Weakening of the T7 Promoter-Polymerase Interaction Facilitates Promoter Release

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During transcription initiation, RNA polymerases retain interactions with their promoters until the RNA is extended to 8–13 nucleotides, at which point the polymerase releases the promoter and moves downstream. It has been shown that release of the T7 promoter is inhibited when the T7 RNA polymerase-promoter interaction is strengthened. Here we asked whether release would be facilitated when the T7 promoter-polymerase interaction is weakened by the introduction of promoter mutations known to reduce promoter activity. Using chemical and enzymatic probes to monitor the position of the polymerase as a function of RNA length, we found that promoter mutations upstream of −4 facilitated release of the polymerase from the promoter, but more downstream mutations did not have such effects. We also found that released complexes turn over more slowly than promoter-bound complexes, indicating that retention of promoter interactions contributes to the dissociation of short RNAs during initial transcription.

T7 RNA polymerase (RNAP) initiates transcription by a process in which it specifically binds to a consensus class III T7 promoter that contains a 23-base pair sequence extending from −17 to +6, melts duplex DNA in the vicinity of the initiation site to form a transcription bubble, and begins RNA synthesis (reviewed in Ref. 1). This initiation process is remarkably similar to that of the multidomain RNAPs from prokaryotic, eukaryotic, and archaeal species (2). When the RNA is <9 nt in length, the T7 initiation complex (IC) is relatively unstable, and short nascent transcripts are continuously synthesized and released. The transition from unstable IC to stable elongation complex (EC) starts when the RNA reaches a length of 8–9 nt and results in promoter release, collapse of the melted promoter region, and displacement of the 5′-end of the nascent RNA (3).

The crystal structure of a T7RNAP IC with a 3-nt transcript reveals that the promoter interaction involves three distinct structural motifs of the T7 RNAP (4, 5). Two of these participate directly in binding the −17 to −5 region of the promoter, which remains duplex throughout the initiation reaction. Residues 93–101 of T7 RNAP recognize the AT-rich −17 to −13 region of the promoter, whereas the promoter recognition hairpin loop (residues 739–770) binds in the major groove of the −11 to −7 region of the promoter. The third motif comprises an intercalating hairpin (residues 231–241) that melts the promoter and stabilizes the upstream edge of the initiation bubble between −5 and −4. The melted template (T) strand downstream of −5 is bound to the palm and finger subdomains by numerous contacts, whereas the single-stranded segment of the non-template (NT) strand downstream of −4 is disordered. The downstream DNA is bent and fit into the enclosed active site pocket and forms three base pairs of RNA-DNA heteroduplex. In the EC, a major conformational change in the N-terminal domain (residues 1–310) allows RNA-DNA hybrid extension to eight base-pairs and disrupts the specific promoter contacts observed in the IC (6–8). Previous biochemical studies (9–11) have shown that the transition from IC to EC usually entails polymerase release from the promoter, although it has been shown that the polymerase can retain promoter contacts and still synthesize long transcripts. The latter can occur if the polymerase is covalently tethered to the promoter (12), or if the promoter-polymerase interaction is strengthened by eliminating the free energy penalty of promoter melting by deletion of the NT strand around the initiation site (13).

The observation that strengthening of the promoter-polymerase interaction inhibits promoter release led us to ask whether weakening of this interaction by mutating the promoter would facilitate release. For example, would it allow the polymerase to release the promoter when the RNA is only 6 or 7 nt in length, rather than the 8 or 9 nt at which release begins on a consensus promoter? Would mutations in the different regions of the promoter (the −17 to −5 binding element, the −4 to −1 melting element, and the +1 to +6 initially transcribed sequence (ITS)) affect release in similar ways? Finally, if complexes release the promoter while the RNA is still relatively short, would they be stable or would they turn over rapidly? Would retention of promoter contacts favor dissociation of the RNA from the IC, or would such dissociation be due solely to the short length of the RNA in these complexes?

To address these questions, we made mutants in different regions of the promoter and examined release using enzymatic footprinting and a chemical nuclease tethered to residue 207 of the polymerase, which lies close to the upstream region of the promoter (−9 to −15) in the IC. Our results indicated that mutations upstream of −5 do facilitate release of the polymerase from the promoter. In contrast, it was found that promoter mutations downstream of −5 do not facilitate release. We also found that released complexes turn over less rapidly than complexes that retain promoter interactions. Thus, retention of promoter contacts contributes significantly to the rapid rate of RNA dissociation from the IC.
Weak Promoter Interaction Facilitates Release

**Table I.** Sequences of promoter DNAs used

| Promoters   | Non-template strand sequence |  |
|-------------|------------------------------|---|
| WT          | CCGAAATTAAGCCTACTATAGGAGAGACTGGAATCGAATGCGACGACGCTGATGCA |  |
| A15G        | CCGAAATTAAGCCTACTATAGGAGAGACTGGAATCGAATGCGACGACGCTGATGCA |  |
| T7G         | CCGAAATTAAGCCTACTATAGGAGAGACTGGAATCGAATGCGACGACGCTGATGCA |  |
| A15G        | CCGAAATTAAGCCTACTATAGGAGAGACTGGAATCGAATGCGACGACGCTGATGCA |  |
| C1G         | CCGAAATTAAGCCTACTATAGGAGAGACTGGAATCGAATGCGACGACGCTGATGCA |  |
| A10C        | CCGAAATTAAGCCTACTATAGGAGAGACTGGAATCGAATGCGACGACGCTGATGCA |  |
| A10C        | CCGAAATTAAGCCTACTATAGGAGAGACTGGAATCGAATGCGACGACGCTGATGCA |  |
| CST         | CCGAAATTAAGCCTACTATAGGAGAGACTGGAATCGAATGCGACGACGCTGATGCA |  |
| C5G         | CCGAAATTAAGCCTACTATAGGAGAGACTGGAATCGAATGCGACGACGCTGATGCA |  |
| T4A         | CCGAAATTAAGCCTACTATAGGAGAGACTGGAATCGAATGCGACGACGCTGATGCA |  |
| A3G         | CCGAAATTAAGCCTACTATAGGAGAGACTGGAATCGAATGCGACGACGCTGATGCA |  |
| G+1C        | CCGAAATTAAGCCTACTATAGGAGAGACTGGAATCGAATGCGACGACGCTGATGCA |  |
| G+2C        | CCGAAATTAAGCCTACTATAGGAGAGACTGGAATCGAATGCGACGACGCTGATGCA |  |

**Experimental Procedures**

RNA Polymerase and Templates—Wild-type and mutant T7 RNAP were purified as described previously (7) and stored in 20 mM Tris-HCl (pH 8.0), 0.5 mM NaCl, 5 mM dithiothreitol, 1 mM EDTA, 50% glycerol. The E207C (−) mutant was constructed by cysteine substitution of Glu-207 in a T7 RNAP in which 7 of the 12 endogenous cysteines had been mutated to serines using PCR-mediated mutagenesis as described previously (7, 14). All DNA oligomers were from Qiagen Corp. and were purified by PAGE. The sequences of all oligomers are presented in Table I. Where indicated, synthetic oligomers were labeled at the 5'-end with [γ-32P]ATP (4000 Ci/mmol, ICN) by T4 polynucleotide kinase (Invitrogen). Maxam-Gilbert G → A ladders of labeled wt T and NT strands were prepared and used as a reference to identify the cleavage positions on DNA templates (14). Double-stranded DNA templates were formed by mixing equimolar amounts of each complementary oligomer in an annealing buffer (20 mM Tris-HCl, pH 8.0, 10 mM NaCl, 5 mM NaCl, 10 mM NaCl, 6 mM MgCl2, 5 mM dithiothreitol, 2 mM Tris-HCl, pH 8.0, 10 mM NaCl, 5 mM dithiothreitol, 2 mM Spermidine, 0.01% Triton X-100. NTPs were added to 0.5 mM, and transcripts were labeled by inclusion of 1% (v/v) 3000 Ci/mmol, 10 mM [α-32P]GTP in the reaction mixture. To form halted complexes, required NTPs with 3'-end terminating nucleotides (3'dNTP, Trilink Biotechnologies) were added to 0.5 mM in the reaction mixture. Reactions were terminated by the addition of an equal volume of stop buffer (90% formamide, 50 mM EDTA, and 0.01% xylene cyanol) and then treated at 90°C for 2 min and chilled in an ice bath. The transcription products were analyzed by electrophoresis in 25% polyacrylamide (22.5% acrylamide, 2.5% bisacrylamide) gels cast in 1× Tris-borate-EDTA buffer containing 7 M urea. The gels were analyzed with a Molecular Dynamics phosphorimaging device.

**DNase I Footprinting and exo III Digestion Assays**—Halted complexes were formed as described above. DNase I footprinting and exonuclease III (exo III) digestion on DNase I reactions containing 10 mM Tris-HCl, pH 8.0, 10 mM NaCl, 6 mM MgCl2, 5 mM dithiothreitol, 2 mM Spermidine, 0.01% Triton X-100. NTPs were added to 0.5 mM, and transcripts were labeled by inclusion of 1% (v/v) 3000 Ci/mmol, 10 mM [α-32P]GTP in the reaction mixture. To form halted complexes, required NTPs with 3'-end terminating nucleotides (3'dNTP, Trilink Biotechnologies) were added to 0.5 mM in the reaction mixture. Reactions were terminated by the addition of an equal volume of stop buffer (90% formamide, 50 mM EDTA, and 0.01% xylene cyanol) and then treated at 90°C for 2 min and chilled in an ice bath. The transcription products were analyzed by electrophoresis in 25% polyacrylamide (22.5% acrylamide, 2.5% bisacrylamide) gels cast in 1× Tris-borate-EDTA buffer containing 7 M urea. The gels were analyzed with a Molecular Dynamics phosphorimaging device.

A Weak Binding Promoter Releases Polymerase Early—To determine whether weakening of the promoter-polymerase interaction would facilitate release, we compared release on a wt promoter to one in which the −15 A:T base pair had been mutated to G:C. This mutation occurs in a region important for polymerase binding (4), and has been previously shown to reduce promoter utilization by 10-fold (15). We therefore expected that this mutant would bind polymerase less strongly than a wt promoter, but would still be a template for transcription. Fig. 1A shows an experiment in which release was measured by DNase I protection. Lanes 1–7 show experiments in which a wt template was used and in which digestion was carried out with either no polymerase present (lane 1) or with polymerases and NTPs allowing RNA extension from 2 to 8 nt (lanes 2–7). Fig. 1A, lanes 8–14, repeats this experiment with the A15G promoter. With the wt promoter, protection extended from the upstream region of the promoter to +9 for RNAs 2–4 nt in length (Fig. 1A, lanes 2–5) and to +11 for RNAs 6 or 7 nt in length (lanes 5 and 6). Upon extension of the RNA to 8 nt, protection of the upstream region of the promoters is lost, and the footprint extends from −3 to +11 (Fig. 1A, lane 7). For the A15G mutant, protection in the 2-nt RNA reaction was negligible (Fig. 1A, lane 9), suggesting that this weak promoter does not stably bind the polymerase when the RNA is only 2 nt in length. For reactions in which RNAs are 3 or 4 nt long, protection is observed from −3 to +9 (Fig. 1A, lanes 10 and 11), but unlike what is seen with the wt template, protection of the upstream region of the promoter is not observed (lane 10) or is weak (lane 11). For reactions with 6–8-nt RNAs, protection extends from −3 to +14 (Fig. 1A, lanes 12–14).

In Fig. 1B, this experiment was repeated using exonuclease III to assess release. The results were broadly similar to what was seen with DNase I protection. For the wt promoter, a block to exo III digestion at −20 is seen with RNAs 2–7 nt in length (Fig. 1B, lanes 3–7), although the block is weak for the 2-nt RNA reaction (lane 3) and is diminished in the 7-nt RNA reaction (lane 7), where we also detected the appearance of a weak block at −5 to −7. Upon RNA extension to 8 nt, the −20 block disappears completely and a strong block is seen at −5 (Fig. 1B, lane 8). On the A15G template, a block at −20 is not detected, except in the 4-nt RNA reaction (Fig. 1B, lane 12) and (very weakly) in the 2-, 3-, and 6-nt reactions (lanes 10, 11, 13). However, blocks are seen at −7 in the 3-nt reaction (Fig. 1B, lane 11) and at −5 to −7 (7-nt reaction, lane 14) and −5 in the 8-nt RNA reaction (lane 15).

The DNase I and exo III results suggest that, on the wt template, most of the polymerase molecules remain bound to the promoter as the RNA is extended from 2 to 7 nt, providing DNase I protection of the upstream promoter region and creating a block to exo III at −20. Upon extension of the RNA to 8 nt, most of the polymerase molecules are released, and protection of the upstream promoter region from DNase I and the exo III block at −20 disappear simultaneously (a fraction of the polymerases may release when the RNA is 7 nt in length).
contrast, on the A15G template, (partial) protection of the upstream promoter region and a −20 block to exo III is seen only when the RNA is 4 nt in length, although protection of downstream promoter regions is seen with RNAs 3–8 nt in length, and downstream blocks to exo III are seen with RNAs 3, 7, or 8 nt in length.

Both DNase I and exo III are potentially perturbing reagents. In particular, exo III overdigestion could “push” the polymerase off the promoter. We have observed such pushing upon extended exo III digestion of both ICs and ECs (9, 16). We therefore also tested the use of a polymerase with a chemical nuclease (FeBABE) tethered to an amino acid near the upstream region of the promoter. We have previously found that cleavage by this reagent is complete in 5 s or less (the limit of manual mixing; Ref. 17), so it should report on the instantaneous occupancy of the promoter by the polymerase. Further, because strong cleavage requires proximity of the nuclease to the DNA, this method should be directly sensitive to movement of the polymerase away from the promoter.

Fig. 2A shows the structures of the T7RNAP IC and EC, with the template (T) and non-template (NT) strands in blue and cyan, respectively, and the residue selected for mutation to Cys and conjugation with FeBABE highlighted in magenta (Glu-207). In the IC, this residue is close to the −9 to −12 region of the NT strand and the −12 to −15 region of the T strand, both of which are highlighted in yellow in Fig. 2A. In the EC, conformational changes in the polymerase and movements of the DNA place residue 207 far from the nucleic acids. Fig. 2B, lanes 2–15 and 16–29, shows cleavage reactions carried out with a polymerase in which FeBABE was conjugated to residue 207 and for RNAs extending from 2 to 8 nt in length (as indicated above each gel lane). Reactions were carried out with labeled T strand (Fig. 2B, lanes 1–15) and NT strand (lanes 16–30) on both wt (lanes 1–8 and 16–22) and A15G (lanes 9–15 and 23–30) promoters. On both promoters, cleavage is observed between −12 and −15 and −9 to −12 on the T and NT strands, respectively, in agreement with the proximity and accessibility of these regions to residue 207 in the IC crystal structure. On the wt template, cleavage is relatively weak in the reaction with the 2-nt RNA (Fig. 2B, lanes 3 and 17), is strong for reactions with 4–6-nt RNAs (lanes 4–6 and 18–20), and then begins to diminish as the RNA is extended to 7 or 8 nt (lanes 7, 8, 21, 22). The intensity of cleavage (in arbitrary units) is presented in Fig. 2C. For the A15G template, cleavage intensity is ~75% of that seen with the wt template in the reactions with 3- and 4-nt RNAs (Fig. 2B, lanes 11, 12, 25, 26) but is reduced, respectively, to 60 and 40% of wt for the 2- and 6-nt reactions (lanes 10, 13, 24, 27), and in the 7- and 8-nt reactions, cleavage on A15G is 30 and 10%, respectively, of that seen with the wt promoter (lanes 14, 15, 28, 29). Overall the FeBABE cleavage, DNase I, and exo III results agree that the polymerase is released earlier from the A15G promoter than from the wt template, although the different methods exhibit quantitative differences in their measures of the fraction of polymerases released at different RNA lengths.

Promoter Release Stabilizes the Transcription Complex—Once a transcription complex extends the RNA to 9 nt and releases the promoter, it becomes much more stable than ICs that retain promoter interactions and readily release short RNAs (18, 19). The instability of ICs could be due to the short length of the RNAs in these complexes or to the fact that they retain promoter interactions. Because the A15G promoter releases the polymerase while the RNA is still relatively short, we wondered whether these released complexes would be as unstable as promoter-bound initiation complexes with RNAs of identical length. To measure this, we carried out transcription reactions in which transcript extension was halted 4, 7, or 8 nt by 3′dNMP incorporation (Fig. 3). When the synthesis of such transcripts follows burst kinetics the steady-state rate of their accumulation reflects the rate at which the halted transcription complexes dissociate and re-initiate synthesis (20). For reactions in which transcription was halted at 4 nt, transcripts were found to accumulate at rates of 1 min−1 and 0.8 min−1 on the wt and A15G promoters, respectively, indicating that the 4-mer ICs turned over rapidly on both templates (Fig. 3, lanes 1, 4, 7, 10, 13, 16, 19, 22). When transcription was halted at 7 nt, synthesis was characterized by burst kinetics, and the steady-state rates of transcript accumulation corresponded to 0.3 min−1 on the wt promoter and 0.06 min−1 on the A15G promoter (Fig. 3, lanes 2, 5, 8, 11, 14, 17, 20, 23). Transcripts halted at 8 nt accumulated at rates of 0.17 min−1 and 0.033 min−1 on the wt and A15G promoters, respectively (Fig. 3, lanes 3, 6, 9, 12, 15, 18, 21, 24). The 7- and 8-mer complexes that form on the A15G promoter therefore turn over 5-fold...
more slowly than the same complexes on the wt promoter. We concluded that release from the promoter stabilizes the transcription complex, even if the RNA is still relatively short.

Mutations in the Polymerase Binding Region of the Promoter Facilitate Release—The T7 promoter can be divided into three parts. The region upstream of −5 remains base-paired throughout the initiation reaction and is important for binding the polymerase via an extensive set of sequence-specific contacts (4). The −4 to −1 TATA element facilitates promoter opening (21), whereas the +1 to +6 ITS is important for efficient progression through initial transcription (mutations in the ITS lead to increased release of short RNAs) (22).

To determine how mutations in these different regions would affect release, we prepared a set of promoters with mutations from −17 to +2. The mutants were selected from information contained in Ref. 15 and included promoters that were 2–3-fold less active than wt (T17G, C12G, A10C, C5G, A3G, G+2C), as well as mutations that were 20-fold (A15C, A10G, C5T) or 30-fold (G11C) less active than wt. Fig. 4 shows NT strand cleavage on these promoters by a polymerase with FeBABE tethered to residue 207. The reactions contained NTPs allowing RNA extension to either 4 or 7 nt. For mutations upstream of −4, there is a direct correlation between the magnitude of the effect of the mutation on promoter utilization and our ability to detect a cleavage signal. Mutations that reduce promoter activity by only 2-fold exhibit cleavage in the IC4 complex similar to a wt promoter (Fig. 4, lanes 3, 9, 21; cleavage of A10C, which is 3-fold less active, was somewhat reduced, as seen in lane 15), whereas cleavage of mutants with 20-fold (lanes 6, 18, 24) or >30-fold (lane 12) reductions was weak or undetectable, indicating low occupancy of these weak promoters by the polymerase. Although the more active promoters could achieve occupancy levels similar to a wt promoter in the 4-nt reactions, the mutations did have an effect on promoter release; cleavage in the 7-nt reactions was reduced by only 20–30% relative to the 4-nt reactions (Fig. 4, lane 4 versus 3, lane 10 versus 9, lane 16 versus 15, and lane 22 versus 21). For the weaker promoters, cleavage signals were too weak for reliable quantification, but inspection of gels indicated more release in the 7-nt reaction than is seen with a wt promoter (Fig. 4, compare lanes 18 and 19).

In the unwinding element, the T4A and A3G mutations reduce promoter activity by 13- and 5-fold, respectively. However, they do not have as drastic an effect on promoter release as the more upstream mutants. For T4A and A3G, cleavage in the 7-nt reactions is reduced by only 20–30% relative to the 4-nt reaction (Fig. 4, lane 28 versus 27 and lane 31 versus 30), a value comparable with what we see for NT strand cleavage with a wt promoter. Fig. 5 shows that mutations in the ITS that reduce promoter activity by 13-fold (G+1C) or 2-fold (G+2C) also affect release differently than the more upstream mutations. For G+1C, cleavage in the 7-nt reaction is identical or even slightly greater than in the 4-nt reaction (Fig. 5, lane 3 versus 2 and...
upstream promoter region is protected only in A3G (Fig. 6, lanes 23 and 24), indicating that this promoter does not release the polymerase as readily as do the other mutant promoters, in agreement with the FeBABE cleavage results (Fig. 5). Note that in the 7- and 8-nt reactions, cleavage between −8 and −10 is strongly enhanced relative to that seen in reactions without polymerase. The enhancement is also seen in Fig. 1A. It has been previously noted that T7RNAP elongation complexes exhibit increased DNase I sensitivity 15–20 nt upstream of the RNA 3′-end (23); this may reflect bending of DNA upstream of the EC.2 Although occupancy of the A15C promoter was too low to provide detectable protection in the 4-nt reaction, protection of −3 to +14 is detected in the 7- and 8-nt reactions with this promoter, as is enhanced cleavage between −8 and −10 (Fig. 6, lanes 7 and 8). Significantly, both protection of −3 to +10 and enhancement of cleavage between −8 and −10 are weaker with A15C than with the more active promoters, indicating that, although complexes with 7- or 8-nt RNAs can be formed on this promoter, they do not accumulate to the same level as with a wt template.

The enhancement of −8 to −10 cleavage in the 7- and 8-nt reactions complicates the interpretation of the DNase I patterns, because in those cases where some polymerases have released and some have not, we observe the combined effects of partial protection and partial enhancement. We interpret the cleavage pattern in the 8-nt A3G reaction (Fig. 6, lane 24), which exhibits both enhanced and protected points of cleavage relative to the no polymerase reaction (lane 21), to reflect release by a small fraction of the polymerases, because the enhancement of cleavage at −10 is much less than is seen with the other promoters. Similarly, the cleavage pattern of the upstream promoter region in the 4-nt reaction with A10C (Fig. 6, lane 14) may reflect a mixture of released and retained complexes.

**DISCUSSION**

The central conclusion of this study is that weakening of the T7 promoter-polymerase interaction by introduction of base changes into the −5 to −17 binding region of the promoter allows the polymerase to release the promoter when the RNA is shorter than the length normally required to trigger release. Mutations in the −4 to −1 TATA sequence, which is melted during initiation (9, 11), do not have such an effect, even when they decrease promoter activity as much as (or more than) mutations in the more upstream region.

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2 Q. Guo and R. Sousa, unpublished results.
Mutations in the ITS also do not appear to facilitate release of the polymerase. In fact, for at least one of the latter mutations, extension of the RNA from 4 to 7 nt actually enhances occupancy of the promoter. There may be at least two explanations for the latter observation. The first is that the introduced mutation destabilizes the association of the RNA with the IC (its mutations have been previously shown to increase the release of short RNAs during initial transcription (22)). Because it has been shown that the presence of the RNA in the IC stabilizes the association of the polymerase (10) with the promoter and that the complex becomes more stable as the RNA is progressively extended (3), it is possible that extension of the RNA from 4 to 7 nt has a proportionately greater stabilizing effect on the IC when the complex is more unstable to begin with. It is also likely that the effect of the mutation becomes progressively less destabilizing as the RNA is extended away from the site of the mutation. For example, with the G+2C promoter and the 4-nt reaction, the mutation is only 2 nt away from the RNA 3′-end, whereas in the 7 nt reaction, it is 5 nt away. The destabilizing effect of the mutation may therefore decrease with its distance from the active site of the enzyme.

Our results also reveal that retention of promoter contacts contributes significantly to the release of RNAs during initial transcription, because we observe that complexes on the A15G promoter with 7- or 8-nt RNAs turn over much less frequently than similar complexes on a wt promoter. That such complexes can be quite stable is perhaps not surprising, because the RNA-DNA hybrid in an elongation complex is 7 base pairs in length (3, 6, 8, 18, 24); therefore, a 7-nt RNA would satisfy this basic requirement. The conclusion that a released complex with a 7-nt RNA is relatively stable is also supported by the observation that such a complex can present a strong block to exo III digestion (Fig. 1B). However, complexes with shorter RNAs are not able to present such strong blocks to exo III. For example, in the 6-nt reaction with A15G, shown in Fig. 1B, lane 13, we observe neither a strong (−20) block, corresponding to a promoter-bound complex, nor a downstream block, corresponding to a released complex. Apparently in this case, the polymerase-promoter association is too weak to resist exo III digestion, but the 6-nt RNA also does not provide for a sufficiently stable elongation complex to block exo III once the polymerase is released. A similar interpretation can be made for the 4-nt reaction in Fig. 1B, lane 11, although in this case, the polymerase-promoter complex provides at least partial resistance to exo III. Interestingly, the 3-nt reaction in Fig. 1B, lane 10, shows a block to exo III around −7, showing that the released complex can block exo III. However, in these reactions oligo-G RNAs can be extended up 14 nt by a process of transcript slippage (25), so it is even possible that these longer RNAs stabilize the released complexes, even though they are only partially complementary to the template.

These exo III results are in qualitative agreement with the DNase I protection patterns and with the FeBABE results, but there are quantitative differences in the detection of promoter release with these methods. For example, with exo III, we detect extensive release on the wt promoter when the RNA is 8 nt long (Fig. 1B, lane 8) at a point that coincides with apparent loss of protection of the upstream promoter region in a DNase I assay (Fig. 1A, lane 7), whereas we detect only partial release at 8 nt with the FeBABE assay (Fig. 2). A recent study (26) using fluorescent probes to characterize initiation on T7 promoters detected little release at 8 nt, 50% release at 9 nt, and complete release at 12–13 nt. exo III digestion is potentially perturbing and is likely to overestimate the fraction of release relative to the other methods (26) but provides additional information on the relative stability of the released complexes. Of the methods used here, the FeBABE cleavage assay is likely to be the least perturbing and is probably the best measure of the point at which release occurs.

We have previously shown (13) that promoter release is inhibited when the T7 promoter-polymerase interaction is strengthened by deletion of the NT strand around the transcription start site. This study establishes the corollary to this: a weakened promoter interaction facilitates release. Tinutt et al. (27) showed previously that, on an Escherichia coli ρ54 promoter, release of the polymerase from the promoter occurs when the RNA reaches 6–7 nt, whereas on a ρ70 promoter, release occurs when the RNA reaches 12–13 nt. They concluded that these differences were probably due to weaker polymerase binding to the ρ54 promoter. It may therefore be generally the case that a weak interaction facilitates the escape of an RNA polymerase from its promoter.

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