Sticky DNA, a Self-associated Complex Formed at Long GAA-TTC Repeats in Intron 1 of the Frataxin Gene, Inhibits Transcription*

Friedreich’s ataxia (FRDA) is an autosomal recessive neurodegenerative disease caused by the expansion of GAA-TTC repeats in the first intron of the frataxin (X25) gene. FRDA patients carrying two expanded GAA-TTC repeats show very low levels of mature frataxin mRNA and protein. A novel type of unusual DNA structure, sticky DNA, was previously found in the expanded GAA-TTC repeats from FRDA patients. To evaluate the effect of sticky DNA on transcription, in vitro transcription studies of (GAA-TTC)n repeats (where \( n = 9-150 \)) were carried out using T7 or SP6 RNA polymerase. When a gel-isolated sticky DNA template was transcribed, the amount of full-length RNA synthesized was significantly reduced compared with the transcription of the linear template. Surprisingly, transcriptional inhibition was observed not only for the sticky DNA template but also another DNA molecule used as an internal control in an orientation-independent manner. The molecular mechanism of transcriptional inhibition by sticky DNA was a sequestration of the RNA polymerases by direct binding to the complex DNA structure. Moreover, plasmids containing the (GAAGGA-TCTTTC)\(_{65}\) repeat, which does not form sticky DNA, did not inhibit in vitro transcription, as expected. These results suggest that the role of sticky DNA in FRDA may be the sequestration of transcription factors.

Friedreich’s ataxia (FRDA),\(^1\) an autosomal recessive neurodegenerative disease, is the most common inherited ataxia and is characterized by progressive gait and limb ataxia, dysarthria, lower limb areflexia, diminished vibration sense, muscle weakness of the legs, and extensor planter response (1–4). The gene responsible for FRDA was mapped to chromosome 9q13-X25 by linkage studies (5, 6). The FRDA gene, X25, contains seven exons (1–6) and encodes a 210-amino acid protein, frataxin (7). The majority (about 98%) of FRDA patients have an expanded GAA-TTC repeat in the first intron of the frataxin gene. Normal alleles have 6–34 repeats of uninterrupted GAA-TTC triplets, whereas FRDA-associated alleles have 66–1700 or more GAA-TTC repeats (7–10). The recessive inheritance and the sequence and the location of the repeat make FRDA unusual among all known triplet diseases (11). A unique molecular mechanism for the etiology of FRDA has been proposed (7, 11).

An inverse correlation between the length of the GAA-TTC repeats and the age of onset and the severity of the disease has been demonstrated (8, 12, 13). FRDA patients carrying expanded GAA-TTC repeats in both alleles have reduced levels of the frataxin protein, and there is an inverse correlation between the size of GAA-TTC repeat and the amount of frataxin protein (14). The reduction in the amount of the frataxin protein in FRDA patients is due to the reduction in the amount of the frataxin mRNA (7, 15, 16). Recent work revealed that the amount of the X25 mRNA is inversely related to the length of the GAA-TTC repeat in vitro (17) and in vivo (18). The formation of three-stranded DNA structures has been proposed to be involved in the transcriptional inhibition by the long GAA-TTC repeat (17–21).

GAA-TTC repeats form triplex structures (reviewed in Refs. 22–25). However, it is uncertain whether the R-R type or the Y-R type of triplex is formed by these repeats related to FRDA (17, 19, 26, 27). Recently, we reported that the long GAA-TTC repeats form R-R triplex at neutral pH in the presence of Mg\(^{2+}\) (19, 20). Since a number of polypurine-polypyrimidine sequences exist in transcription regulatory regions in eukaryotic genes, triplexes may have an important role in the regulation of gene expression (19, 27). In fact, intermolecular and intramolecular triplexes are known to inhibit transcription (reviewed in Ref. 28). Two mechanisms have been proposed to explain the transcriptional inhibition by triplexes. Formation of a triplex may block the transcription elongation by RNA polymerase (29–31) or may interfere with the binding of transcription factors to sequences that control transcription (32–34).

Recently, Grabczyk and Ussin proposed another model, in which an RNA polymerase advancing within a long GAA-TTC repeat causes the transient formation of an R-R type of triplex, which then creates a pause site (35). A novel unusual DNA structure, sticky DNA, was described for the FRDA-related long GAA-TTC repeat (19). A correlation was observed between the length of the GAA-TTC repeats and the formation of sticky DNA, which suggested the involvement of this structure in the etiology of FRDA. Sticky DNA is formed by the association of two long GAA-TTC repeats in the R-R triplex configurations. A strand-exchanged model, in which the pyrimidine strands of two R-R triplexes were exchanged with each other, was proposed to explain this extremely stable struc-
tured. Since the sticky DNA structure is a self-associated complex, sticky DNA may inhibit transcription and may be involved in the reduction of the frataxin X25 mRNA in FRDA patients.

Herein, we report that sticky DNA effectively inhibits in vitro transcription by the T7 and SP6 RNA polymerases. This inhibitory effect is apparently due to the sequestration of the RNA polymerases by direct binding to sticky DNA.

EXPERIMENTAL PROCEDURES

Plasmds—The GAA-TTC repeats were originally cloned and inserted into the pSP63 vector (Life Technologies, Inc.) as described by Ohshima et al. (18). For bacterial transcription studies, the pGEM-3Zf(+) vector (Promega), which contains the T7 and the SP6 promoters, was used. To subclone GAA-TTC repeats in one orientation, in which the repeating rGAA RNA is formed by T7 RNA polymerase, various lengths of GAA-TTC repeats were excised by BamHI–EcoRI digestion from pSP63-based plasmids and inserted into the BamHI–HindIII sites of the pGEM-3Zf(−) vector. To obtain the pGEM-3Zf(−)-based plasmids in the other orientation in which the repeating rUUC RNA is formed by T7 RNA polymerase, the GAA-TTC repeats were excised by EcoRI–PstI digestion from the pSP63-based plasmids and inserted into the same sites of the pGEM-3Zf(−) vector. For eukaryotic transcription studies, the pCR3.1 vector (Invitrogen), which contains the cytomegalovirus immediate early promoter, was used. The GAA-TTC inserts were excised by EcoRI–XhoI digestion from the pSP63-based plasmids. To subclone the GAA-TTC repeats in one orientation so that the repeating rGAA RNA is formed, the excised GAA-TTC repeats were inserted into the EcoRI–XhoI sites of the pCR3.1 vector. For subcloning in the other orientation in which the repeating rUUC RNA is formed, the excised GAA-TTC fragments were inserted into the EcoRI–XhoI sites of the pCR3.1 vector.

The repeating hexanucleotide sequence (GAAGGAAGTCCTTC)65 was reported by Ohshima et al. (20). This repeating sequence was also subcloned into the pGEM-3Zf(−) vector for bacterial transcription and into the pCR3.1 vector for eukaryotic transcription as described above.

All plasmids were transformed into Escherichia coli SURE strain by CaCl2 method (36). The transformants were cultured in 1 liter of LB medium at 37 °C to an A600 of ~0.5 (logarithmic phase). Plasmids were isolated by the alkaline lysis method and purified by CsCl density gradient centrifugation (36). Three phenol/chloroform extractions were performed followed by precipitation with ethanol and resuspension in 10 mM Tris-HCl (pH 7.9), 6 mM MgCl2, 2 mM spermidine, 10 mM dithiothreitol) supernatant. The pellets were reconstituted with 400 μM each of rATP, rGTP, and rCTP; 17 μM [α-32P]rUTP (Amersham Pharmacia Biotech); 40 μM rNasin (Promega); and 1, 10, or 50 units of SP6 RNA polymerase (New England Biolabs). The RNA product was purified and analyzed as described above.

Eukaryotic in Vitro Transcription Using HeLa Nuclear Extract—Eukaryotic in vitro transcription was carried out with the HeLa nuclear extract in vitro transcription system (Promega) (39) as follows. A mixture of 100 ng of the XhoI-linearized pCR3.1-based plasmid and 100 ng of the XhoI-linearized pCR3.1 vector was transcribed in a 25-μl volume with 400 μM each of rATP, rGTP, and rUTP; 16 μM rCTP; 0.17 μl of [α-32P]rUTP (Amersham Pharmacia Biotech); and 1 μl (1.6 units) of HeLaScribe nuclear extract (Promega) in HeLa nuclear extract 1× buffer (20 mM Hepes (pH 7.9 at 25 °C), 100 mM KCl, 0.2 mM EDTA, 0.5 mM dithiothreitol, and 20% glycerol) supplemented with 3 mM MgCl2. Reactions were performed at 30 °C for 4 h and terminated by adding 175 μl of HeLa nuclear extract stop mix (0.3 μl Tris-HCl (pH 7.4 at 25 °C), 0.3 mM sodium acetate, 0.5% SDS, 2 mM EDTA, and 3.0 μg/ml tRNA). The RNA product was purified by extraction with phenol/chloroform/isomyl alcohol (25:24:1), precipitated with ethanol, dissolved in formamide loading buffer, and analyzed in a 4% polyacrylamide gel containing 7 M urea. The gels were exposed to x-ray film at room temperature after drying.

Densitometric Analyses—Quantitation of autoradiographs was carried out with ImageQuant™ (Molecular Dynamics, Inc., Sunnyvale, CA) on a Molecular Dynamics densitometer.

RESULTS

Effect of Length of GAA-TTC Repeats on Transcription—Since the FRDA patients carrying two long GAA-TTC repeats have very low levels of mature frataxin transcripts (7, 15, 16), it was important to examine the ability of sticky DNA to inhibit transcription. Recent work showed that the long GAA-TTC repeats inhibit transcription in vitro (17) and in vivo (18). To analyze the effect of sticky DNA on transcription, various lengths of GAA-TTC repeats ranging from 9 to 150 repeats were subcloned into the pGEM-3Zf(−)-vector in both orientations (Fig. 1). In the pGEM-3Zf(−)-based plasmids, the GAA-TTC repeats can be transcribed from the T7 and the SP6 promoters in the presence of the T7 or the SP6 RNA polymerases, respectively. First, we used the well characterized T7 RNA polymerase system to analyze the effect of the sticky DNA structure on polyadenylate in vitro transcription (37).

The effect of the GAA-TTC repeat lengths on the transcription by T7 RNA polymerase was studied. The pGEM-3Zf(−) vector without an insert was used as the internal control template. All plasmids were linearized by HindIII for the termination of run-on transcription. A mixture of the HindIII-linearized GAA-TTC-containing plasmid and the HindIII-linearized pGEM-3Zf(−) vector was transcribed by 10 units of T7 RNA polymerase. The expected sizes of the full-length repeating rGAA RNAs from 693 to 1116 nucleotides and those of the repeating rUUC were from 690 to 1113 nucleotides. The full length of the internal control RNA was expected to be 60 nt. When the repeating rGAA RNA was formed, the internal control showed similar amounts of 60-nucleotide-long RNA, indicating that each reaction had the same activity to T7 RNA polymerase (Fig. 2A). However, the amount of the repeating rGAA RNA was significantly decreased as the number of
GAA-TTC repeats increased (Fig. 2A). From the incorporated radioactivities, we estimated that, for the long repeat tracts, ∼1.5 r(GAA)\textsubscript{150} RNA molecules were produced from a single template DNA. However, for the shorter tracts, ∼5.0 r(GAA)\textsubscript{n} RNA molecules were produced from a single template DNA. This suggested that the (GAA-TTC)\textsubscript{150} repeat inhibited transcription by ∼29% compared with the (GAA-TTC)\textsubscript{n} repeat (data not shown). We observed a similar effect of the GAA-TTC repeat lengths on the formation of the repeating rUUC RNA (Fig. 2B). Thus, as the number of GAA-TTC repeats increased, the amount of the repeating r(UUC)\textsubscript{n} RNA decreased. The number of the r(UUC)\textsubscript{150} RNA molecules produced from a single template was estimated to be ∼1.5, whereas ∼3.4 r(UUC)\textsubscript{n} RNA molecules were produced from a single template DNA (data not shown). These data are in agreement with results which were previously reported in vitro by Bidichandani et al. (17) and in vivo by Ohshima et al. (18).

Furthermore, for the formation of the repeating rGAA RNA, slower migrating bands were observed among the transcripts for all lengths of GAA-TTC repeats (Fig. 2A). Incubation at 65°C for 5 min in a formamide loading buffer decreased the amount of the slower migrating bands and increased the amount of the full-length transcripts. However, the slower migrating bands could not be completely eliminated under all conditions tested (data not shown). In contrast, there was no slower migrating band in the formation of the repeating rUUC RNA (Fig. 2B). The temperature and the sequence dependence of the appearance of the slower migrating bands suggest that these bands arise due to the formation of an unusual structure.

This structure may be either a R-R-Y type of DNA-RNA triplex or a secondary structure composed exclusively of the repeating rGAA RNA.

The effect of the (GAAGGA-TCCCTTC)\textsubscript{65} hexamer repeat on transcription was also examined using the T7 RNA polymerase system. The amount of the r(GAAGGA)\textsubscript{150} RNA was significantly higher than that of the equivalent length of the repeating rGAA RNA (Fig. 2A). The formation of the r(UCCUUC)\textsubscript{65} RNA also showed a higher amount of full-length RNA compared with the equivalent length of the repeating rUUC RNA (Fig. 2B). This result indicates that the GAAGGA-TCCCTTC hexamer repeat does not inhibit transcription in vitro. These data are consistent with previous in vivo observations (20).

**Effect of Sticky DNA on Transcription—**To determine the effect of the sticky DNA structure on transcription, the (GAA-TTC)\textsubscript{150}-containing plasmids (pRW4204 and pRW3545) were linearized by HindIII, and the linear band and the retarded band were separated by agarose gel electrophoresis and eluted. These species were transcribed in vitro using 10 units of T7 RNA polymerase. The formation of the r(GAA)\textsubscript{150} RNA from the gel-isolated linear template showed a similar amount of the full-length transcript compared with that from the HindIII-digested pRW4204, which was not gel-isolated (Fig. 3A). However, the formation of the r(GAA)\textsubscript{150} RNA from the gel-isolated sticky DNA template showed a dramatic reduction in the amount of the full-length transcript (Fig. 3A). The amount of r(GAA)\textsubscript{150} RNA from the sticky DNA template was about 8.8% of that from the linear template (Fig. 3A). A similar reduction of the amount of transcription from the sticky DNA template was seen in the formation of the r(UUC)\textsubscript{150} RNA (Fig. 3B); the amount of the r(UUC)\textsubscript{150} RNA from the sticky DNA template was about 0.4% of that from the linear template (Fig. 3B). These results show that the sticky DNA structure strongly
inhibits T7 RNA polymerase transcription in an orientation-independent manner.

Surprisingly, a reduction in the amount of transcription was also observed in the transcription of the internal control template. The amount of the control 60-nt RNA in the transcription of sticky DNA was about 6.5% of the transcription of the linear template in the formation of the repeating rGAA RNA (Fig. 3A) and about 20% in the formation of the repeating rUUC RNA (Fig. 3B). This result suggests that the sticky DNA structure can inhibit transcription not only in cis but also in trans.

Fig. 4 shows the analysis of the transcriptional inhibition by sticky DNA, using different amounts of T7 RNA polymerase. When 1 unit of T7 RNA polymerase was used, an inhibitory effect of the sticky DNA was observed on the transcription of both the (GAA)\textsubscript{150} containing plasmid and the internal control, although the amount of the full-length RNA was much less compared with the amount of RNA synthesized by 10 units of polymerase (Fig. 4). However, when 50 units of T7 RNA polymerase was used, the transcriptional inhibition by the sticky DNA was overcome in the transcription of both the (GAA)\textsubscript{150} containing template and the internal control template (Fig. 4). This suggests that the excess of T7 RNA polymerase neutralizes the transcriptional obstacle (sticky DNA) within the long GAA-TTC repeat. Alternately, the T7 RNA polymerase extract may contain unknown factors that abolish the sticky DNA structure.

To distinguish between these two possibilities, the sticky DNA template was analyzed after transcription using the different amounts of T7 RNA polymerase. The linear band and the retarded band were isolated from the HindIII-digested pRW4204 in an agarose gel and end-labeled by [\(\alpha\text{-}\text{P}\text{]}\text{dATP}]. The in vitro transcription reaction was carried out on the [\(\alpha\text{-}\text{P}\text{]}\text{dATP}-labeled template DNA with 10 or 50 units of T7 RNA polymerase in the absence or presence of rNTPs. In the absence of rNTPs, T7 RNA polymerase can bind to the T7 promoter but cannot start the RNA synthesis (40). However, in the presence of rNTPs, T7 RNA polymerase can bind to the promoter and extend the RNA chain (40). Then the template DNA was analyzed by agarose gel electrophoresis after the transcription reaction.

The gel-isolated linear DNA had no retarded band, and its structure was unaffected by incubation with either 10 or 50 units of T7 RNA polymerase in the absence of rNTPs, as expected (Fig. 5A, left). However, the gel-isolated sticky DNA template had a substantial amount of the retarded band and small amount of the linear band. The structure of the sticky DNA template was also unaffected by incubation with 10 or 50 units of T7 RNA polymerase in the absence of rNTPs (Fig. 5A, right). These results indicate that the T7 RNA polymerase extract was not contaminated by factors that could abolish the sticky DNA structure. Thus, the binding of the T7 RNA polym-
The positions of the linear and the retarded band are shown in the upper panel of each gel. The amount of RNA formed by T7 RNA polymerase was determined by densitometric analysis of the autoradiograph. The amount of RNA formed by T7 RNA polymerase was increased to 50 units (Fig. 5, right). The amount of the smear increased when the amount of rNTPs was increased to 50 units (Fig. 5, left). The amount of the smear decreased and a smear appeared above the linear band (Fig. 5, right). In the transcription reaction done using 50 units of T7 RNA polymerase, the amount of the smear increased and a smear appeared above the linear band (Fig. 5, right). In the transcription reaction done using 10 units of T7 RNA polymerase, the amount of the smear decreased and a smear appeared above the linear band (Fig. 5, left).

On the other hand, when the transcription of the linear template was carried out with 10 units of T7 RNA polymerase in the presence of rNTPs, the amount of the linear template decreased, and a smear appeared above the linear band (Fig. 5, left). The amount of the smear increased when the amount of rNTPs was increased to 50 units (Fig. 5, right). When the linear DNA template was transcribed with rNTPs, the amount of the smear increased, and the amount of the linear band increased (Fig. 5, right). In the transcription reaction done using 10 units of T7 RNA polymerase, the amount of the smear increased, and a smear appeared above the linear band (Fig. 5, left). However, a small amount of the retarded band still remained (Fig. 5, right).

In the transcription reaction done using 50 units of T7 RNA polymerase, where the inhibitory effect of the sticky DNA had been eliminated (Figs. 3 and 4), the retarded band was entirely absent, and the higher molecular weight smear pattern was observed (Fig. 5, right). These data indicate that transcription with an excess of GAA-TTC repeat in the pSPL3 vector, was linearized by XmnI, and the linear band and the retarded band were isolated in an agarose gel electrophoresis. As a negative control, the Ndel-linearized pSPL3 vector was prepared. Then 10 units of T7 RNA polymerase were preincubated with one of these linearized pSPL3-based plasmids and used for transcription of the SapI-linearized pGEM-3Zf(−) vector (Fig. 1A). The expected size of the full-length transcript was 332 nt.

When the T7 RNA polymerase was preincubated with the Ndel-linearized pSPL3 vector, a large amount of the 332-nt RNA was observed (Fig. 6, left lane). Preincubation of T7 RNA polymerase with the linear pRW3822 did not have a significant effect; the amount of the 332-nt RNA was 92.9% of the control (Fig. 6, middle lane). This suggests that the long GAA-TTC sequence itself does not have the capacity to sequester the T7 RNA polymerase. However, when the T7 RNA polymerase was preincubated with the sticky DNA formed by pRW3822, the amount of the 332-nt RNA was reduced to 4.1% of the negative control (Fig. 6, right lane). This dramatic result shows that the sticky DNA structure rather than just the long GAA-TTC sequence has the capacity to bind the T7 RNA polymerase. Hence, the sticky DNA structure sequesters the T7 RNA polymerase and reduces the amount of available T7 RNA polymerase, thereby resulting in the transcriptional inhibition of the T7 RNA polymerase even in the absence of transcription.

To test the requirement of transcription in the T7 RNA polymerase sequestration by the sticky DNA, we used the pSPL3-based plasmid (18), which does not contain the T7 promoter, as a competitor. First, pRW3822, which contains the (GAA-TTC)_{150} repeat in the pSPL3 vector, was linearized by XmnI, and the linear band and the retarded band were isolated in an agarose gel electrophoresis. As a negative control, the Ndel-linearized pSPL3 vector was prepared. Then 10 units of T7 RNA polymerase were preincubated with one of these linearized pSPL3-based plasmids and used for transcription of the SapI-linearized pGEM-3Zf(−) vector (Fig. 1A). The expected size of the full-length transcript was 332 nt.

The sizes of the 1-kilobase pair DNA ladder are shown on the left of each gel. The positions of the linear and the retarded band are shown on the right.
Sticky DNA Inhibits Transcription

Fig. 7. Effect of GAA\textsuperscript{2}TTC repeat length and sticky DNA on \textit{in vitro} transcription by SP6 RNA polymerase. A, effect of length of GAA\textsuperscript{2}TTC repeats on SP6 RNA polymerase transcription. A mixture of EcoRI-digested GAA\textsuperscript{2}TTC-containing plasmids (Fig. 1B, right column) and the EcoRI-digested pGEM-3Zf(--)-vector was used for transcription by 10 units of SP6 RNA polymerase, and the RNA produced was analyzed in a 4% polyacrylamide gel containing 7M urea. The number of GAA\textsuperscript{2}TTC repeats is shown at the top of the gel. 1kb, 1-kilobase pair ladder; hexamer, (GAAGGA\textsuperscript{2}TTC\textsuperscript{2})\textsubscript{150} repeat. The sizes are shown on the left, and the expected positions of full-length RNA from each template DNA are shown on the right. B, effect of the sticky DNA structure on SP6 RNA polymerase transcription. The gel-isolated linear and retarded bands were used for \textit{in vitro} transcription by 1, 10, or 50 units of SP6 RNA polymerase and analyzed in a 4% polyacrylamide gel containing 7M urea. The gel to the right shows a longer exposure of the analysis of transcription by 1 unit of SP6 RNA polymerase. The type of template DNA and the amount of SP6 RNA polymerase are shown at the top of the gel.

with the SP6 RNA polymerase from SP6 phage (38). First, we examined the effect of the GAA\textsuperscript{2}TTC repeat lengths on SP6 RNA polymerase transcription. To analyze the formation of the repeating rGAA RNA, the pGEM-3Zf(--)-vector (which were previously used to produce the repeating rUUC\textsuperscript{2}-based plasmids) were isolated (17). No inhibition of transcription was observed with an increase in the GAA\textsuperscript{2}TTC repeat length. The expected lengths of the transcripts were observed in all cases (data not shown). Also, the GAAGGA\textsuperscript{2}TTC\textsuperscript{2} hexamer repeat did not inhibit transcription as expected from the prokaryotic \textit{in vitro} (Fig. 2) and \textit{in vivo} transcription experiments (18).

The most likely explanation of the apparent inability of sticky DNA to inhibit eukaryotic transcription stems from the frequency of utilization of the DNAs as templates. In the T7 and SP6 RNA polymerase systems, each DNA template was used 2–5 times by the RNA polymerases. Alternatively, in the eukaryotic transcription system, only a small fraction (less than 1%) of all template DNAs were ever used for transcription. Thus, since it is not possible to isolate pure sticky DNA without contaminating linear DNA (Fig. 5, \textit{Isolated RB}), this presence of a small amount of linear DNA would probably be used selectively as a template in the eukaryotic transcription system.

DISCUSSION

The effect of the sticky DNA structure on transcription was investigated using the T7 and SP6 RNA polymerase systems. We found that sticky DNA inhibits bacterial \textit{in vitro} transcription effectively in an orientation-independent manner. This inhibition was observed not only in the GAA\textsuperscript{2}TTC-containing template but also in the internal control template. The molecular mechanism of this inhibitory effect is the sequestration of the RNA polymerase. However, two molecular mechanisms are possible to explain the sequestration data. One possibility is that RNA polymerase is paused within the sticky DNA structure formed by the long GAA\textsuperscript{2}TTC repeats and causes the reduction in the amount of available RNA polymerase. The other possibility is that sticky DNA itself has a property to bind RNA polymerases without transcription. Our results (Fig. 6)
supported the latter possibility. The tripod structure has been known to inhibit transcription by blocking transcription elongation or by interfering with binding of a trans-acting factor (28–35, 42). Thus, the sticky DNA exhibits a novel mechanism to inhibit transcription.

The sticky DNA structure was hypothesized to be an inter-molecular bitriplex based on R-RY triplexes (19). In this model, the structure has long single-stranded regions containing TTC repeats as well as the three-stranded regions (19). Therefore, RNA polymerase may bind to the single-stranded or the triple-stranded regions. Several factors are known to associate with either the single-stranded or the triple-stranded DNA. The *Drosophila* GAG factor is known to bind a tripod, although the affinity for a R-RY triplex (19). In this model, the structure has long single-stranded regions containing TTC triplexes (19). In this model, *the sticky DNA exhibits a novel mechanism to inhibit transcription.*

Interactions between two polypurine-polypyrimidine tracts, called a T-loop, were reported previously (46, 47). Since the formation of the T-loop requires acidic pH and the presence of spermine, this structure is probably different from sticky DNA (19). However, both the sticky DNA and the T-loop are based on the triplex configuration and contain a single-stranded and a single-stranded region. Recently, Ashley and Lee showed that the T-loops inhibit transcription by *Escherichia coli* RNA polymerase I (48). The molecular mechanism of the inhibitory effect of the T-loop is unclear, but it may be the same as found herein for sticky DNA. Namely, these structures may inhibit *E. coli* RNA polymerase I transcription by a sequestration mechanism.

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