Template Strand Switching by T7 RNA Polymerase*

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T7 RNA polymerase (RNAP) is able to traverse a variety of discontinuities in the template (T) strand of duplex DNA, including nicks, gaps, and branched junctions in which the 3′ end of the T strand is not complementary to the non-template (NT) strand. The products represent a faithful copy of the T strand, with no insertions or deletions. On double-stranded templates having protruding 3′ ends the polymerase is able to insert the free 3′ end of the NT strand and to utilize this as a new T strand (“turn around transcription”), resulting in the anomalous production of high molecular weight transcripts.

The capacity of T7 RNAP to bypass interruptions in the T strand depends upon the stability of the elongation complex. Sequences that are expected to stabilize a local RNA:DNA hybrid (such as the presence of a C6 tract in the T strand) dramatically reduce dissociation of the RNAP while still allowing the enzyme to insert a new 3′ end. Similar effects on RNAP release are observed when the enzyme reaches the end of a template (i.e. when synthesizing runoff products), resulting in markedly different yields of RNA product during multiple rounds of transcription.

T7 RNA polymerase (RNAP)† is a single subunit enzyme that is able to carry out all of the steps in the transcription process without the need for auxiliary factors (for review see Ref. 1). The crystal structure of the enzyme has been solved to a resolution of 3.5 Å (2), and it is therefore highly attractive as a model for studies of the mechanism of RNA synthesis (for review see Refs. 3 and 4).

During the early stages of transcription, T7 RNAP engages in multiple cycles of initiation in which short RNA products are synthesized and released without movement of the RNAP away from the promoter (5–9). Once the enzyme has initiated transcription and cleared the promoter, the ternary elongation complex is highly processive and is able to synthesize long RNA chains (>15,000 nt) until it encounters a termination signal or reaches the end of the template. The transition from an unstable initiation complex to a stable elongation complex (EC) is accompanied by isomerization of the complex (6, 10, 11) and may involve wrapping of a flexible “thumb” domain (12, 13) and possibly a “specificity loop” (1, 14) around one or both strands of the template, leading to partial closure of the DNA binding cleft.

The non-template (NT) strand of the DNA does not appear to be required for initiation or for the early stages of transcription, as Maslak and Martin (15) have shown that T7 RNAP can initiate transcription at a promoter that is single-stranded in the initiation region (from −6 and downstream), and Milligan et al. (7) have shown that the enzyme can transcribe a single-stranded DNA template once it has initiated transcription from a double-stranded promoter. However, Zhou et al. (16, 17) have suggested that the stability of the EC may require the presence of duplex DNA at the leading edge of the elongation complex, as an elongation complex that is halted just upstream from a region in which the NT strand is missing appears to be unable to resume transcription.

A number of studies to determine the importance of the integrity of the template (T) strand to processive transcription have been carried out. Although there are conflicting reports in the literature with regard to the ability of T7 RNAP to bypass nicks in the T strand (18, 19), Zhou et al. (16, 17) observed that T7 RNAP is able to bypass gaps of 1–24 nt, resulting in a faithful copy of the T strand in the regions preceding and following the gap. Although this result demonstrated that the enzyme is able to thread the 3′ end of a paired T strand into the active site, the RNAP was unable to switch to a free oligomer of single-stranded DNA, leading these authors to conclude that the DNA template must be double-stranded at the leading edge of the EC in order for template switching to occur (17). However, an alternate explanation for this observation is that if the downstream oligomer is not tethered in the vicinity of the EC (because it is not complementary to the NT strand) its local concentration is low, and switching to the new T strand is inefficient.

In this work, we have explored the ability of T7 RNAP to traverse nicks and gaps in the template strand and also to jump across branched junctions in which the leading or trailing edges of the T strand are not complementary to the NT strand. We found that the RNAP is able to traverse all of these discontinuities. Our results do not support a requirement that the DNA must be double-stranded at either the leading or trailing edge of the elongation complex in order for strand switching to occur but suggest an important role for the NT strand in tethering the T strand in the vicinity of the EC.

EXPERIMENTAL PROCEDURES

DNA Templates and RNA Polymerase—DNA oligomers were synthesized by Macromolecular Resources (Colorado State University) and purified by low pressure reverse phase chromatography. To prepare synthetic templates, the indicated combinations of oligomers were mixed together (final concentration 0.5 μM, each oligomer) in 40 μL of GHT buffer (30 mM K-HEPES, pH 7.8, 100 mM potassium glutamate, 15 mM Mg(OAc)₂, 0.25 mM EDTA, 0.05% Tween 20 (20)), and the samples...
were heated to 70 °C for 10 min and cooled slowly to room temperature (2–3 h). The templates were either used immediately or stored at −20 °C.

T7 RNAP having a His₆-amino-terminal-lead (encoded by pBH161) was purified as described previously (21). All plasmids were constructed by standard procedures (22).

**Transcription Assays**—Unless otherwise noted, transcription was carried out in a volume of 10 μl in GHT buffer (see above) containing 0.5 mM ATP, CTP, GTP, and UTP (Amersham Pharmacia Biotech Ultrapure); 2 μCi of [α-32P]ATP or GTP (specific activity of 800 Ci/mmol; NEN Life Science Products); 10–20 ng RNA polymerase, and 50 ng synthetic DNA or 1 μg of plasmid DNA as template. Reactions in which ITP replaced GTP contained 0.5 mM ITP and 2 mM GMP to allow efficient initiation (23). Reactions were incubated at 37 °C for 10 min and terminated by the addition of 10 μl of stop buffer, and the products were resolved by electrophoresis in polyacrylamide gels containing 7 M urea (21). The radioactivity in each electrophoretic species was quantified by exposing the gel to a PhosphorImager™ screen (Molecular Dynamics) using a Storm 860 scanner and ImageQuant version 4.2a software (Molecular Dynamics). The efficiency of gap or branch jumping was calculated as follows: efficiency = (long runoff product/short runoff product + long runoff product) taking into account the base composition of individual transcripts.

**RNA Sequence Determination**—Transcription reactions were carried out as described above except that the volume was increased to 100 μl, and 10 units of RNase inhibitor (Boehringer Mannheim) were added. After 1 h at 37 °C, samples were treated with 1 unit of DNase (RNase-free, Promega) for 20 min and extracted with phenol and chloroform. RNA was precipitated at −70 °C in the presence of 70% isopropanol and 0.3 M sodium acetate and resolved by electrophoresis in a 1 M sodium acetate in TE (0.01 M Tris-HCl, pH 7.4; 0.1 mM EDTA), and incubated at 37 °C for 10 min and terminated by the addition of 10 μl of stop buffer, and the products were resolved by electrophoresis in polyacrylamide gels containing 7 M urea (21). The radioactivity in each electrophoretic species was quantified by exposing the gel to a PhosphorImager™ screen (Molecular Dynamics) using a Storm 860 scanner and ImageQuant version 4.2a software (Molecular Dynamics). The efficiency of gap or branch jumping was calculated as follows: efficiency = (long runoff product/short runoff product + long runoff product) taking into account the base composition of individual transcripts.

**Template Strand Switching by T7 RNA Polymerase**—The ability of the polymerase to jump across a branch in the template (in which the 3’ end of the T strand at the distal edge of the discontinuity is not complementary to the NT strand) was examined as shown in Fig. 1C. In this experiment, T strand oligomers having unpaired 3’ tails 4–16 nt long were positioned either immediately after the 5’ end of the upstream T strand oligomer (lanes 9, 11, 13, 15, and 17) or after a gap of 1 nt (lanes 10, 12, 14, 16, and 18). The polymerase is able to transcribe through all of these junctions, resulting in the synthesis of transcripts that are longer than the product from a fully double-stranded template (lane 1, 47 nt) by an increment that corresponds to the length of the branch minus the size of the gap (if present). The efficiency of branch jumping drops with increasing length of the branch, but a plot of the data (Fig. 1D) suggests that a basal level of branch jumping may remain even with longer branches. Further experiments with templates having longer branches will be required to confirm this.

The products arising from transcription across the branch junctions were discrete and homogeneous and of a size expected for an exact copy of the branched T strand. The accuracy of transcription across such a junction was confirmed by sequencing a cDNA copy of the “jump” transcript produced from template 11, which has a branch of 8 nt. The results demonstrate that the transcript represents an accurate copy of the T strand on either side of the discontinuity and that T7 RNAP does not insert or delete nucleotides when it traverses such a junction (Fig. 2).

**Anomalous Transcription of Templates with Protruding 3’ Ends Is Due to Branch Jumping**—T7 RNA polymerase is known to synthesize anomalous, high molecular weight products from templates that have protruding 3’ ends (24). This phenomenon depends upon the structure at the termini, but not the sequence (24–26), and is not due to initiation at the ends of the DNA, as it requires the presence of a promoter in the template (24, 26). In view of the finding that the RNAP is able to insert a free 3’ end when it reaches the end of a T strand, we asked whether the synthesis of these unusual products might arise from the same phenomenon (Fig. 3). Although transcription of templates that have a blunt end results in the synthesis of the expected runoff products (lanes 1 and 2), transcription of templates that have a protruding 3’ end gives rise to additional RNAs that would result from insertion of the 3’ end of the NT strand and subsequent transcription of that strand (lanes 3–5). If the terminus of the template upstream from the promoter has a blunt or recessed 3’ end, the process ceases there (lanes 4 and 5, 71 nt). However, if the upstream terminus has a protruding 3’ end (lane 3) the polymerase can again insert the free end, re-transcribing the template strand. Repeated cycles of transcription around the DNA by this mechanism (“turn around transcription”) result in the synthesis of a ladder of products and the accumulation of high molecular weight RNAs that do not enter the gel. As noted elsewhere, the production of these spurious products can be avoided through the use of T7 RNAP mutants such as del172-3 (25, 26).

**Effects of G·C Tracts on Branch Jumping and Runoff Transcription**—In the experiment described in Fig. 1, a C₆ tract was
included in the T strand to enhance the formation of a stable DNA duplex in the vicinity of the discontinuity when paired with a complementary G:C tract in the NT strand. To determine how the presence of this G:C “clamp” affects transcription, we positioned the G:C tract either before or after the discontinuity (Fig. 4). In a completely duplex DNA template (one having no discontinuities in the T strand) only full-length runoff products (47 nt) were synthesized, with no evidence of pausing near or within the G:C tract (Fig. 4B, lane 1). When the G:C tract was placed downstream from the discontinuity (as in Fig. 1) the characteristic production of short RNAs (24 nt) that terminate at the proximal side of the discontinuity was observed, along
Template Strand Switching by T7 RNA Polymerase

Table I

| Number | IDa | Sequenceb |
|--------|-----|-----------|
| 1      | MR3 | 5’TGGAAAAATTTATAGGACCTCTATCTTATAGGAGACCACATAACCTTTGTCCTTG3’ |
| 2      | MR2 | 5’CTGAACTATTTATAGGACCTCTATCTTATAGGAGACCACATAACCTTTGTCCTTG3’ |
| 3      | MR37| 5’TGGAAAAATTTATAGGACCTCTATCTTATAGGAGACCACATAACCTTTGTCCTTG3’ |
| 4      | MR38| 5’TGGAAAAATTTATAGGACCTCTATCTTATAGGAGACCACATAACCTTTGTCCTTG3’ |
| 5      | MR39| 5’TGGAAAAATTTATAGGACCTCTATCTTATAGGAGACCACATAACCTTTGTCCTTG3’ |
| 6      | MR40| 5’TGGAAAAATTTATAGGACCTCTATCTTATAGGAGACCACATAACCTTTGTCCTTG3’ |
| 7      | MR41| 5’TGGAAAAATTTATAGGACCTCTATCTTATAGGAGACCACATAACCTTTGTCCTTG3’ |
| 8      | MR42| 5’TGGAAAAATTTATAGGACCTCTATCTTATAGGAGACCACATAACCTTTGTCCTTG3’ |
| 9      | MR43| 5’TGGAAAAATTTATAGGACCTCTATCTTATAGGAGACCACATAACCTTTGTCCTTG3’ |
| 10     | MR44| 5’TGGAAAAATTTATAGGACCTCTATCTTATAGGAGACCACATAACCTTTGTCCTTG3’ |
| 11     | MR45| 5’TGGAAAAATTTATAGGACCTCTATCTTATAGGAGACCACATAACCTTTGTCCTTG3’ |
| 12     | MR46| 5’TGGAAAAATTTATAGGACCTCTATCTTATAGGAGACCACATAACCTTTGTCCTTG3’ |
| 13     | MR47| 5’TGGAAAAATTTATAGGACCTCTATCTTATAGGAGACCACATAACCTTTGTCCTTG3’ |
| 14     | MR48| 5’TGGAAAAATTTATAGGACCTCTATCTTATAGGAGACCACATAACCTTTGTCCTTG3’ |
| 15     | MR49| 5’TGGAAAAATTTATAGGACCTCTATCTTATAGGAGACCACATAACCTTTGTCCTTG3’ |
| 16     | MR50| 5’TGGAAAAATTTATAGGACCTCTATCTTATAGGAGACCACATAACCTTTGTCCTTG3’ |
| 17     | MR51| 5’TGGAAAAATTTATAGGACCTCTATCTTATAGGAGACCACATAACCTTTGTCCTTG3’ |
| 18     | MR52| 5’TGGAAAAATTTATAGGACCTCTATCTTATAGGAGACCACATAACCTTTGTCCTTG3’ |
| 19     | MR53| 5’TGGAAAAATTTATAGGACCTCTATCTTATAGGAGACCACATAACCTTTGTCCTTG3’ |
| 20     | MR54| 5’TGGAAAAATTTATAGGACCTCTATCTTATAGGAGACCACATAACCTTTGTCCTTG3’ |
| 21     | MR55| 5’TGGAAAAATTTATAGGACCTCTATCTTATAGGAGACCACATAACCTTTGTCCTTG3’ |
| 22     | MR56| 5’TGGAAAAATTTATAGGACCTCTATCTTATAGGAGACCACATAACCTTTGTCCTTG3’ |
| 23     | MR57| 5’TGGAAAAATTTATAGGACCTCTATCTTATAGGAGACCACATAACCTTTGTCCTTG3’ |
| 24     | MR58| 5’TGGAAAAATTTATAGGACCTCTATCTTATAGGAGACCACATAACCTTTGTCCTTG3’ |
| 25     | MR59| 5’TGGAAAAATTTATAGGACCTCTATCTTATAGGAGACCACATAACCTTTGTCCTTG3’ |
| 26     | MR60| 5’TGGAAAAATTTATAGGACCTCTATCTTATAGGAGACCACATAACCTTTGTCCTTG3’ |
| 27     | MR61| 5’TGGAAAAATTTATAGGACCTCTATCTTATAGGAGACCACATAACCTTTGTCCTTG3’ |

aLaboratory reference number.
bSequences that are not complementary to MR37 are indicated in bold; homopolymeric tracts >6 nt are underlined.

![Diagram](image1)

Fig. 2. Accuracy of transcription across a branch junction.

Transcripts arising from templates 1 or 11 (see Fig. 1A) were copied into cDNA using Moloney murine leukemia virus reverse transcriptase, amplified by polymerase chain reaction, and cloned into a plasmid vector. The sequence of the cDNA in the region corresponding to the branch junction was determined from two separate clones from each template and is shown in brackets. The results demonstrate that the RNA synthesized from this template (which includes a branch of 8 nt) is a faithful copy of the upstream and downstream T strands, with no insertions or deletions (see Table I for sequences of the two T strand DNA oligomers).

![Diagram](image2)

Fig. 3. Strand switching at protruding 3’ ends results in anomalous production of high molecular weight products. Templates constructed by annealing the synthetic oligomers indicated were transcribed by T7 RNAP, and the products were analyzed as described in Fig. 1. The lane numbers above the autoradiogram refer to the templates in the left panel; transcripts are identified by length in the margin. Note that the presence of a protruding 3’ end on the NT strand in templates 3–5 results in the synthesis of transcripts that are larger than 15 nt, the size of the RNA that is expected from transcription to the end of the T strand (cf. template 2). Repeated strand switching on a template that has protruding 3’ termini at both ends (template 3) results in the synthesis of high molecular weight (HMW) RNA that fails to enter the gel.

with production of the jump products (47 or 51 nt; Fig. 4B, lanes 2 and 3). In contrast, when the G:C clamp was placed prior to the discontinuity, production of the short RNA was not detected, whereas synthesis of full-length runoff (jump) products continued to be observed (Fig. 4B, lanes 4 and 5). Under the latter conditions, the apparent efficiency of branch and gap jumping approaches 100%, as the jump transcripts are practically the only products observed.

The results above suggest that the presence of a G:C clamp at the end of a template stabilizes the ternary complex, preventing dissociation of the RNAP while still allowing branch or gap jumping to occur. To examine this, we incubated RNAP with templates that either had a terminal G:C clamp or did not
and then determined the availability of free enzyme by the subsequent addition of a second DNA template (Fig. 4C). We found that the presence of a G:C clamp at the terminus of the primary template inhibited the production of runoff transcripts from this template (24 nt) and greatly diminished the synthesis of products from the second template (83 nt; lane 7). This phenomenon was not observed with templates that lacked a terminal G:C clamp (lanes 1 and 6). The low level of RNA synthesis observed in the presence of the template having a G:C clamp is not due to a failure of this template to initiate RNA synthesis or to a general inhibition of the RNAP, as this template directs the synthesis of equivalent amounts of poly(G) products when GTP is present as the sole substrate (Fig. 4D).

Under these conditions, repeated cycles of initiation and slippage of the nascent RNA result in the characteristic formation of a ladder of products 2–14 nt in length (5). This result demonstrates that the template is fully competent to carry out the early stages of transcription and that the deficit in RNA synthesis occurs only later in transcription, when the RNAP has reached the end of the template.

A possible explanation for this effect is that the formation of a stable RNA:DNA hybrid at the end of the template stabilizes the transcription complex and prevents enzyme turnover. To examine this, we determined the effect of substituting ITP for GTP in the transcription reaction (as I:C base pairs are less stable than G:C base pairs). As shown in Fig. 5, substitution of ITP for GTP resulted in increased release of short products (30 nt) from a template having a G:C clamp positioned before a branch junction while still allowing branch jumping to occur (lanes 4 and 5).

Effects of Alternate Structures at the DNA Terminus on Product Yield—In view of the observations above, we explored the effects of other variations in structure and sequence at the ends of a DNA template on the yield of RNA products (Fig. 6). In these experiments, the activity of templates that ended in various homopolymeric tracts was compared with that of a control template that ended with the sequence 5'-GACTAC-3' (in the NT strand), either when the T strand was complementary to the NT strand or was unpaired. As before, the presence of a C6 tract in the T strand dramatically reduced the yield of transcripts, whether paired with a G6 tract in the NT strand (lane 2) or not (lane 5). In contrast, the presence of an A6 tract in the T strand stimulated product yield (lanes 3 and 6), consistent with the lower stability of A:T base pairs as compared with G:C base pairs.

The presence of an A6 tract in the T strand resulted in premature release of products and a lower overall yield as compared with results obtained with a T6 tract (compare lanes 4 and 7 with lanes 3 and 6). Although the release of the transcripts prior to reaching the end of the template is consistent with the hypothesis that the formation of a stable RNA:DNA hybrid at the terminus of the template inhibits runoff transcription.
ent with the remarkably low stability of rU:dA base pairs (27), the decreased yield of product is more difficult to explain. It has been observed that during the early stages of initiation T7 RNAP aborts transcription more frequently before or after incorporating a UMP residue, and it has been suggested that the weak interactions of the rU:dA base pair may hinder the correct orientation of the base in the active site, resulting in slower catalysis (5, 8). Thus, even though dissociation of the transcription complex would be enhanced when transcribing through a poly(dA) tract, the increased time required for the polymerase to reach the point of release would result in a slower turnover rate and hence a lower yield.

DISCUSSION

We have shown that T7 RNAP is able to bypass a variety of interruptions in the template strand and that the sequence at the end of the strand affects the stability of the complex and its ability to traverse the discontinuity. Our observation that the enzyme can cross gaps of 1–2 nt in the T strand is in agreement with a previous report demonstrating jumping of gaps up to 24 nt in length (17). However, in that report, the authors concluded that template switching by T7 RNAP occurred only if the 3' end of the T strand was complementary to the NT strand (i.e., was present in the form of a DNA duplex). In this work, we have shown that T7 RNAP may also insert the 3' end of an unpaired T strand when it is located in a branch junction. Under these circumstances, the 3' end of the T strand is present at a high local concentration (as it is tethered to the EC via its complementarity to the downstream region of the NT strand), and this may account for failure on the part of previous investigators to detect jumping of T7 RNAP to a second molecule added in trans. We have also found that T7 RNAP is able to traverse branch junctions in which the T strand is unpaired before the discontinuity (i.e., has a 5' tail) or in which there are unpaired regions on both sides of the discontinuity (having both 3' and 5' tails), although with reduced efficiency in the latter case (30). In contrast to these results, Nudler et al. (28) have reported that Escherichia coli RNAP may also switch to a new template strand requirement for double-stranded DNA for template switching by T7 RNAP suggests that there may be important structural and functional differences between the bacterial and phage enzymes.

There are conflicting observations regarding the importance of the NT strand to the processivity and stability of the T7 RNAP elongation complex. Some experimental results suggest that the polymerase sequesters primarily the T strand and that a continuous encirclement of both strands may not be required for processivity. For example, polymerases that approach one another from opposite directions over the same transcription region are able to pass one another with no apparent pausing or termination, and we have observed processive transcription on single-stranded templates over distances as great as 800 nt (29). Furthermore, cross-linking of psoralen mono-adducts or of psoralen-derivatized oligonucleotides inhibits transcription.

2 B. He, A. Kukarin, D. Temiakov, S. T. Chin-Bow, D. L. Lyakhov, M. Rong, R. K. Durbin, and W. T. McAllister, submitted for publication.
by T7 RNApol when the cross-link is to the T strand but not to the NT strand (31, 32), and we have observed similar results in the case of a protein cross-linked to the T or NT strands.\(^3\)

Although these results indicate that the presence of the NT strand is not required for the stability (and hence processivity) of an actively transcribing complex, Zhou et al. (17) reported that an elongation complex that is halted just upstream from a region in which the NT strand is missing is unable to resume transcription when the remaining substrates are added and suggested that the NT strand may be required for the stability of such a complex. In work to be reported elsewhere, we have found differences in the stabilities and properties of halted complexes versus actively transcribing complexes, and we have shown that the presence of the NT strand is more important to the stability of the former complexes than to the latter.\(^4,5\)

The effects of various configurations and sequences at the ends of the DNA template on product yield and enzyme release indicate that the stability of an RNA:DNA hybrid is important in maintaining the association of the ternary complex and that under certain circumstances product release and complex dissociation may be rate-limiting. Thus, for example, we have found that a terminal C\(_6\) tract in the T strand inhibits dissociation and that this effect may be relieved by substituting ITP for GTP in the reaction. We have observed similar effects on templates that end in a variety of G:C-rich sequence contexts, indicating that it is the stability of the RNA:DNA hybrid, and not its sequence, that causes this effect (30).

Due to the particularly low stability of rU:dA base pairs (27), the premature dissociation of complexes observed at the end of a template having a terminal A\(_8\) tract in the T strand (Fig. 6) is expected. However, the lower yield of transcripts from such a template is not consistent with a lowered hybrid stability (which should increase the turnover rate and hence product yield). As noted above, a possible explanation for this phenomenon is that the low stability of rU:dA base pairs may result in a decreased rate of elongation, thus increasing the time required to synthesize an rU:dA hybrid long enough to destabilize the complex and promote dissociation. In exploring this phenomenon, we examined the effects of a longer dA tract at the end of the T strand (an A\(_{10}\) tract) and found that very few polymerases were able to reach the end of this template (Fig. 6, lane 11). Under these conditions the longest products synthesized appeared to have incorporated only 8 U residues, suggesting that this is the maximum length of an extended rU:dA hybrid that the enzyme can tolerate when approaching the end of the DNA or that the enzyme slips to the end of the template after the incorporation of 7–8 consecutive UMP residues (33).

In previous work, we examined the effects of extended poly(dA) tracts in the T strand when these are embedded within a transcription unit (i.e., are not located at the end of the template) and found that they destabilize T7 RNApol, sometimes causing termination but also resulting in slippage of the transcription complex on the template (33). These effects were greatly enhanced at lower concentrations of UTP, and under these conditions the slippage products were observed to approach a limit size that would correspond to the incorporation of 8–10 consecutive UMP residues (33). These observations may have relevance to the process of termination. Class I termination signals for T7 RNApol (e.g., T\(_6\)) as well as rho-independent termination signals for E. coli RNApol encode RNAs with a stable stem-loop structure followed by a run of uridylate residues. It seems likely that these U runs function both to destabilize the transcription complex as well as to slow the passage of the enzyme through the signal (34).

The picture that emerges from these and other studies of T7 RNApol is of a transcription complex that is held together by dynamic interactions between the RNApol, the DNA template, and the RNA product. As the enzyme approaches the end of the template strand, potential contacts with downstream regions of the template are lost, resulting in decreased stability of the complex.\(^6\) At this point, the stability of the RNA:DNA hybrid becomes critical to the continued maintenance of the complex. If the strength of the hybrid is high (e.g., in a G:C-rich sequence context) the complex is slow to dissociate, allowing the enzyme to position a new T strand 3′ end (if available) in the active site and to resume transcription. If the strength of the hybrid is low (e.g., in an rU:dA context), dissociation is more likely to occur before the enzyme can resume transcription.

Under normal conditions of transcription in which the enzyme is highly processive (i.e., the presence of an intact duplex DNA template downstream, the absence of a pause or termination signal, and non-limiting concentrations of substrate), changes in the strength of the local RNA:DNA hybrid generally do not decrease the stability of the EC below the threshold that would result in dissociation. Thus, the RNApol does not usually terminate in extended T:A-rich regions nor does it pause in G:C-rich tracts (see Ref. 33 and this work). The contribution of the RNA:DNA hybrid to complex stability is likely to become of particular importance when the EC is destabilized or is halted for some reason (e.g., upon reaching the end of the template, or in the context of a pause/termination signal (35))\(^5\). In work to be reported elsewhere, we have found that the structure and stability of the T7 transcription complex is dynamically determined and that the properties of a halted EC are quite different from an EC that is involved in active transcription.\(^4,5\) Efficient release of the complex at the ends of the template is stimulated in the presence of T7 lysozyme (which enhances recognition of certain types of termination signals (26, 35)) or by the use of T7 RNApol mutants with altered termination properties.\(^6\)

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