Tiny abortive initiation transcripts exert antitermination activity on an RNA hairpin-dependent intrinsic terminator

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ABSTRACT

No biological function has been identified for tiny RNA transcripts that are abortively and repetitiously released from initiation complexes of RNA polymerase in vitro and in vivo to date. In this study, we show that abortive initiation affects termination in transcription of bacteriophage T7 gene 10. Specifically, abortive transcripts produced from promoter φ10 exert trans-acting antitermination activity on terminator TΦ both in vitro and in vivo. Following abortive initiation cycling of T7 RNA polymerase at φ10, short G-rich and oligo(G) RNAs were produced and both specifically sequestered 5- and 6-nt C + U stretch sequences, consequently interfering with terminator hairpin formation. This antitermination activity depended on sequence-specific hybridization of abortive transcripts with the 5’ but not 3’ half of TΦ RNA. Antitermination was abolished when TΦ was mutated to lack a C + U stretch, but restored when abortive transcript sequence was additionally modified to complement the mutation in TΦ, both in vitro and in vivo. Antitermination was enhanced in vivo when the abortive transcript concentration was increased via overproduction of RNA polymerase or ribonuclease deficiency. Accordingly, antitermination activity exerted on TΦ by abortive transcripts should facilitate expression of TΦ-downstream promoter-less genes 11 and 12 in T7 infection of Escherichia coli.

INTRODUCTION

Transcription complexes of RNA polymerase are relatively unstable at the initiation versus elongation stage, repetitiously releasing short RNA transcripts and restarting initiation during the so-called ‘abortive initiation cycling’ step. Release of short transcripts has been observed in virtually all in vitro transcription reactions with various RNA polymerases (1–3), even at saturating concentrations of ribonucleotides (4), although the maximum sizes of abortive transcripts differ among RNA polymerases. For example, bacteriophage T7 and SP6 RNA polymerases generate abortive transcripts of up to 13 (5) and 6 nt (3), respectively, while E. coli RNA polymerase and human RNA polymerase II release transcripts up to 17 (6) and 8 nt (7), respectively. These are smaller than noncoding RNAs of 18–30 nt that mediate downregulation of gene expression (8,9).

Successful escape from abortive initiation cycling into processive elongation in vitro is as infrequent as one in tens or hundreds of reactions (10,11). The low possibility of promoter clearance and abundant production of abortive transcripts are not limited to in vitro reactions, as in vivo accumulation of abortive transcripts was recently detected in E. coli (12). However, no biological function has been identified for these abortive transcripts to date. In this study, we show for the first time that abortive transcripts from phage T7 promoters interfere with an intrinsic (or factor-independent) T7 terminator, TΦ, facilitating read-through of the terminator sequence and expression of TΦ-downstream genes. These tiny abortive transcripts may be the smallest among all the coding and noncoding RNAs that play a molecular role.

The phage T7 terminator, TΦ, can stop transcription with no requirement for additional factors via the typical class I intrinsic termination mechanism induced by specific features of RNA, including a stable hairpin structure and the immediately following oligo(U) sequence (13). This mechanism is distinct from that of another class II intrinsic termination activated upon specific DNA sequence recognition by T7 RNA polymerase (14). Regulation of terminators is classified into two categories. In an ‘antitermination’ mechanism, a regulatory molecule inhibits termination, allowing expression of downstream genes. By an ‘attenuation’ mechanism,
terminator-downstream gene expression is attenuated through facilitation of termination (15,16).

In both the antitermination and attenuation mechanisms, specific regulatory molecules interact with either RNA polymerase or transcript RNA to affect termination. Translating or stalling ribosome, RNA-binding proteins and tRNA interact with transcripts and alter RNA secondary structures to affect termination (16). In this study on T7 terminator Tϕ (Figure 1A), we show that tiny abortive transcripts produced from T7 promoters interact with transcript RNA (Figure 1B) and sequester 5- and 6-nt regions of the terminator hairpin-forming sequence (Figure 1C).

Expression of T7 genes 11 and 12 encoding tail tubular proteins depends entirely on read-through at Tϕ, since the two genes are located immediately downstream of Tϕ, but no promoter exists upstream (17). Accordingly, the intrinsic terminator, Tϕ, may be affected by abortive initiation, and this antitermination mechanism induced by abortive transcripts facilitates the production of T7 tail tubular proteins in T7 infection of E. coli.

MATERIALS AND METHODS

DNA templates

The pKM01 plasmid was constructed by replacing a 140-bp XbaI/BglI fragment of pET3 (18) with the oligonucleotide linkers, 5’-CTAGAGGATCCGAGCCCGGT and 5’-TAGGTACCCGGGTCTCGGATCCT-3’. A biotinylated 167 bp KM01 template was obtained from pHM01 by polymerase chain reaction with the forward primer, 5’-CGCGGTAGAGGATCGAGA-3’, and reverse primer, 5’-biotin-CCGGATATAGTTCCTCCTTCA-3’ that was biotinylated at the 5’ end, in 35 cycles of denaturation at 94°C, annealing at 57°C and extension at 72°C, each for 30 s.

Transcription reactions

In stepwise walking reactions (19), elongation complexes (ECs) stalled at 77 residues upstream (position T–77) from the termination site and containing 15 nt-long RNAs were obtained by incubating the biotinylated template KM01 (125 nM) bound to streptavidin-coated magnetic beads in 160 μl transcription buffer (40 mM Tris–HCl, pH 7.9, 6 mM MgCl2, 100 mM KCl and 10 mM dithiothreitol) containing 500 μM ATP, 500 μM GTP, 50 μM CTP, 40 μM of RNasin (Promega) and 2000 U of T7 RNA polymerase at room temperature for 20 min. ECs at position T–77 advanced to position T–74 in a reaction with 80 μCi of [α-32P]UTP (800 Ci/mmol, Perkin-Elmer). Radiolabeled ECs further progressed to downstream positions via repeated washing and incubation in transcription buffer containing 0.5 μM rNTPs. For incorporation of inosine monophosphate (IMP) into RNA transcripts, ECs were incubated with 5 μM inosine triphosphate (ITP, Ambion).

Both multi- and single-round transcription reactions were performed in 20 μl transcription buffer containing 200 μM rNTPs and 4 U of RNasin at room temperature. For pulse-chase labeling, incubation was continued with addition of 2 μCi [α-32P]UTP (800 Ci/mmol) for 3 min. The reaction was terminated by adding 20 μl loading buffer (12 M urea, 10 mM ethylenediaminetetraacetate (EDTA) and 0.1% bromophenol blue). Reaction products were separated using 8 M urea–12% polyacrylamide gel electrophoresis. Gels were dried and scanned with a Phosphor image analyzer Fuji BAS 3000, and band intensities quantified using TINA 2.0 software.

Ribonuclease protection assay

The radioactive probe was synthesized in 200 μl transcription buffer containing 0.5 mM NTP, 5 μCi of [α-32P]UTP (800 Ci/mmol), 5 pmol linear DNA, 40 U of RNasin and 200 U of T7 RNA polymerase at 37°C for 4 h and dissolved in a 600 μl hybridization solution (40 mM PIPES, 80% formamide, 0.4 M NaCl and 1 mM EDTA). RNA samples isolated from 20 ml E. coli culture using RiboPure Bacteria Kit (Ambion) were dissolved in 30 μl hybridization solution containing probe RNA. The RNA mixture was heated at 85°C for 5 min and incubated at 45°C for 16 h. Upon addition of 300 μl digestion buffer containing ribonucleases A and T1 (Ambion), incubation was continued at 37°C for 60 min. Digestion was terminated by incubation with 10 μl of 20% sodium dodecyl sulfate (SDS) and 2.5 μl of 20 mg/ml protease K at 37°C for 15 min. RNA was extracted using phenol, chloroform and isoamyl alcohol, prior to precipitation using ethanol, and dissolved in 10 μl loading buffer.

Figure 1. Antitermination at the T7 intrinsic terminator Tϕ. (A) Putative secondary structure of Tϕ RNA. Termination occurs mostly at the underlined G residue (position T). The 5’ end sequence of transcripts is also shown. (B) Model of antitermination mediated by abortive transcripts. Tiny abortive transcripts are released from initiation complexes and sequester critical parts of the terminator RNA hairpin to facilitate read-through at Tϕ. (C) Destabilization of the Tϕ terminator hairpin by hybridization of the abortive transcript oligo(G) with 6-nt bottom left and/or 5-nt top left part of the hairpin.
Products were analyzed on 8 M urea–12% polyacrylamide gels.

RESULTS
Tiny abortive transcripts interfere with intrinsic termination at Tφ

The linear template, KM01, contained a transcription unit from promoter φ10 to terminator Tφ, both cloned from phage T7 gene 10. Transcription by T7 RNA polymerase was terminated mainly at a G residue (position T) next to the oligo(U) sequence of Tφ (Figure 1A). Termination efficiency, monitored in time course pulse-labeling experiments of multi-round transcription, gradually decreased over reaction time with higher concentrations of T7 RNA polymerase (Figure 2A and B). The decrease curve approached a plateau level of 24% in a reaction with 80 U of RNA polymerase (Figure 2B, triangles).

In contrast, after ECs were stalled at position T–33 and purified from aborted and released transcripts in a stepwise polymerase walking assay using biotinylated DNA template and streptavidin-coated magnetic beads (19), termination efficiency was as high as 78% in a chase reaction of single-round transcription. Termination efficiency of Tφ was substantially reduced in multi-round transcription, possibly due to accumulation of released transcription products, which was enhanced with increasing polymerase concentrations and incubation times. The released products included 92-nt terminated transcripts, 115-nt read-through runoff transcripts and short abortive transcripts of various lengths (PR1, Figure 2C, lane 1).

When these transcripts were made nonradioactive, purified (PR1) and added to a single-round transcription reactions of ECs stalled at position T–33 and carrying 59-nt radioactively labeled RNA, termination efficiency in a chase reaction was 58% (Figure 2D, bar 2), which

Figure 2. Antitermination mediated by tiny abortive transcripts. (A) Decreasing termination efficiency with increasing reaction times and increasing concentrations of T7 RNA polymerase. In multi-round transcription reactions (20 µl), biotinylated DNA template (50 nM) was pre-incubated with 20, 40 or 80 U of T7 RNA polymerase and 200 µM NTPs at room temperature for varied time periods before radiolabeling of transcripts by incubation with 2 µCi of [α-32P]UTP (800 Ci/mmol) for 3 min. Terminated (T) and read-through runoff (R) transcripts were quantified in duplicate, and average termination efficiencies (TE) shown for each lane. (B) Plot of experimental data of (A) and abolishment of antitermination due to the prior presence of external C + U-rich RNA. Termination efficiencies (y-axis) measured in (A) are plotted against reaction time (x-axis). An experiment (inverted triangles) was additionally performed with 1 µM RNA of ACCCCUU and 80 U of T7 RNA polymerase. (C) Diverse transcripts produced at various NTP compositions. All four NTPs were present in the ‘PR1’ experiment, GTP and ATP in ‘PR2’ and GTP only in ‘PR4’, with each NTP at a concentration of 200 µM (+). In ‘PR3’, the concentration of GTP was 200 µM and ATP was 20 µM (±). Biotinylated KM01 template (50 nM) was transcribed with [γ-32P]GTP and 80 U of T7 RNA polymerase at room temperature for 30 min. Released RNA transcripts, including short products of various sequences and long terminated (T) and read-through (R) products, were purified using streptavidin-coated magnetic beads and resolved on denaturing 20% polyacrylamide gels. (D) Antitermination effects of various RNAs on Tφ. Termination efficiencies were measured in single-round transcription reactions of radiolabeled ECs stalled at position T–33 with 200 µM NTPs at room temperature in the absence (NC) or presence of nonradioactive transcripts produced in PR1, PR2, PR3 or PR4 experiments. These experiments were additionally performed in the presence of chemically synthesized RNA GGGGG (SR1, a product of reiterative transcription), GGGAGA (SR2, a product of template-dependent transcription) and ACCCCUU (SR3, a sequence in the left bottom of the terminator hairpin). In the last lane, SR3 RNA and PR1 transcripts were together added to the assay.
was 20% lower than the control reaction (78%) with no addition of transcripts (Figure 2D, bar 1). Accordingly, TΦ termination was disrupted by the transcription products in a trans-acting manner.

When short transcripts produced in a multi-round transcription reaction with nonradioactive GTP and ATP (PR2, Figure 2C, lane 2) were added to the single-round reaction of radiolabeled EC at position T−33, termination efficiency was 59% (Figure 2D, bar 3), similar to that obtained with all short and long transcripts (58%, Figure 2D, bar 2). The data indicate that short transcripts, rather than long transcripts, affect TΦ termination efficiency.

The short transcripts included diverse abortive initiation cycling products (Figure 2C, lane 2). Template-dependent transcription produced G-rich RNA sequences (such as G2, G3, 5′-GGGA-3′, GGGAG, GGGAGA) and reiterative transcription generated oligo(G) RNAs of various lengths (G4, G5, G6, etc.) due to slippage of RNA polymerase, as described previously (4,5). Accordingly, all or some of these tiny nascent products of abortive initiation cycling weakened TΦ in a trans-acting manner.

Oligo(G) RNA weakened TΦ more effectively than the other short transcripts. Oligo(G) sequences were dominant over the template-dictated G-rich transcripts in the presence of low or no ATP and high GTP levels (PR3 and PR4, Figure 2C, lanes 3 and 4, respectively), and reduced TΦ termination efficiency in trans by 28% (Figure 2D, bar 4 versus 1) and 30% (Figure 2D, bar 5 versus 1), significantly higher extents than template-dictated transcripts.

The results were reproduced with chemically synthesized RNAs. RNA of G5 (SR1) reduced the TΦ efficiency by 34% (Figure 2D, bar 6 versus 1), which was more effective than the RNA sequence, GGGAG (SR2), which induced only 12% reduction (Figure 2D, bar 7 versus 1). In contrast, the C + U-rich RNA ACCCCUU (SR3) did little affect termination (Figure 2D, bar 8 versus 1), although the sequence was complementary to a bottom right part of the terminator hairpin. Furthermore, when the G-rich short RNA (of PR1 products) was mixed with the SR3 RNA, little reduction in efficiency was observed (Figure 2D, bar 9 versus 1), indicating that the G-rich RNA was effectively sequestered by the C + U-rich SR3 RNA from inactivating TΦ. Accordingly, we propose that G richness in abortive transcripts is crucial in trans-antitermination of TΦ.

Tiny abortive transcripts interfere with terminator hairpin formation

Next, we examined whether abortive transcripts inactivate TΦ by destabilizing the terminator RNA hairpin structure rather than altering RNA polymerase conformation, since oligo(G) RNAs can hybridize with three different parts of the TΦ hairpin stem (Figure 3A), specifically, CCCCUU between positions T−38 and T−33 (at a bottom stem), CCUCU between positions T−28 and T−24 (including a top stem) and UCUUU between positions T−16 and T−13 (in a center stem). The resulting destabilized structures were simulated by incorporating IMP at specific G-residue positions, consequently weakening the top, middle and bottom stems separately.

When the bottom stem was weakened by IMP incorporation at positions T−12 to T−7, termination efficiency in single-round chase reactions was as low as 9.3% (Figure 3B). IMP incorporation at a top stem of T−19 to T−17 also led to a substantial reduction in termination efficiency (33% termination; Figure 3C). Furthermore, IMP incorporation at both the bottom and top stems almost abolished termination (4.3% termination; structure not shown). In contrast, IMP at the center stem of T−32 to T−29 did not affect termination efficiency to a significant extent (71% termination, Figure 3D). Our results indicate that the bottom and top stems are critical for TΦ activity while the central stem is dispensable.

A short RNA sequence (ACCCCUU, SR3) corresponding to a bottom left part of the stem between positions T−39 and T−33 was chemically synthesized and added to multi-round reactions. TΦ efficiency was only slightly reduced over a 2-h pre-incubation period (Figure 2B, inverted triangles). This C + U-rich RNA not only did not exert antitermination but also sequestered G-rich abortive transcripts from exerting antitermination, as observed in the single-round transcription experiments (Figure 2D, bar 9). Accordingly, we propose that sequence complementarity with the left (5′) side of the terminator stem, not the right (3′) side, is critical for antitermination, probably because the 5′ side is generated earlier and is more accessible to external RNA than the 3′ side.

Furthermore, a modified terminator (Figure 3E) in which two base pairs in the bottom stem were reversed (bold) and three base pairs in the top stem were deleted (boxed) displayed initially lower termination efficiency (46%) than its wild-type counterpart (78%) due to the modification of two critical parts, which did not decrease further with longer pre-incubation times (Figure 3F, rectangles), in contrast to wild-type terminator efficiency (Figure 2A and B). The absence of trans-acting antitermination with the partially active modified terminator could be explained by the fact that abortive G-rich RNA could not hybridize with the 5′ (left side) part of the bottom or top stem in the modified terminator.

To establish whether hybridization between abortive transcripts and the 5′ part of the stem is necessary for antitermination, the modified TΦ terminator template was additionally altered in the φ10-proximal abortive-initiation region, so that sequence complementarity was restored in 8 base pairs between the two modified regions (Figure 3E, estimated ΔG = −10.2 kcal/mol). Termination efficiency of multi-round transcription reactions decreased with incubation time (from 36% initially to 17% in 2 h). Antitermination activity on modified TΦ was observed with abortive transcripts of complementarily modified sequences (Figure 3F, triangles). The results collectively suggest that G-rich abortive transcripts from T7 promoter φ10 inactivate the T7 intrinsic terminator TΦ by specifically sequestering a C + U-stretch in the bottom left and/or top left stem of the terminator RNA hairpin.
Dependence of T\textsubscript{\textphi} antitermination on sequence-specific RNA hybridization

We further examined whether abortive transcripts from T7 promoters other than \textphi10 also inactivate T\textsubscript{\textphi}. The T7 genome DNA has 17 promoters for T7 RNA polymerase (20), and short template-dictated transcripts of abortive initiation cycling are diverse in sequence and length (up to \textphi17 8 nt) (4,5). Firstly, 8-nt abortive RNAs, expected from all T7 promoters, were chemically synthesized and their antitermination effects separately measured in single-round transcription reactions. The effects were diverse (Figure 4A), ranging from no influence to a 69% reduction in termination efficiency, compared with the control reaction (82% termination) with no addition of short RNA. Among the 8-nt RNAs, the strongest antitermination effect was exerted by GGAGGUAC RNA from the promoter \textphi1.5 (13% termination), followed by GGGAGAGG RNA from promoters \textphi17 and \textphiOR (29% termination).

Next, 13-nt abortive RNAs were synthesized and tested. The effects of these transcripts did not differ significantly from those of 8-nt RNAs, except for those from promoter \textphi6.5. The 13-nt RNA (GGGAGAUAGGGGC) from \textphi6.5 induced a 77% reduction, whereas 8-nt RNA (GGGAGAUAC) induced only a 5% reduction (Figure 4A). The results clearly indicate that abortive transcripts of \textphi6.5 (13 nt) and \textphi1.5 (8 nt) exert very strong trans-acting antitermination activity on T\textsubscript{\textphi} (5 and 13% termination, respectively), possibly due to the formation of 7 and 6 consecutive base pairs with the top left stem of T\textsubscript{\textphi}, respectively (Figure 4B).

Plotting of the termination efficiencies measured in the presence of various short RNAs against the free energy changes estimated for the most stable intermolecular duplexes formed between external short RNA and the 5' (left-side) half RNA of terminator T\textsubscript{\textphi} (Figure 4A) revealed a statistically significant \(P = 2.3 \times 10^{-8}\) positive linear correlation \(r^2 = 0.83\). In contrast, no correlation was evident \(r^2 = 0.083, P = 0.25\) when abortive RNAs were annealed to the 3' (right-side) half of T\textsubscript{\textphi} RNA, explaining why a short RNA (ACCCCUU) capable of base pairing with a bottom right part of the stem did not hamper terminator hairpin formation (Figure 2B, inverted triangles). In contrast, synthetic RNA (AAGGGGCU) fully complementary to the bottom left part of the stem completely abolished termination (Figure 4A), confirming that this region is essential.

![Figure 3. Antitermination-sensitive parts of T\textphi hairpin RNA destabilized by IMP incorporation.](image-url)
for Tφ termination and external RNA hybridization with the 5′ side, but not the 3′ side, is effective in antitermination.

Transcription from eight T7 promoters (φ1.3, φ3.8, φ6.5, φ9, φ10, φ13, φ17 and φOR) starts with 5′-GGG, and oligo(G) RNAs that are more potent than template-dictated RNAs are additionally produced by reiterative transcription from these promoters. Therefore, their trans-acting antitermination activities may be stronger than that shown in Figure 4A. For example, termination efficiency was significantly lower with all short transcripts from φ10 (59% termination, Figure 2D, bar 3), compared with that of the 8-nt RNA sequence (GGGAGACC) from φ10 (70% termination, Figure 4A).

The degree of trans-acting antitermination depended not only on the sequences and lengths of abortive transcripts but also on their amounts. Increasing amounts of the 8-nt RNA (GGAGGUAC) from φ1.5 induced an exponential decrease in Tφ termination efficiency (Figure 4C). The results of single-round transcriptions were consistent with those of multi-round transcriptions (shown in Figure 2A and B). The data indicate that abortive transcripts from specific T7 promoters exert trans-acting antitermination activity on Tφ upon sufficient accumulation.

In vivo antitermination of terminator Tφ

Finally, we examined whether sufficient quantities of abortive transcripts accumulate to inhibit Tφ activity in vivo, since they are subject to degradation by ribonucleases. As abortive transcripts have recently been detected within E. coli, antitermination of Tφ was examined in several E. coli strains containing different amounts of T7 RNA polymerase producing variable levels of abortive transcripts. In vivo termination efficiency of Tφ was estimated by measuring the amounts of terminated and read-through RNA using the ribonuclease protection assay. A radiolabeled probe of 114 nt was designed to hybridize with the read-through RNA at 108 nt (from position +127 to +234 with respect to the transcription initiation site +1) and with the terminated RNA at 85 nt (+127 to +211) (Figure 5A).

In E. coli BL21(DE3), one copy of the T7 RNA polymerase gene is incorporated in the chromosome under control of an isopropyl β-D-thiogalactopyranoside (IPTG)-inducible lacUV5 promoter, and polymerase levels are positively and linearly correlated with IPTG concentrations (21). BL21(DE3) was transformed with pET3a containing the promoter φ10, terminator Tφ and a replication origin of pBR322, and the lacUV5 promoter was induced with varying concentrations of IPTG. Termination efficiency was 95% at 10 μM IPTG, and decreased to 82% at 1 mM IPTG (Figure 5B, rectangles). Clearly, Tφ antitermination in vivo is correlated with the concentrations of IPTG used for induction of T7 RNA polymerase and, consequently, concentrations of abortive transcripts.

Another plasmid, pSAT7, also contains the T7 RNA polymerase gene under control of the lacUV5 promoter, but a replication origin of p15A, which renders pSAT7 compatible with pET3a. Upon co-transformation of BL21(DE3) with pSAT7 and pET3a, higher levels of T7 RNA polymerase and abortive transcripts are expected to be produced, compared to transformation with pET3a alone. The termination efficiency decreased at increasing concentrations of IPTG and was 66% at 1 mM IPTG.
**DISCUSSION**

To our knowledge, this is the first study to establish a biological function for abortive transcripts that are generally considered nugatory nonfunctional products of inefficient initiation of transcription. The abortive transcripts are diverse in size and sequence, but smaller than the 18–30 nt noncoding transcripts of RNA interference.

Our data show that tiny abortive transcripts (up to 8 or 13 nt) produced from several phage T7 promoters exert trans-acting antitermination activity on Tφ, a typical intrinsic T7 terminator of RNA hairpin-oligo(U), in a sequence-specific manner.

Termination efficiency was constant (around 49%) at variable IPTG concentrations (Figure 5D, rectangles), consistent with in vitro data showing no antitermination (Figure 3F, rectangles). In contrast, when BL21(DE3) was transformed with the other pET3a variant that was complementarily modified at both Tφ and ϕ10-proximal abortive regions (Figure 3E), termination efficiency diminished from 50% to 20% (Figure 5D, triangles), reflecting in vitro data showing antitermination (Figure 3F, triangles). These results confirm that trans-acting antitermination occurs in a sequence-specific manner, both in vivo and in vitro.
The underlying mechanism of T7 antitermination involves direct intermolecular sequence-specific interactions between terminator RNA and abortive transcripts, altering the terminator RNA secondary structure rather than RNA polymerase conformation. G-rich transcripts accumulating due to abortive initiation cycling at T7 promoters could effectively sequester 5- and 6-nt C+U stretches of T7 terminator RNA, preventing the formation of a terminator hairpin (Figure 1C). RNA that formed a more stable duplex with the 5′ half of the terminator exerted stronger interference activity on T7 than that forming a less stable duplex (Figure 4A). Since the correlation between the stability of intermolecular RNA duplex and reduction in termination efficiency was high \((r^2 = 0.83)\) and statistically significant \((P = 2.3 \times 10^{-8})\), it is more likely that RNA secondary structures, rather than RNA polymerase conformations, are modulated via interactions with the abortive transcripts.

Furthermore, antitermination was abolished when transcription occurred in the presence of an external C+U-rich RNA that could sequester the G-rich abortive transcripts (Figure 2B, inverted triangles and Figure 2D, bar 9) or when the terminator hairpin was modified to lack a critical C+U-stretch sequence (Figures 3E, 3F, rectangles and 5D, rectangles), but was restored both in vitro (Figure 3F, triangles) and in vivo (Figure 5D, triangles) when the abortive region sequence was changed to complement the modified terminator sequence (Figure 3E). Antitermination was enhanced in vivo when T7 RNA polymerase production was increased in E. coli (Figure 5B, asterisks versus rectangles) or degradation of abortive transcripts was reduced in an endonuclease-lacking E. coli strain (Figure 5B, triangles versus asterisks). Thus, antitermination of an intrinsic terminator via read-through by abortive transcripts was demonstrated both in vitro and in vivo.

In the T7 genome, T7 is positioned immediately upstream of the promoter-lacking genes 11 and 12 encoding tail tubular proteins (17), and expression of the two genes is entirely dependent on read-through at T7. The termination efficiency of T7 was very high (95%) in vitro at low concentrations of IPTG (Figure 3B, rectangles). If 5% read-through is not sufficient for abundant production of tail tubular proteins, the T7 terminator needs to be substantially inactivated during the course of T7 infection of E. coli. We propose a mode of trans-acting antitermination by abortive initiation transcripts as a possible mechanism for T7 inactivation (Figure 1B). G-rich abortive transcripts produced from T7 promoters \(\phi 1.5\) and \(\phi 6.5\) and oligo(G) RNA produced from \(\phi 1.3,\) \(\phi 3.8,\) \(\phi 6.5,\) \(\phi 9,\) \(\phi 10,\) \(\phi 13,\) \(\phi 17\) and \(\phi OR\) could induce strong antitermination of T7.

The timing of T7 antitermination is determined by the length of time it takes to accumulate T7-inactivating abortive transcripts to an effective concentration of antitermination. According to Figure 4C, several \(\mu M\) 8-nt abortive transcripts from the promoter \(\phi 1.5\) should be sufficient to induce an antitermination effect. The T7 lysozyme would facilitate antitermination by enhancing the concentration of abortive transcripts. T7 lysozyme accumulating late in T7 infection stimulates abortive initiation by T7 RNA polymerase (22), making it more likely that high levels of T7-inactivating abortive transcripts accumulate at a late stage during T7 infection of E. coli.

In summary, abortive initiation can affect intrinsic termination, as tiny transcripts produced by abortive initiation cycling from certain T7 promoters effectively inactivate the T7 intrinsic terminator, T7, in vivo and in vitro, interfering with formation of the terminator RNA hairpin. This antitermination mechanism depends on sequence-specific hybridization of G-rich abortive transcripts with 5- and 6-nt C+U-stretch sequences of terminator RNA, raising the possibility that this mode of antitermination occurs at other intrinsic RNA-hairpin terminators as well. Furthermore, during the course of T7 infection of E. coli, accumulation of T7-inactivating abortive transcripts may facilitate expression of downstream genes encoding tail tubular proteins.

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REFERENCES
1. Lescure, B., Williamson, V. and Sentenac, A. (1981) Efficient and selective initiation by yeast RNA polymerase B in a dinucleotide-primed reaction. *Nucleic Acids Res.*, 9, 31–45.
2. Carposious, A.J. and Graila, J.D. (1980) Cycling of ribonucleic acid polymerase to produce oligonucleotides during initiation in vitro at the lac UV5 promoter. *Biochemistry*, 19, 3245–3253.
3. Nam, S.C. and Kang, C.W. (1988) Transcription initiation site selection and abortive initiation cycling of phage SP6 RNA polymerase. *J. Biol. Chem.*, 263, 18123–18127.
4. Martin, C.T., Muller, D.K. and Coleman, J.E. (1988) Processivity in early stages of transcription by T7 RNA polymerase. *Biochemistry*, 27, 3966–3974.
5. Guajardo, R., Lopez, P., Dreyfus, M. and Sousa, R. (1998) NTP concentration effects on initial transcription by T7 RNAP indicate that translocation occurs through passive sliding and reveal that divergent promoters have distinct NTP concentration requirements for productive initiation. *J. Mol. Biol.*, 281, 777–792.
6. Hsu, L.M. (2002) Promoter clearance and escape in prokaryotes. *Biochem. Biophys. Acta*, 1577, 191–207.
7. Luse, D.S. and Jacob, G.A. (1987) Abortive initiation by RNA polymerase II in vitro at the adenovirus 2 major late promoter. *J. Biol. Chem.*, 262, 14990–14997.
8. Yeung, M.L., Bennasser, Y., Le, S.Y. and Jeang, K.T. (2005) siRNA, miRNA and HIV: promises and challenges. *Cell Res.*, 15, 935–946.
9. Carthew, R.W. and Sontheimer, E.J. (2009) Origins and mechanisms of miRNAs and siRNAs. *Cell*, 136, 642–655.
10. Lopez, P.J., Guillerez, J., Sousa, R. and Dreyfus, M. (1997) The low processivity of T7 RNA polymerase over the initially transcribed sequence can limit productive initiation in vivo. *J. Mol. Biol.*, 269, 41–51.
11. Vo,N.V., Hsu,L.M., Kane,C.M. and Chamberlin,M.J. (2003) In vitro studies of transcript initiation by Escherichia coli RNA polymerase. 3. Influences of individual DNA elements within the promoter recognition region on abortive initiation and promoter escape. *Biochemistry*, 42, 3798–3811.
12. Goldman,S.R., Ebright,R.H. and Nickels,B.E. (2009) Direct detection of abortive RNA transcripts in vivo. *Science*, 324, 927–928.
13. d’Aubenton Carafa,Y., Brody,E. and Thermes,C. (1990) Prediction of rho-independent Escherichia coli transcription terminators. A statistical analysis of their RNA stem-loop structures. *J. Mol. Biol.*, 216, 835–858.
14. Sohn,Y. and Kang,C. (2005) Sequential multiple functions of the conserved sequence in sequence-specific termination by T7 RNA polymerase. *Proc. Natl Acad. Sci. USA*, 102, 75–80.
15. Kumarevel,T. (2007) Structural insights of HutP-mediated regulation of transcription of the hut operon in Bacillus subtilis. *Biophys. Chem.*, 128, 1–12.
16. Gollnick,P. and Babitzke,P. (2002) Transcription attenuation. *Biochim. Biophys. Acta*, 1577, 240–250.
17. Carter,A.D., Morris,C.E. and McAllister,W.T. (1981) Revised transcription map of the late region of bacteriophage T7 DNA. *J. Virol.*, 37, 636–642.
18. Studier,F.W., Rosenberg,A.H., Dunn,J.J. and Dubendorff,J.W. (1990) Use of T7 RNA polymerase to direct expression of cloned genes. *Methods Enzymol.*, 185, 60–89.
19. Sohn,Y., Shen,H. and Kang,C. (2003) Stepwise walking and cross-linking of RNA with elongating T7 RNA polymerase. *Methods Enzymol.*, 371, 170–179.
20. Studier,F.W. and Rosenberg,A.H. (1981) Genetic and physical mapping of the late region of bacteriophage T7 DNA by use of cloned fragments of T7 DNA. *J. Mol. Biol.*, 153, 503–525.
21. Samul,R. and Leng,F. (2007) Transcription-coupled hypernegative supercoiling of plasmid DNA by T7 RNA polymerase in Escherichia coli topoisomerase I-deficient strains. *J. Mol. Biol.*, 374, 925–935.
22. Stano,N.M. and Patel,S.S. (2004) T7 lysozyme represses T7 RNA polymerase transcription by destabilizing the open complex during initiation. *J. Biol. Chem.*, 279, 16136–16143.