Abortive Cycling and the Release of Polymerase for Elongation at the σ54-dependent glnAp2 Promoter*

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Transcription initiation at the σ54-dependent glnAp2 promoter was studied to follow the state of polymerase as RNA synthesis begins. σ54 polymerase begins transcription in abortive cycling mode, i.e. after the first bond is made, approximately 75% of the time the short RNA is aborted and synthesis must be restarted. Polymerase is capable of abortive initiation until it reaches a position beyond +3 and before +7, at which stage polymerase is released from its promoter contacts and an elongation complex is formed. Initial elongation is accompanied by two transcription bubbles, one moving with the polymerase and the other remaining at the transcription start site. The σ54-associated polymerase shows an earlier and more efficient transition out of abortive initiation mode than prior studies of σ70-associated polymerase.

σ54 is an alternative bacterial transcription factor that directs transcription of specific subsets of genes (reviewed by Magasanik (1989), Kustu et al. (1989), and Merrick (1993)). σ54 is the only sigma factor that is not a member of the σ70 family of proteins, as defined by sequence similarity (Merrick and Gibbons, 1985; Lonetto et al., 1992). Regulation of σ54-dependent promoters differs from regulation of σ70-dependent promoters (Gralla, 1991; Collado-Vides et al., 1993) despite the fact that both sigma factors bind the same core RNA polymerase. All known σ54-dependent promoters are controlled by activator proteins rather than by repressors (Collado-Vides et al., 1993). The activators are generally enhancer-binding proteins (Reitzer and Magasanik, 1986), which work from locations that are too remote to activate transcription in σ70-dependent systems.

Certain steps in the transcription cycle at σ54 promoters differ from analogous steps at σ70 promoters. σ54 can bind certain promoters without being part of holoenzyme (Buck and Cannon, 1992), whereas σ70 cannot (Dombroski et al., 1992). The two holozymes recognize different sequence elements (see review by Merrick, 1993). In addition the σ54 polymerase elements are located in different positions near −12 and −24 (Moret and Buck, 1989). ATP hydrolysis is required to form open complexes at the σ54 promoters (Popham et al., 1989; Weiss et al., 1991) but not at σ70 promoters.

Recently we identified another difference; σ54 can remain bound to a promoter after RNA polymerase begins transcription elongation (Tintut et al., 1995). This is in contrast to the well-established σ70 transcription cycle, in which σ70 is released when an elongation complex is formed. The observation has led us to attempt to investigate the transition from open promoter complex to elongation complex for σ54 holoenzyme. The process is well known for σ70 promoters (Hansen and McClure 1980; Carposiss and Gralla, 1985; Krummel and Chamberlin, 1989). At those promoters RNA synthesis begins with an abortive cycling pathway, in which the polymerase-bound holoenzyme synthesizes short RNAs (Carposiss and Gralla, 1980). Subsequently, a longer RNA of length 10 or 11 is made, and at this point polymerase is released from the promoter (Carposiss and Gralla, 1985; Krummel and Chamberlin, 1989). σ70 is released both from the polymerase and from the DNA, and the complex becomes fully committed to elongation.

In this paper we investigate the transition of σ54 holoenzyme from open complex to elongation complex at the Escherichia coli glnAp2 promoter. Popham et al (1989) showed that open complexes formed at an analogous promoter are capable of synthesizing short, probably abortive, RNAs. We confirm this finding in a different context and go on to characterize the transition from abortive to productive transcription. The results indicate that several important aspects of this transition differ from those observed in prior studies of σ70 systems.

EXPERIMENTAL PROCEDURES

Materials

Core polymerase was purchased from Epicentre Technologies (Madison, WI), and σ54 and NtrC were purified as described (Tintut et al., 1995). NTPs and O-methyl-GTP were purchased from Pharmacia Biotech Inc. Dinucleotide UpA, 3′-dATP, DNase I, K^304_O, and carboxymethyl phosphate was purchased from Sigma. The competitor DNA is a 160-base pair fragment carrying the Rhizobium meliloti nifH promoter (Tintut et al., 1995).

Methods

Footprinting—The following common procedures were performed for both DNase I and K^304_O footprinting experiments. In order to form a closed complex, core RNA polymerase (10 μl) and σ54 (20 μl) were mixed with 0.5 mM supercoiled plasmid carrying the glnAp2 promoter (pLR1; Reitzer and Magasanik, 1986) in footprinting buffer (25 mM Tris acetate, pH 8.0, 8 mM magnesium acetate, 10 mM potassium chloride, 1 mM dithiothreitol, 3.5% [w/v] polyethylene glycol; Buck and Cannon, 1992). The reaction was incubated for 20 min at 37°C. For open complex formation, NtrC (40 μM), CBP (10 μM), and ATP (4 mM) were included in the above reaction for 20 min. Before transcription initiation, the competitor DNA, R. meliloti nifH (48 μl) was added for 3 min to bind free excess proteins. In order to stall the polymerase at different positions along the template DNA, different nucleotide combinations (0.5 mM each) were added for 1 min as indicated in Table I. In some reactions, ATP was substituted with 3′-dATP (0.5 mM) as a source of hydrolysis, where indicated. The samples were probed with footprinting reagent in a 40-μl reaction as follows.

For DNase I digestion, 2 μl of DNase I (0.45 μg/ml, including 45 μM MgCl_2 and 22.5 μM CaCl_2) was added for 30 s at 37°C, followed by an addition of 2 μl of 0.5 mM EDTA to stop the reaction. For K^304_O footprinting, 4 μl of 92.5 μM K^304_O was added for 1 min at 37°C, and
RESULTS

The initial goal was to monitor the progress of a54 helixosome as it transcribed from the glnAp2 promoter. To do that, the polymerase was stalled at different positions along the template as it attempted to move downstream during transcription initiation. The stalling was accomplished by omitting a subset of the required nucleotides. The expected positions of stalling are shown in Table I along with the nucleotide combinations used. Three assays were used to characterize these stalled complexes: DNase I footprinting to locate the promoter elements, abortive initiation to assay abortive transcripts, and permanganate probing to locate melted regions.

Plasmid pLR1, which contains the a54-dependent promoter glnAp2 with upstream NtrC enhancer sites, was used (Reiterz and Magasanik, 1986). Primer extension foot-printing methods, which allow probing of both DNA strands of the same sample, were used (see Gralla (1985)). This method also allows the use of supercoiled DNA, which may be an important parameter in this system.

DNase I Footprinting

Top Strand—First, known closed and open complexes (Ninfa et al., 1987; Popham et al., 1989) are probed with DNase I to establish control patterns. To form a closed complex, template DNA was incubated with purified core RNA polymerase and a54. To form an open complex, ATP and enhancer NtrC (phosphorylated with carbamyl phosphate; Feng et al., 1992) were added in addition to core and a54. Each sample was divided in half, and both the top and bottom strands of DNA were probed to observe footprints.

The footprint pattern of the top strand of the glnAp2 promoter is shown in Fig. 1A. Lane 1 shows the protection pattern of a closed complex formed from a54 and core polymerase. The closed complex footprint covers from –34 to –2 (compare lane 1 versus the lane 2 control pattern). This encompasses both the –12 and –24 promoter recognition elements but not the transcribed region, in agreement with prior experiments (Popham et al., 1989). When phosphorylated NtrC and ATP were also present, the open complex formed extends into the transcribed region to position +23 (lane 3), covering at total of approximately 57 bases.

Next, the holoenzyme was allowed to transcribe to different positions by adding different combinations of nucleotides (see Table I). In order to prevent binding by new polymerases from solution, free proteins were titrated with a competitor promoter DNA. The competitor contains the a54-dependent tightly binding R, meliloti nifH promoter (Buck and Cannon, 1992) carried on a 160-base pair fragment. A 100-fold excess of this competitor was added after open complexes were formed with glnAp2 DNA, but before the addition of nucleotides, which caused the polymerase to begin RNA synthesis. The lack of protection in control lane 2, where competitor was mixed with glnAp2 before addition of proteins, confirms that a sufficient amount of the competitor is present to titrate excess proteins.

In the first experiment dinucleotide primer UpA and CTP were added to the open complex to allow the polymerase to form a single bond, creating the product UpApC, where “A” is the initiating nucleotide (see “Transcription” below) (Popham et al., 1989). This corresponds to incorporating the nucleotide at position +2 in the natural mRNA. Under these conditions the result shows no change in the DNase protection pattern compared to the open complex (lane 4 versus lane 3). Not surprisingly, the polymerase can make the first bond of the mRNA without moving along the DNA.

By contrast, the polymerase did change its interaction with DNA when GTP was added to allow transcription as far as the +7 position (see Table I). This is demonstrated by the shortened protection seen in lane 5. The polymerase now fully protects the DNA only from –2 to +23 with additional partial protection as far as –11 upstream and perhaps to –27 downstream. The overall protection pattern corresponds to less than a 40-base pair region, compared to open and first bond-making complexes, both of which protect approximately 57 base pairs. The comparison indicates that when the polymerase transcribes to the +7 position, it is released from its upstream promoter contacts. The important promoter element near –24 is no longer protected, and the protection near the –12 element is quite weak.

Next, the polymerase is allowed to transcribe to position +18 (see Table I). The primary protection (lane 6) now extends from +3 to +23 and is not as complete as in other cases. In addition there is partial protection from +23 to +39. Thus, the stalled
polymerase covers a total of 36 bases, approximately the same extent as seen at the +7 position, but the protection is weaker. Finally, when all four nucleotides are present no protection is seen (lane 7), as expected for a polymerase that has moved to downstream positions.

Bottom Strand—The top strand footprinting results above indicate that the polymerase breaks free of the promoter and exhibits a shortened footprint sometime after reaching position +2 and before reaching position +7. The bottom strands of the same DNA samples were probed for purposes of comparison (Fig. 1B). On this strand the open complex protection extends downstream to +15 (lane 3). Because the primer is in the near upstream region, this border is not well defined; footprinting using a labeled glnAp2 promoter fragment showed that the upstream boundary protection is approximately at −35 (data not shown). This yields an approximately 50-base pair footprint, which is slightly shorter than seen on the top strand. When polymerase was allowed to produce UpApC, the footprint did not change (lane 4), as also seen on the top strand.

As also observed on the top strand, a drastic change in the footprint occurs when polymerase is allowed to move to position +7 (lane 5). The protection is strong from −13 to +22. The result is similar to that from the top strand in that polymerase no longer protects the critical −24 promoter element. A transition indicative of loss of promoter interaction has occurred on both strands. The footprints shorten a little more when transcription is halted at +18 (lane 6), at which positions from −3 to +31 are partially protected.

In addition, lanes 5, 6, and 7, corresponding to stalling at +7, at +18, and steady-state transcription, show partial protection of the region upstream from the start site (lesser intensity in these lanes compared to the control lane 2). This is a reflection of the weak DNase protection due to σ not being released from this region, as shown by Tintut et al. (1995).

A pictorial summary of the DNase I footprinting results is presented in Fig. 7. The main point at this stage is that a transition releasing polymerase from the promoter elements has occurred before polymerase reaches position +7.

**Transcription**

The purpose of experiments in this section is to compare the transcriptional state of the polymerase to the physical state studied in the DNase footprinting experiments. The goal is to learn if abortive transcription occurs and, if so, at what position it ends as the polymerase transits to elongation mode.

The production of UpApC was used as a measure of abortive initiation. This product is made using dinucleotide primer UpA and 32P-labeled CTP as the only source of nucleotides (Fig. 2). When all required abortive transcription components were present, an abortive initiation product was seen (lane 7 with ATP). The specificity of this reaction was confirmed by showing its dependence on various required components. No signal is seen with the omission of NtrC (lane 1), carbamyl phosphate to phosphorylate NtrC (lane 2), σ54 (lane 3), core polymerase (lane 4), ATP (lane 5), and UpA (lane 6). Parallel experiments...
(data not shown) to produce long transcripts under these same conditions confirmed the specificity of glnA transcription.

Next we characterized the amount of abortive initiation that occurs when polymerase is permitted to move to various positions (see Table I) as described in the DNase footprinting experiments. The goal is to learn at what position the polymerase ceases to make large amounts of abortive initiation products. Open complexes were formed, and a subset of nucleotides containing radioactive CTP (see Table I) were added. The reactions were allowed to proceed for 10 min to achieve the steady state conditions. Reaction products were loaded directly onto an acrylamide gel, separated by electrophoresis, and detected by autoradiography.

In the presence of dinucleotide UpA and labeled CTP, the polymerase produced a large amount of abortive product UpApC (see band indicated by the arrow in Fig. 3A, lane 1). When the polymerase was allowed to move as far as position +7, a much smaller amount of abortive product was made (much lighter band in the same position of lane 2, quantified below). In addition small amounts of slightly longer products were made, as expected; we have not definitively characterized the size of these longer products. The data indicate that few abortive products are made when polymerase is allowed to move as far as position +7.

When additional nucleotides were present (see Table I) to promote further downstream movement of the polymerase, the amount of abortive product was only slightly lessened (lane 3; band in same position denoted by arrow). Two major longer products were also produced under these conditions. Calibration using short RNAs (data not shown) suggests that these two products correspond to RNAs of the expected length, 19, and also of length 22; the 22-mer probably occurs from the misincorporation of the omitted UTP, followed by stalling at the next position of UTP where two UTOPs must be incorporated. This has been seen previously (Carpousis and Gralla, 1985). Finally, when all four nucleotides were present, the amount of abortive product was essentially unchanged (lane 4).

This qualitative analysis indicates that a transition from a frequent abortive initiation state to a lower abortive state has occurred between position +2 and +7 (lane 1 versus lane 2).

**Note:**
The +7 position is the same point at which DNase footprinting revealed a physical change in the state of the transcription complex.

One further combination of nucleotides was used to narrow the position at which the transition occurs. By the use of UpA, CTP, and 3-methyl-GTP the polymerase could be stalled at position +3. The result (Fig. 3B, lane 6) indicates that the polymerase stalled at +3 still produces large amounts of abortive products (quantified below). That is, the amount is comparable to that seen when transcription is stalled at +2 (lane 7) but much more than that seen when transcription is stalled later (lane 7). Thus the transition out of frequent abortive mode seems to occur after the polymerase reaches +3 but before it reaches +7.

For each of several samples where the polymerase stalled at positions +2, +3, +7, +18, and +18, the amount of abortive products and productive transcripts were quantified using a Phosphorimager. From the quantitative data, the relative molar amount was determined by normalizing to the number of radiolabeled CTPs incorporated (Table II). Note that the longer products incorporate more than one radioactive cytidine, and thus their molar amount is over-represented on the autoradiographs. The molar excess of abortive RNA UpApC (compared to productive RNA, 18 + 19 nucleotides long, produced in a parallel experiment) was determined. The data (Table II) showed that during the 10-min abortive initiation period, a 50-fold excess of abortive product UpApC was made from polymerase stalled at the +2 and +3 positions.

The relative amount of abortive products associated with stalling at each position is plotted in Fig. 4 to display the transition from frequent abortive phase to more stable elongation complex. When transcription is artificially stalled at position +2, the amount of abortive product UpApC made is approximately 15-fold higher than the amount of abortive product made at position +7. This huge overproduction of UpApC is maintained when polymerase is artificially stalled at position +3. Apparently, a transition out of abortive mode has occurred at position +7, as the polymerase aborts transcription rarely after reaching this position. This much lower production of UpApC is maintained under conditions in which polymerase can reach position +18 and, indeed, under conditions of free transcription. The quantitative analysis confirms that a transition away from abortive mode has occurred between positions +3 and +7.

**Table II**

<table>
<thead>
<tr>
<th>Stalling position</th>
<th>RNA size</th>
<th>Relative RNA amount</th>
<th>Abortive probability</th>
</tr>
</thead>
<tbody>
<tr>
<td>+2</td>
<td>3</td>
<td>52</td>
<td>ND</td>
</tr>
<tr>
<td>+3</td>
<td>3</td>
<td>48</td>
<td>0.75</td>
</tr>
<tr>
<td>+7</td>
<td>3</td>
<td>3.5</td>
<td>0.75</td>
</tr>
<tr>
<td>+18</td>
<td>3</td>
<td>2</td>
<td>0.7</td>
</tr>
<tr>
<td>&gt; +18</td>
<td>19 + 22</td>
<td>2.5</td>
<td>ND</td>
</tr>
</tbody>
</table>

The size and amount of RNA produced under each of five conditions is shown. The data are normalized to the amount of productive RNA (19 and 22 nucleotides long) as shown by the asterisk. The abortive probability refers to the probability that the RNA of indicated length will be released rather than elongated. It is calculated from the ratio of the amount of the individual RNA to the total amount of all RNA of that length and longer. ND, not determined.
A key parameter in the synthesis of productive transcripts is the probability of aborting synthesis at a particular position (Carpousis, 1983; Carpousis and Gralla, 1985). The probability can be defined as the amount of RNA shorter than a certain length divided by the sum of all RNA products made (abortive plus longer). For example, the data showed that UpApC constitutes approximately 75% of all RNAs made under various conditions (Table II). That is, in several experiments where the polymerase was stalled at position +7 or +18, the probability of aborting synthesis at +2 was calculated to be 0.75. Thus, approximately three out of four times, polymerase aborts synthesis after forming UpApC and must restart; the remaining time, it goes forward to make productive transcript. This predicts that only a small (roughly 2-fold) excess of abortive product will be seen during productive transcription, as is observed. Note that huge amounts of abortive products are only produced when polymerase is artificially stalled at a position that is not far enough to be associated with the transition to elongation mode. Significant amounts of abortive transcripts have been seen previously under productive transcription conditions, but this is probably a consequence of the use of low concentrations of CTP, GTP, and UTP (Popham et al., 1989).

In order to confirm that this abortive phase is not restricted to dinucleotide-primed synthesis, we repeated selected experiments using the normal initiating nucleotide ATP. Abortive transcripts could not be detected directly because the presence of the triphosphate end caused them to migrate within a region dominated by radioactive products from the unincorporated labeled CTP. Thus after transcription the samples were subjected to calf intestine alkaline phosphatase to cleave off the 5'-phosphate groups (Jacob et al., 1994) before loading onto gels. Under these conditions abortive product can be seen. Lane 1 of Fig. 5 shows that a large amount of abortive product is made when ATP and labeled CTP are used to form pppApC. When the same experiment is done in the presence of additional GTP and UTP to allow elongation, the result shown in lane 2 is obtained. The much reduced amount of abortive product under these conditions is similar to the result obtained with dinucleotide-primed synthesis. This confirms that abortive initiation can occur using the natural initiating nucleotide ATP.

**KMnO₄ Footprinting**

Both DNase footprinting and abortive transcription experiments indicate that a transition out of the abortive initiation phase occurs prior to RNA synthesis reaching the +7 position. We now follow the movement of the melted DNA bubble during this same transition. Previous experiments indicated that even after the transition occurs the promoter start site can remain transiently bound by σ54, which keeps the start site in a single-stranded state (Tintut et al., 1995). We now investigate single-stranded regions present during the transition from abortive mode to elongation mode.

In order to follow the single-stranded regions in this transition, the exposed single-stranded DNA was probed with KMnO₄. KMnO₄ reacts selectively with single-stranded thymines and has been used previously to detect the melted transcription bubble at this promoter (Sasse-Dwight and Gralla, 1988, 1990). In these experiments the bottom strand of the glnAp2 promoter was probed. As a control, open complexes, formed as described above, yield a strong permanganate signal (Fig. 6, lane 2) corresponding to bottom strand thymines at positions −9 and +1. As expected, this reactivity was absent in closed complexes (Fig. 6, lane 1).

Next, samples were probed with permanganate using the same conditions described above to move the polymerase to various positions along the template. No change in pattern was observed when complexes forming product UpApC were probed (Fig. 6, compare lane 3 with the open complex signal of lane 2). Recall that at this stage the polymerase has not moved (as assayed using DNase footprinting; Fig. 1) and is in abortive mode (as assayed by abortive initiation; Fig. 3A).

By contrast, when the polymerase is stalled at position +7, new permanganate hypersites extended to positions +7 and +9 (Fig. 6, lane 4). Recall that under these conditions the polymerase has been largely released from abortive mode (see above). When polymerase is stalled further downstream in the +18 to +21 region, additional permanganate-sensitive sites are seen corresponding to thymines within this region (lane 5). As expected, no hypersites are seen in the transcribed region when polymerase transcribes in the presence of all nucleotides (lane 6). The results provide evidence that a melted bubble moves downstream with the elongating polymerase. The differences in intensities of permanganate-sensitive bands is probably due to different environments surrounding thymines at different positions.

Note that under all conditions the transcription start site remains open (Fig. 6, bracket indicating bands in lanes 2–6). This is in agreement with prior experiments, not involving abortive initiation, analogous to those in lanes 2, 5, and 6 but probing the top strand with permanganate (Tintut et al., 1995).
The result supports the view that, as polymerase is released from the promoter, the original open complex bubble splits into two bubbles; one moves with the elongating polymerase and the other remains behind with $\sigma^{54}$.

**DISCUSSION**

These experiments describe the transition from abortive initiation mode to elongation mode for $\sigma^{54}$ holoenzyme at the glnA promoter (see summary in Fig. 7). The holoenzyme makes the first mRNA bond without moving from the promoter. After making this short RNA product, the data show that there is a 75% chance that the synthesis will be aborted and started anew. Thus, even when all elongation substrates are present, this abortive product accumulates in modest excess over long RNA. However, longer abortive products do not accumulate in significant amount under these conditions, leading to relatively efficient productive transcription.

The polymerase is capable of abortive initiation when RNA synthesis reaches position +3 but loses this property prior to position +7 (Fig. 4). At this stage, footprinting shows that the polymerase is released from the contacts that hold it to the promoter region (see summary in Fig. 7). This leads to a reduction in the size of the protected region by approximately 20 base pairs. Permanganate probing shows that the transcription bubble has moved forward to cover the position that is transcribed. Thus the polymerase appears to have reached elongation mode prior to +7 position of the glnA gene. Probing of complexes stalled further downstream also show elongation complexes with characteristic of shortened footprints and transcription bubbles covering the point of synthesis.

This pathway may be compared to the analogous pathway used by the common $\sigma^{70}$ RNA polymerase holoenzyme. The comparison reveals both similarities and differences. The main similarity is that both pathways require the polymerase to pass through an abortive initiation mode prior to being committed to transcription elongation (Carpousis and Gralla, 1985). The molecular basis for this requirement is not known, but it was speculated previously to be related to the primer-independent nature of mRNA initiation (Carpousis and Gralla, 1985). The lack of primers, common to RNA polymerases but not DNA polymerases, may cause difficulty in initiating RNA synthesis. The abortive mode can be thought of as a phase in which a primer is created with some difficulty. For example, cycling to produce abortive RNAs may occur because these short RNAs are associated with the “loose product site” and are not translocated to the “tight product site” (see Chamberlin (1992–1993)) until a stable ternary complex is formed (Mustaev et al., 1994). Alternatively, the need to break strong contacts between polymerase and promoter may also retard the transition to elongation phase.

There are several differences in this pathway compared to prior studies, which used the $\sigma^{70}$ form of polymerase. One important difference is that the $\sigma^{54}$-dependent glnA promoter involves a relatively efficient transition out of abortive cycling mode. The data show that approximately 25% of the glnA2 RNA 5’ ends that are produced will end up in productive transcripts. In the two $\sigma^{70}$ cases studied, the probabilities are much lower: 2% atlacUV5 (Carpousis and Gralla, 1980; Carpousis, 1983) and less than 8% atT7A1 promoters (Krummel and Chamberlin, 1989). This difference is largely a consequence of what happens after the first bond of the mRNA is formed. Although the $\sigma^{54}$ polymerase is capable of abortive initiation up to position +7, it aborts primarily after forming the first bond. That is, the release of abortive products at glnA2 during transcription is observed primarily from polymerases that have formed a single bond. By contrast, in prior studies at $\sigma^{70}$ promoters, longer RNA products are also aborted.

A second difference is that polymerase is released from the contacts that hold it to the promoter sooner in the $\sigma^{54}$ case studied here than in the $\sigma^{70}$ cases studied previously (Carpousis and Gralla, 1985; Krummel and Chamberlin, 1989). As discussed above, at glnA2 this occurs after formation of the

\[ \text{L. Hsu and M. J. Chamberlin, personal communication.} \]
second bond but prior to formation of the sixth bond. In the prior cases elongation mode is not attained until the mRNA is approximately twice as long, and abortive cycling occurs at several positions. One possible cause of these differences might be a lower affinity of core polymerase for several positions. One possible cause of these differences might be a lower affinity of core polymerase for several positions. One possible cause of these differences might be a lower affinity of core polymerase for several positions. One possible cause of these differences might be a lower affinity of core polymerase for several positions.

Another difference is that the initial transcription bubble is split into two parts only during initiation by σ54 holoenzyme. That is, as RNA synthesis begins the initial bubble separates into two bubbles; one moves downstream with the polymerase and the other remains transiently over the start site. We show elsewhere that this is because σ54 remains promoter-bound during initial transcription due to its stronger affinity for DNA (Tintut et al., 1995).

The mechanism of σ54-dependent transcription has been described as a hybrid between prokaryotic and eukaryotic mechanisms (Gralla, 1991; North et al., 1993). If this analogy extends to the pathway studied here, then one might expect eukaryotic mRNA synthesis to go through an analogous, quite brief, abortive to elongation transition (see Jacob et al. (1994), and references therein). Indirect experiments support this possibility, but more direct experiments will be required to test this further. σ54 is also used more commonly in other bacteria, and it will be useful to learn if there are promoter- or species-specific differences in the mechanism of transcription initiation by σ54 holoenzyme.

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2 Y. Jiang, M. Yan, and J. D. Gralla, unpublished data.
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