Abortive Initiation of Transcription at a Hybrid Promoter

AN ANALYSIS OF THE SLIDING CLAMP ACTIVATOR OF BACTERIOPHAGE T4 LATE TRANSCRIPTION, AND A COMPARISON OF THE $\sigma^{T_0}$ AND T4 GP55 PROMOTER RECOGNITION PROTEINS

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Bacteriophage T4 late promoters are transcribed by an RNA polymerase holoenzyme comprising the Escherichia coli core, E, the phage gene 55-encoded promoter recognition subunit, gp55, and the gene 33-encoded co-activator, gp33. Transcriptional initiation is activated by the T4 gene 45-encoded sliding clamp, which is loaded onto DNA at enhancer-like sites by its clamp-loader. Correct initiation of transcription at late promoters in basal mode requires only RNA polymerase core and gp55 (E-gp55). Dinucleotide-primed abortive initiation of basal and activated T4 late transcription has been compared. Only the trinucleotide non-productive transcript is made at a high rate; all other short transcripts are made at rates of less than one molecule per productive transcript. Gp45 increases abortive trinucleotide synthesis along with productive transcription, although the proportion of productive transcripts is also elevated. Nevertheless, this increase accounts for only a small part of the activation of T4 late transcription that is generated by its activator and co-activator. The pattern of production of short transcripts differs subtly between basal and enhanced transcription, indicating that linking the RNA polymerase with its sliding clamp activator only generates minor changes in the transition from abortive to productive RNA chain elongation. The T4 late promoter is converted to a strong $\sigma^{T_0}$ promoter by inserting an appropriate $\sim$35 promoter element. A direct comparison at such a hybrid promoter shows $\sigma^{T_0}$ and gp55 generating qualitatively and quantitatively different patterns of abortive initiation at the same start site.

DNA-dependent RNA polymerases are obliged to carry out prodigious feats of processive polymerization when they generate long transcripts, because release of the nascent RNA chain at any step of elongation is essentially irreversible. In contrast, the first steps of RNA chain elongation by these enzymes are tentative. Short transcripts are rapidly generated and released in a repetitive process in which the enzyme does not leave the transcriptional start site (1–3). It appears therefore that the generation of these abortive transcripts is part of the process of undocking polymerase from the promoter. The distribution of very short transcripts generated at different Escherichia coli promoters differs, in some instances strikingly (4), reflecting large-scale differences in the ability to clear different promoters that are caused by start site-proximal DNA sequence.

Undocking from the promoter takes place in two fundamentally different ways. For the bacterial polymerase autonomously recognizing and opening a promoter, leaving that site is also autonomous. The eukaryotic nuclear RNA polymerases are recruited to their promoters by homologous transcription initiation machineries through protein-to-protein contacts, and undocking from the promoter breaks those contacts. When it departs CAP-activated promoters, E. coli RNA polymerase undoubtedly also senses contacts, in that instance between its $\alpha$-subunit and the activator, at an early step of promoter clearance. Many other activators of bacterial transcription must generate similar circumstances. It is important to know whether these events confer characteristic signatures on the pattern of generation of abortive transcripts. In the case of transcriptional activators, it is also necessary to examine whether activation changes the relative yield of productive and abortive transcripts.

The experiments that are reported here concern bacteriophage T4 late promoters, whose activation is generated by the gp45 sliding clamp of the T4 DNA polymerase holoenzyme. The unique mechanism of this activation requires loading of gp45 onto DNA at entry sites such as primer-template junctions or single-stranded DNA gaps by its clamp loader, the gp44-gp62 complex. Once loaded onto DNA, the donut-shaped gp45 trimer slides along DNA by one-dimensional diffusion. The ligands of gp45 include two phage T4-encoded subunits of the T4 late RNA polymerase holoenzyme: the late promoter-recognition protein gp55 and the gp33 co-activator of late transcription (5–7). It is through these interactions that gp45 becomes part of the transcription initiation complex at the open T4 late promoter. In the T4-infected E. coli cell, transcription of the late genes requires gp55, gp33, and gp45. Replication-dependence and replication-coupling of late transcription in vivo is thought to be related to the requirement for continuously reloading gp45 onto DNA (8).

Correct and productive initiation of transcription at T4 late promoters is also generated in vitro by E. coli RNA polymerase core enzyme, E, and gp55 (9). In the experiments that are described below, we compare this basal transcription, in which RNA polymerase operates autonomously, with activated transcription, in which RNA polymerase is constrained by its sliding clamp, in regard to abortive initiation of transcription.

The $\sigma$-family subunits of bacterial RNA polymerases can also play an important role in determining how the transcription complex leaves the promoter. For example, a start-proximal downstream binding site on the non-transcribed DNA strand

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for region 2.4 of E. coli $\sigma^{70}$ generates an extended promoter-proximal pausing event that is an essential component of the regulation of bacteriophage λ late transcription (10, 11). It is conceivable, therefore, that individual $\sigma$ proteins generate characteristic patterns of abortive transcription. Examining this issue requires a promoter at which two $\sigma$ proteins can direct initiation of transcription at the identical nucleotide. We have modified a T4 late promoter for this purpose and report a comparison of abortive initiation generated with E. coli $\sigma^{70}$ and T4 gp55.

**EXPERIMENTAL PROCEDURES**

**Proteins and Chemicals**—DNA exonuclease III, Taq DNA polymerase, restriction enzymes, DNase I, protease K, nucleotides, as well as RNA and DNA oligonucleotides were purchased. T4 AsiA protein was generously provided by M. Ohshamouch and E. N. Brody. The purification of T4 gp32, gp45, and gp44-62 complex from overproducing bacteria was described and referenced previously (12, 13). E. coli strain RL721, which encodes an RNA polymerase $\beta'$ subunit with a hexahistidine affinity tag at its C-terminal 29 amino acids, was provided by R. Landick and K. Severinov. We also thank J. Abelson for a gift of tr- and t6-encoded proteins.

**Purification of $\beta'$-(His)6 Tagged E. coli RNA Polymerase Core**—E. coli RL721 was lysed and processed for chromatography on Ni-NTA agarose essentially as described for the native purification of the yeast transcription protein $\beta'$ (138–594) (14). The material eluting from Ni-NTA agarose with buffer B (40 mM Tris, pH 8.0, 7 mM MgCl$_2$, 10% (v/v) glycerol, 0.01% (v/v) Tween 20, 7 mM $\beta$-mercaptoethanol, 0.5 mM phenylmethylsulfonfluoride) also containing 100 mM NaCl and 200 mM imidazole was applied to Bio-Rex 70, washed stepwise with buffer C (40 mM Na-Hepes, pH 7.8, 100 mM NaCl, 0.05 mM ZnSO$_4$, 0.1 mM EDTA, 10% (v/v) glycerol, 0.01% (v/v) Tween 20, 10 mM $\beta$-mercaptoethanol, and 0.5 mM phenylmethylsulfonfluoride), and buffer C supplemented successively with 100, 150, 200, and 250 mM NaCl. RNA polymerase eluted in buffer C + 500 mM NaCl. Bio-Rex chromatography with stepwise elution was then repeated, yielding purified RNA polymerase core, whose concentration was determined from its optical density at 280 nm (weight extinction coefficient = 5.5 x 10$^{5}$ cm$^{-1}$ g$^{-1}$).

DNA—Plasmid pRT510-C+18 (15), a 32 bp derivative of pH310 (16), contains a derivative of the T4 gene 23 late transcription unit essentially as described for the native purification of the yeast transcription protein $\beta'$ (138–594) (14). The material eluting from Ni-NTA agarose with buffer B (40 mM Tris, pH 8.0, 7 mM MgCl$_2$, 10% (v/v) glycerol, 0.01% (v/v) Tween 20, 7 mM $\beta$-mercaptoethanol, 0.5 mM phenylmethlysulfonfluoride) also containing 100 mM NaCl and 200 mM imidazole was applied to Bio-Rex 70, washed stepwise with buffer C (40 mM Na-Hepes, pH 7.8, 100 mM NaCl, 0.05 mM ZnSO$_4$, 0.1 mM EDTA, 10% (v/v) glycerol, 0.01% (v/v) Tween 20, 10 mM $\beta$-mercaptoethanol, and 0.5 mM phenylmethlysulfonfluoride), and buffer C supplemented successively with 100, 150, 200, and 250 mM NaCl. RNA polymerase eluted in buffer C + 500 mM NaCl. Bio-Rex chromatography with stepwise elution was then repeated, yielding purified RNA polymerase core, whose concentration was determined from its optical density at 280 nm (weight extinction coefficient = 5.5 x 10$^{5}$ cm$^{-1}$ g$^{-1}$).

**RESULTS**

**The Pattern of Transcript Accumulation**—The T4 late transcription unit from pRT510-C+18 (5), which has been used for these experiments, has the consensus T4 late TATA box –10 site, TATAAATA, and the transcriptional start region of T4 gene 23 (which encodes the major phage head protein), reconstructed to generate transcripts with a 17-nt C-less 5' end (Fig. 1a). When open complexes at this promoter are presented with the dinucleotide primer GpA, ATP, UTP, and GTP, they generate nascent 17-mer transcripts that are arrested before CCC . . . (bp 18–20) and can be elongated subsequently with the missing nucleoside.

Linear DNA was prepared for activated transcription by digestion with exonuclease III to create a primer-template junction downstream of the promoter that serves as the DNA- loading site for the T4 gp45 sliding clamp transcriptional activator. DNA loading of gp45 by the T4 gp44-62 complex requires dATP (or ATP) hydrolysis and was done in the presence of an excess of T4 gp32 single-stranded DNA-binding protein. The T4 late promoter is recognized by the E. coli RNA polymerase core enzyme, E, in combination with the T4 late promoter-specific $\sigma$-family initiation protein gp55. Transcription in basal mode was done with Egp55 only, and was also examined under conditions of partial repression by gp33. Gp45-activated (enhanced mode) transcription required gp33 and gp44-62 complex in addition to gp55.

Fig. 1b shows the transcripts generated in an enhanced mode transcription experiment. DNA was incubated with all proteins at 25 °C for 3 min, then GpA, ATP, GTP, and UTP were added for 15 min. The [32P]-UMP-labeled oligonucleotides generated during this time were treated with alkaline phosphatase to convert [a-32P]UTP to $^{32}$P, before being resolved on a 25% polyacrylamide gel. Initial phosphodiester bond formation was dependent on priming by the dinucleotide GpA when ATP, UTP, and GTP were kept at 5 μM, as expected (data not shown).

The distribution of products was dominated by two bands, B and N, with smaller quantities of other components. Band N is the nascent 17-mer transcript; it was extended by 1 nt in the presence of 3'-O-methyl-CTP. (It was also slowly extended in the absence of 3'-O-methyl-CTP by error incorporation of UMP, yielding band N', barely apparent in Fig. 1b.)

The identities of other bands were established in two ways: 1) extraction of material from the gel and resolution by thin layer chromatography with non-radioactive markers (GpA gpApUpA, etc.; data not shown); 2) abortive transcription primed with the same series of (longer) oligoribonucleotides and one [a-32P]NTP, or with GMP and [a-32P]ATP. These experiments established that band B contains the trinucleotide GpApU and pentamer GpApUpA, band A the tetramer GpApUpApA, band C the hexamer GpApUpApUpA. Bands D and E constitute the 7-mer GpApUpApUpGpA, and 8-mer GpApUpA-pApUpGpApApGpApA, respectively (data not shown). The ladder series of 9-mer to 17-mer bands can be counted up from band F to band E constitute the 7-mer GpApUpApUpGpA, and 8-mer GpApUpA-pApUpGpApApGpApA, respectively (data not shown). The ladder series of 9-mer to 17-mer bands can be counted up from band F to

Evidence that band B also contains GpApUpApU penta-
mer was provided by the observation that the principal U-labeled transcript generated with GpAUpA primer and labeled UTP co-migrates with band B (data not shown). This pentanucleotide was identified as a minor product in band B from an experiment like Fig. 1b by eluting material and further separation by thin layer chromatography. By quantifying the distribution of radioactivity, we determined that the 5-mer product constituted ;0.5% of the 3-mer product in band B (on a molar basis). The arrested transcription products also included an ;24-nt transcript (band O, Fig. 1b) whose presence has not been determined except to show that it is arrested before C, since it was extended by 1 nt in the presence of 3′O-methyl-CTP.

The Source of Minor Transcripts—Since the transcripts in bands D to M are all present in small quantities relative to the arrested 17-mer transcript, we examined whether they were in fact generated by initiation at the T4 late promoter with Egp55. This was done in two ways, first, by comparing RNA synthesis by RNA polymerase core, E, and by basal as well as enhanced transcription by Egp55. For each enzyme, the products generated with GpA, ATP, GTP, and [α-32P]UTP are shown before further addition at the left (the pulse), after subsequently adding 3′O-methyl-CTP in the middle, and after subsequent addition of all four ribonucleoside triphosphates at the right (the chase).

Fig. 1. The short transcripts. a, the T4 late promoter in pRT510-C+18 and its TATA-less “No −10” variant. b, transcripts of pRT510-C+18 made with GpA, ATP, GTP, and [α-32P]UTP. Transcription in enhanced mode (see “Experimental Procedures”). Proteins and DNA were preincubated for 3 min at 25 °C before transcription for 15 min. c, comparison of transcripts generated by E. coli RNA polymerase core, E, and by basal as well as enhanced transcription by Egp55. Details as in panel c.
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next experiments examine whether attachment of the T4 late RNA polymerase to the sliding clamp transcriptional activator changes the distribution of transcripts, especially of non-chaseable (abortive and/or dead-end) transcripts. Only minor differences in the products generated during the transcription pulse were noted (Fig. 3a). In basal as in activated transcription, the distribution of shorter transcripts was dominated by band B. Only the 6-mer (band C) was produced in quantities comparable with 17-mer. The proportion of 3-mer to 17-mer was approximately 2-fold higher for basal than for activated transcription (Fig. 3a). A comparison of the size and abundance distributions among the non-chaseable transcripts for basal and enhanced transcription (Fig. 3b) shows, somewhat surprisingly, a greater proportion of 6-mer, 7-mer, and 8-mer relative to 3-mer transcripts in enhanced transcription. On a per-17-mer basis, the yields of all these non-chased products were low: 0.22, 0.19, and 0.06 molecules for the 6-mer, 7-mer, and 8-mer, respectively, in unenhanced transcription and 0.84, 0.50, and 0.14, respectively, for enhanced transcription (after correcting for products generated by core enzyme alone; average of four determinations).

Further examination of the data in Figs. 1c, 2, and 3 shows that the fraction of 6-mer to 9-mer transcripts that can be chased into longer products was 4–6-fold greater for basal transcription than for enhanced transcription. Transcripts of this length that are arrested before C do not come from the late promoter in pRT510-C+18. The significance of this result is that gp45 also increases specificity on this DNA template, probably by activating transcription at the T4 promoter more efficiently than at nonspecific sites.

The time course of accumulation of 17-mer and band B transcripts is compared in Fig. 4. Activation of transcription by gp45 clearly increased the yield of the abortive 3-mer and productive 17-mer transcripts alike, but also increased the relative proportion of 17-mer to 3-mer products. If every open promoter complex were converted to ternary transcription complexes arrested at bp 17, the production of GpAp*U should eventually cease. The fact that this did not happen indicates that some RNA polymerase molecules did not leave the promoter, either in enhanced or unenhanced transcription.

Comparing RNA Polymerase Core Enzymes—Short oligonucleotides are generated in transcription not only by abortive or arrested chain elongation, but also by hydrolytic retraction of RNA chains. In E. coli RNA polymerase, hydrolytic retraction requires GreA or GreB protein (18, 19). To establish whether the abortive and other short transcripts show indications of contribution from RNA chain retraction, we compared our purified E. coli RNA polymerase with enzyme reconstituted from its α, β, and β′ subunits (generously made available by A. Goldfarb, E. Nuñer, and K. Severinov). We noted only one minor difference in enhanced transcription by these two enzymes: a late promoter-specific 8-mer non-chaseable transcript that was generated by the purified E. coli RNA polymerase at a low rate (Fig. 4b), was not generated by the reconstituted enzyme (data not shown).

The RNA polymerase α subunit is modified after phage T4 infection in its C-proximal domain, at Arg-265 by ADP-ribosylation. This C-terminal domain is far removed from the catalytic site (20) and it does not participate in activation of T4 late transcription by gp45 (21). T4-modified RNA polymerase also contains a T4-encoded subunit, RpbH (22), which has been shown to confer salt sensitivity on ternary complexes formed with T4-modified RNA polymerase (23) and could conceivably affect the initiation phase of transcription. Our T4-modified RNA polymerase core, prepared from T4-infected cells, is contaminated with residual amounts of α70. Because the T4 late
promoter in pRT510-C+18 also contains a −35 consensus TT-GACA element (Fig. 1a), it is also transcribed by E-σ²⁰ RNA polymerase (5). To suppress such σ²⁰-mediated transcription, the T4-modified RNA polymerase was supplemented with the σ²⁰ antagonist AsiA (24–26) along with all the components required for T4 late transcription. Total and abortive transcripts generated by the unmodified and phage T4-modified RNA polymerase core enzymes in gp55-dependent enhanced late transcription did not differ significantly (data not shown).

Comparing σ-Proteins: σ²⁰ and gp55—The insertion of a consensus −35 site at the T4 late promoter in pRT510-C+18 (Fig. 1a) makes this a strong promoter for the E. coli E-σ²⁰ holoenzyme. We compared σ²⁰- and gp55-generated abortive and productive transcripts in the experiment that is summarized in Fig. 5. Transcription in the presence of GpA, ATP, GTP, and UTP yielded the 17-mer transcript, indicating initiation at the same site with Egp55 and E-σ²⁰. Nevertheless, E-σ²⁰ yielded short and non-chased transcripts in an entirely different pattern. Much less band B transcript was produced relative to 17-mer by E-σ²⁰ than in enhanced transcription by Egp55, and the yield of band D transcript, the 6-mer, relative to band B transcript (predominantly 3-mer) was much higher for E-σ²⁰ than for Egp55. Control transcription of “No −10” DNA (Fig. 5, two bottom traces) indicated that these E-σ²⁰ transcripts came almost exclusively from the intended site.

**DISCUSSION**

One non-productive transcript, the trinucleotide, is made at an exceptionally high rate when the T4 late holoenzyme, Egp55, initiates transcription at the T4 late promoter (with the dinucleotide GpA) (Fig. 1). Addition of that first nucleotide to GpA is clearly non-processive, since production of all other transcripts is very low compared with the yield of GpApU (Figs. 1 and 4). Adding three more nucleotides drastically changes
GpA, ATP, GTP, and \[5\text{ min and samples were taken at the indicated times after addition of}\]

The polymerase and DNA were preincubated for 5 min and RNA synthesis proceeded for 6 min. For the chases, RNA synthesis then continued for 5 min in the presence of all four NTPs (at 1 mM each). The patterns of transcription by \(E\sigma^{70}\) and \(E\cdot\sigma^{70}\) transcripts of “No – 10” DNA, as control.

processivity: an estimate is arrived at by assuming that all 6-, 7-, and 8-mer transcripts are abortive rather than arrested, the yields of these products relative to 17-mer signifying a processivity greater than \(-0.7\) for addition of the 7th and 8th nucleotides. The rarity of 4-mer and 5-mer transcripts (relative to 6-mer) also implies an especially low processivity confined to the first step of chain elongation. It is interesting to compare these findings with the situation encountered in transcription of the \(SUP4\) tRNA\(^{TV}\) gene by yeast RNA polymerase III, which is brought to its promoter by the DNA-bound transcription factor IIIIB. The yield of all the abortive pol III transcripts, including the initially formed pppApA, is low, implying a relatively high processivity (greater than \(-0.5\)) even for the earliest step of RNA chain elongation, and showing that translocation of the polymerase or of its transcription bubble along the DNA template (27) is not a prerequisite of processive RNA chain elongation.

Transcriptional activation by the gp45 sliding clamp clearly increases abortive production of GpApU along with productive 17-mer synthesis (Figs. 1c and 4). One might have considered, \textit{a priori}, that transcriptional activation by gp45 could operate primarily to relieve a block on the transition from abortive to productive transcription. That is evidently not the case; although the proportion of 17-mer transcript to abortive GpApU is increased by the activator and co-activator, the change is quantitative (up to \(-3\)-fold in Figs. 3 and 4) rather than absolute, and clearly cannot account for more than a small part of the very large transcriptional activation achievable \textit{in vitro} (28, 29). A contrasting example of activator action is provided by recent experiments on regulation of T4 middle genes (30). T4 middle promoters are under positive control of the MotA and AsiA proteins. MotA binds to its “Mot box” DNA site 30 bp upstream of the transcriptional start. AsiA, the “anti-sigma” protein first identified by Stevens (26) binds to \(\sigma^{70}\), blocks T4 early transcription by \(E\cdot\sigma^{70}\), and is the essential co-activator of MotA for transcription at T4 middle promoters (reviewed in Ref. 24). MotA and AsiA generate their activation of middle transcription partly by recruiting RNA polymerase to the promoter and partly by facilitating promoter escape. In the absence of AsiA, there is substantial abortive initiation at a middle promoter, but almost no production of full-length transcripts (30). The contrasting results presented here specify that facilitating the escape from the T4 late promoter can only be a small part of transcriptional activation by gp45 and gp33. The sliding clamp, which interacts directly with gp55 and gp33 (6–8) appears to be a device designed to bring RNA polymerase to the promoter. Whether it also facilitates subsequent steps leading to promoter opening is the subject of ongoing work.

We also examined the effect of gp33, which suppresses basal transcription by \(E\cdot gp55\) (12), on the generation of short transcripts. Gp33 suppressed GpApU synthesis along with production of 17-mer RNA (data not shown). Evidently, the suppression of basal transcription by gp33 is also not primarily generated by blocking the transition from abortive to productive transcription.

All other short transcripts are made by \(E\cdot gp55\) at a rate of less than one molecule per productive transcript (Fig. 3) during the time interval in which all the productive transcripts are generated (Fig. 4). A proportion of these other (longer) short transcripts are not extended upon provision of all four ribonucleoside triphosphates. Because they are relatively rare, we have not determined whether they are truly abortive (released from polymerase and DNA) or arrested (remaining in a ternary transcription complex but failing to elongate promptly despite the presence of the appropriate substrate). The patterns of production of these short non-chaseable transcripts show subtle differences between enhanced and basal transcription. There appears to be greater production of 6-mer and 7-mer transcript per 17-mer transcript in enhanced transcription at the T4 late promoter relative to basal transcription, perhaps reflecting a gp45 interaction with RNA polymerase in transition from the start site-confined holoenzyme to the elongation complex. It is interesting to note a somewhat similar effect at

\begin{footnote}{P. Bhargava and G. A. Kassavetis, manuscript in preparation.}\end{footnote}
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middle promoters: in addition to their key effects on promoter clearance, MotA and AsiA also generate 8-, 10-, 11-, and 12-mer transcripts as a sort of promoter-leaving signature of their transcriptional activation (30).

If all open promoter complexes were capable of leaving the transcriptional start site and moving down the DNA template, production of the abortive GpApU transcript should cease once all 17-mer nascent transcripts have accumulated. This is not quite what happens, although there is an initial burst of production of trinucleotide. The ultimate continuation of trinucleotide production suggests that a fraction of polymerase molecules do not or cannot leave the promoter. The experiments that are presented here are not well suited for measuring this proportion accurately, but we estimate it as not greater than 15–20%. A considerably higher proportion of similarly incompetent RNA polymerase molecules were noted in preparations of E. coli σ70-holoenzyme (31).

E. coli RNA polymerase generates short oligonucleotides hydrolytically by a process that is controlled by the GreA and GreB proteins. We have shown that this action does not make a substantial contribution to the generation of short oligonucleotides by Egp55 at the T4 late promoter, noting only the 8-nt non-chaseable transcript as possibly being generated by GreB-mediated hydrolysis. The post-infection modification of RNA polymerase core (in its α subunit) and attachment of the RpbA subunit had no effect on the generation of abortive transcripts.

The distribution and abundance of abortive transcripts, and the ability of polymerase to undock and form a stable, productive elongation complex varies markedly between promoters, due not only to the influence of initially transcribed sequence, but also responding to differences of polymerase affinity for the vicinity of the transcriptional start to KMnO₄ at the promoter (5). Differences of abortive initiation between Eσ70 at its cognate glnP2 promoter and Eσ70 at the lac UV5 and T7A1 promoters suggested to Tintut and co-workers (33) that differences of affinity of polymerase core for different σ-subunits may also influence the abortive phase of transcription. These comparisons involve situations in which the effects of promoter sequence, initially transcribed sequence, and polymerase constitution are convoluted. The construction of the hybrid prT510 promoter allowed us to make a head-to-head comparison of σ70 and gp55 driving abortive initiation at exactly the same site. To our knowledge, this is the first such direct comparison between σ proteins. Gp55 and σ70 generate different signatures in abortive initiation, Eσ70 holoenzyme producing a smaller proportion of non-chased short transcripts relative to the productive 17-mer than did Egp55. Moreover, the non-chased transcripts made by Eσ70 were differentiated by a relatively high yield of 6-mer relative to the 3-mer (and 5-mer) band B (Fig. 5). There is a clear correlation here with previously observed differences of σ70-directed and gp55-directed opening of this promoter (5): the reactivity of DNA in the vicinity of the transcriptional start to KmO₄ at the promoter is differently distributed when Eσ70 and Egp55 form open complexes at the hybrid promoter in prT510. The difference from σ70 is due to gp55 itself rather than gp33 and gp45 (compare Figs. 5 and 2), also consistent with the fact that basal and gp45-activated open promoter complexes of Egp55 are not distinguishable by permanganate footprinting (5). That σ70 could influence abortive initiation is consistent with the location of its segment 3.2 in the vicinity of the transcriptional start site (34, 35). Since gp55 is much smaller than σ70 and highly diverged from it in amino acid sequence, it may not reach to every site that is occupied by σ70 and may make different contacts with the core enzyme.

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