished). In at least this one example, a *bona fide* hairpin functionally substituted for the palindrome modeled on the 7SL RNA gene.

As is already well established, a palindrome is not required for termination (1,3), but our results indicate that such sequences can stimulate transcription and we naturally suspect that this stimulation results from some aspect of transcriptional termination. La mediates many of the complex effects that sequences associated with termination have upon overall transcriptional activity, making it at least a good candidate to serve as the *trans*-acting factor responsible for the effects of these different terminator structures.

La is required for initiation by pol III, is involved in clearing the template for subsequent rounds of transcription and transiently associates with the nascent transcript (12). Sequence near the terminator affects both the rate of template clearance and the affinity of La for the nascent transcript (9). A detailed investigation of the mechanism by which *cis*-acting termination signals stimulate transcription is beyond the intention of this investigation. However, it is noteworthy that substitutions which eliminate the 7SL-like palindrome also sensitize transcription to the addition of La and that compensatory mutations which restore dyad symmetry in this region correspondingly desensitize transcription to the addition of La. Thus, these preliminary correlations at least support a possible role for La in mediating these effects As one speculative explanation, a terminal hairpin might alter the association of La with the 3′-end of the nascent transcript, thereby resulting in the effects observed here. As an alternative explanation that draws upon precedents from prokaryotic termination (22,23), a hairpin might cause transcriptional pausing, which might then kinetically facilitate the effects of La upon termination.

The detailed mechanisms by which palindromic sequences effect termination and, consequently, template activity remain to be determined. However, the dyad symmetry of such elements should be recognized as a component of the sequence context for a terminator.

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