Published in final edited form as:
Science. 2013 June 28; 340(6140): 1577–1580. doi:10.1126/science.1237934.

Mechanism of Eukaryotic RNA polymerase III transcription termination

Soren Nielsen, **Yulia Yuzenkova**, and **Nikolay Zenkin***
Centre for Bacterial Cell Biology, Institute for Cell and Molecular Biosciences, Newcastle University, Baddiley-Clark Building, Richardson Road, Newcastle upon Tyne, NE2 4AX, UK

Abstract

Gene expression in organisms involves many factors and is tightly controlled. Although much is known about the initial phase of transcription by RNA polymerase III (Pol III), the enzyme that synthesizes the majority of RNA molecules in eukaryotic cells, termination is poorly understood. Here, we show that the extensive structure of Pol III – synthesized transcripts dictates the release of elongation complexes at the end of genes. The poly-T termination signal, while not causing termination in itself, causes catalytic inactivation and backtracking of Pol III, thus committing the enzyme to termination and transporting it to the nearest RNA secondary structure, which facilitates release. Similarity between termination mechanisms of Pol III and bacterial RNA polymerase suggests that hairpin-dependent termination may date back to the common ancestor of multi-subunit RNA polymerases.

Termination of transcription is an obligatory step following synthesis of the transcript, which leads to dissociation of RNA polymerase (RNAP) and the transcript from the template DNA. However, apparently different mechanisms are utilized by evolutionary conserved multi-subunit RNAPs from bacteria, archaea, and three eukaryotic RNAPs to terminate transcription (1-3). Pol III terminates after synthesis of a poly-U stretch (4, 5), and most studies have focused on the efficiency of recognition of the poly-T (on the non-template strand) termination signal (6). Both upstream and downstream sequences were shown to influence efficiency of recognition (7). However, the events leading to termination on the poly-T signal, i.e. dissociation of Pol III from the template, are not known.

We investigated this problem by using assembled elongation complexes, a technique successfully used to investigate various RNAPs (8-11). These complexes, assembled with purified RNAP, synthetic complementary template and non-template DNA strands and RNA, allow skipping the step of initiation and, therefore, excluding any accessory factors from the reaction. Complexes were immobilized on streptavidin beads via biotin on the 5′ end of the non-template strand (scheme in Fig. 1A). The RNA in complexes was radioactively labeled by incorporation of radioactive NMP (12). First, we analyzed transcription through poly-T signals of various lengths by purified *S. cerevisiae* Pol III. As seen from Fig. 1A, at poly-T signals longer than 5 nucleotides, transcripts finishing at the end the poly-T signal were formed. On long poly-T signals (12T), transcription was stopping predominantly after 6^{th}-10^{th} T (T_{12} template in Fig. 1B, lane 10). No stopping was observed on homopolymeric tracts other than poly-T (Fig. S1).

*Correspondence to: n.zenkin@ncl.ac.uk.

One Sentence Summary: Formation of the secondary structure of the transcript facilitates termination of transcription by RNA polymerase III

Supplementary Materials: www.sciencemag.org Materials and Methods Supplementary Text Figures S1-S8 Table S1 References (*23-27*)

Europe PMC Funders Author Manuscripts

We tested, if transcripts ending with a poly-U stretch were released from the template as a result of termination. This can be done by analysis of transcripts in the supernatant and immobilized fractions of the reaction ("super" and "beads" fractions, respectively, in scheme of Fig. 1B). As seen from Fig. 1B, while RNAs resulting from transcription to the end of template (Run off products) were released in the supernatant, transcripts ending at the poly-T signal were not released and remained part of the elongation complex, independently of the length of the transcript or sequences surrounding the poly-T signal (12). Most of the transcripts remained in the complex even after prolonged (30 minutes) incubation. The inability of Pol III and RNA to leave DNA was not due to the deficiency in the upstream DNA duplex restoration or to formation of an extended RNA-DNA hybrid (13) (Fig. S2A, B). These results indicate that Pol III pauses rather than terminates at the poly-T signal.

We analyzed transcription of the full length 5S and $tRNA^{Tyr}$ (*SUP4*) genes. Given the length of this gene, as a template, we used streptavidin-beads-immobilized double-stranded PCR products with a single-stranded extrusion at the 3′ end of the template strand and an RNA primer complementary to this extrusion (14) (see scheme in Fig. 2A and (12)). As expected, transcription ended in the poly-T signal, however the full length 5S and $tRNA^{Tyr}$ RNAs were readily released from the template (Fig. 2A, lanes 1-3 and 7-9, respectively). After just one minute of transcription almost all full length RNAs were found in the supernatant. Note that biotin for immobilization of these complexes was on the 5′ end of template strand, which slowed down release of the Run off products.

Transcript release during bacterial transcription termination is facilitated by an RNA hairpin that forms behind the poly-U tract (15). We noted that all transcripts synthesized by Pol III, as per their functions (structural or transfer RNAs), have very extensive secondary structures, so that the poly-U tract is always preceded by RNA hairpins and/or stems (Fig. S3 and Table S1). In contrast, the templates used above (Fig. 1 A, B) do not code for stable secondary structures. We therefore hypothesized that, as in the case of bacterial termination, termination by Pol III may also be facilitated by an RNA hairpin/stem, in this case, provided by the structure of RNA itself. To test this hypothesis, we changed the sequence of the 5S and $tRNA^{Tyr}$ genes to eliminate formation of RNA secondary structures close to poly-U stretch of the transcript (5S-HP and $tRNA^{Tyr}$-HP templates). As seen from Fig. 2A (lanes 4-6 and 10-12), the release of the transcripts ending in the poly-U signals of the mutant genes was indeed abolished.

To test the requirement for the RNA secondary structure during Pol III termination in the presence of transcription factors, we analyzed transcription in more native conditions by using *S. cerevisiae* nuclear lysates and promoter containing DNA templates. Given that any alterations to the secondary structure of $tRNA^{Tyr}$ destroy the internal promoter of Pol III, an unstructured spacer was introduced between the body of the $tRNA^{Tyr}$ and poly-U stretch. In full agreement with the results obtained with purified Pol III, the secondary structure preceding the poly-U tract was essential for termination (Fig. 2B).

To directly test the role of an RNA hairpin in termination by Pol III, we changed the sequence of template T_{12}, which did not allow for release (Fig. 2C, lanes 1-3), so, that the synthesized transcript formed a 9 bp long hairpin before the poly-U stretch (HP/T_{12} template in Fig. 2C). This indeed led to the release of the transcript ending in the poly-T signal, *i.e.* to termination (Fig. 2C, lanes 4-6). Addition of a short RNA complementary to the hairpin-less transcripts upstream of (but not far away from; see Fig. S4) the poly-U stretch, which mimics a termination hairpin (16), also resulted in efficient termination (Fig. 2C, lanes 7-9). These results also confirmed that transcript release was caused by RNA secondary structure close to the poly-U of the transcript, rather than the sequence of nucleic acids upstream of the poly-T signal. We also constructed a template, which coded for helix I of the 5S gene,

positioned upstream of the poly-T signal (HP_{5S}/T_{12} template in Fig. 2C). Note that this helix is formed by the most proximal 5′ and 3′ parts of the full-length 5S RNA (scheme in Fig. 2A). The shoulders of helix I were connected by a short loop. This hairpin also caused efficient termination in the poly-T signal (Fig. 2C, lanes 10-12).

For efficient termination by bacterial RNAP, the termination RNA hairpin has to be immediately upstream the poly-U stretch (1, 17). Consistently, a hairpin immediately upstream the poly-U tract also causes termination by Pol III (Fig. 2C). To test the requirements for the distance between hairpin and poly-U tract, we introduced unstructured spacers of different length between them (Fig. 2D). Surprisingly, we found that distance as large as ~12 base pairs between the poly-U stretch and the hairpin still allows for efficient termination (Fig. 2D, lanes 1-3). Longer spacers result in diminished termination (Fig. 2D, lanes 4-9; see also Fig. S4). These results suggest that an RNA hairpin formed within ~12 nt upstream of the poly-U stretch is sufficient for termination of the poly-T-paused complex.

The ability of the termination hairpin to act on the paused complex at a distance of ~20 nt from the 3′ end of the transcript, suggests that the paused complex should slide backwards to approach the hairpin. We analyzed the geometry of the paused elongation complexes carrying 8U (EC^{8U}) and 10U (EC^{10U}) tracts on the 3′ ends of their hairpin-less transcripts. Pure EC^{8U} and EC^{10U} were formed on T_8 and T_{10} templates (12), respectively, by transcription in the presence of UTP with subsequent washing away of unincorporated substrates and Mg^{2+}. To map the position of Pol III active center, we used the ability of RNAP active center to immobilize Fe^{2+} ion (instead of the native Mg^{2+}), which, by generating hydroxyl radicals, induces cleavage of the transcript in the vicinity of the active center (18). As seen from Fig. 3A, transcripts in EC^{8U} and EC^{10U} were cleaved in the 5′ proximal part of the poly-U tract (lanes 4, 9), indicating that the active center of Pol III has shifted backwards from the 3′ end of RNA. Protection from RNase A (a single-strand specific ribonuclease, which cleaves phosphodiester bonds after pyrimidines) was also consistent with a backtracked conformation, as bodies and poly-U tracts of the transcripts of EC^{8U} and EC^{10U} were mostly protected from RNase A (Fig. 3A, lanes 2 and 7, Fig. S5). In agreement with the length of the secondary channel (19), the very 3′ end proximal Us in EC^{10U} were exposed to RNase A, while all Us of the 8U tract of EC^{8U} were protected (Fig. 3A, lanes 2, 7, Fig. S5). Backtracking of the termination complex thus explains a loose (anywhere within ~12 nt) requirement for the positioning of the termination hairpin/stem upstream of the poly-T signal.

The backtracked conformation of the EC^{polyU} is consistent with the inability of the complex to extend the transcript (Fig. 3B, lanes 4, 5 and 9, 10). Note, however that this backtracking is unusual because the highly efficient hydrolytic activity of Pol III active center (14), that can rescue backtracked complex, is switched off (Fig. 3B, lanes 2, 3 and 7, 8; compare to lanes 12, 13 and Fig. S6B). As follows from our results, upon backtracking for long distances this activity is diminished ensuring the formation of a “dead-end” complex, whose only fate would be to terminate. On other sequences polymerizing and hydrolytic activities of Pol III are not affected (Fig. 3B, lanes 11-15; Fig. S6).

The distance between the Pol III active center and the RNA secondary structure required for termination is 7 nucleotides (Fig. S7, Supplementary Text), which notably resembles bacterial termination. Like the genes used in our study, most of the Pol III transcripts contain no or very short spacer between the poly-U signal and the nearest secondary structure (Table S1). Therefore the sole function of poly-T signal on these templates is to pause Pol III at ~7 nucleotides from the nearest secondary structure (Supplementary Text, Fig. S8). However, some transcripts synthesized by Pol III contain longer spacers between the poly-U tract and the nearest RNA duplex (Fig. S3 and Table S1) suggesting that the deep backtracking on the

poly-T signal of these genes is required to bring Pol III closer to the nearest secondary structure (Fig. S8). Note also that, in the case of short spacers, the 3′ penultimate RNA duplex (e.g. helix IV of 5S and TψC arm of tRNA) could also cause termination should the 3′ proximal hairpin fail to fold (Fig. S8).

Backtracking on the poly-T signal results in a strong (G, C rich) RNA/DNA hybrid within the termination complex, which however does not influence termination. Efficient hairpin-dependent termination can also be achieved on poly-G track (Fig. S7). Therefore, termination by Pol III does not require weak RNA-DNA hybrid as was postulated earlier (15), and the formation of an RNA hairpin/stem is sufficient to dislodge the paused elongation complex from the template. Backtracking and the presence of a non-homopolymeric RNA/DNA hybrid within the backtracked termination complex also excludes a possibilities of RNA/DNA hybrid shearing (20) or forward translocation (21). The results are consistent with the recently proposed “allosteric” mechanism of termination (22), when the RNA hairpin allosterically “opens” RNAP and leads to its dissociation from the template, though we cannot exclude the possibility that hairpin-dependent destruction of the Pol III elongation complex takes place via a different route.

The above results argue that the poly-T signal itself may not be sufficient for elongation complex dissolution. In our preliminary results (SUN and NZ, unpublished), we observed archaeal RNAP, which was proposed to terminate on the poly-T signal without involvement of additional factors (3), also fails to dissociate on the poly-T signal, while RNA hairpin preceding the poly-U tract is sufficient to cause termination. This suggests that archaeal RNAP may also utilize RNA-duplex-dependent termination, the mechanism which thus may date back to Last Universal Common Ancestor (LUCA) of the three kingdoms of life. Termination caused by structures embedded in the functional body of the transcript provide a simple factor-independent mechanism for dissolution of the elongation complex and may serve as a checkpoint for proper folding of RNA, which has been essential for the ribozymes of the LUCA, and remains essential for structural and transfer RNAs synthesized by Pol III.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank Jeff Roberts, Evgeny Nudler, Peter E. Geiduschek and Jeremy Brown for critical reading of the paper. We are grateful to George Kassavetis, Rob van Nues and Nicholas Proudfoot for experimental advises. This work was supported by UK Biotechnology and Biological Sciences Research Council, BBSRC under SysMO initiative and the European Research Council [ERC-2007-StG 202994-MTP].

References and Notes

1. Gusarov I, Nudler E. The mechanism of intrinsic transcription termination. Mol Cell. 1999; 3:495. [PubMed: 10230402]
2. Richard P, Manley JL. Transcription termination by nuclear RNA polymerases. Genes Dev. Jun 1.2009 23:1247. [PubMed: 19487567]
3. Santangelo TJ, Cubonova L, Skinner KM, Reeve JN. Archaeal intrinsic transcription termination in vivo. J Bacteriol. Nov.2009 191:7102. [PubMed: 19749050]
4. Matsuzaki H, Kassavetis GA, Geiduschek EP. Analysis of RNA chain elongation and termination by Saccharomyces cerevisiae RNA polymerase III. J Mol Biol. Jan 28.1994 235:1173. [PubMed: 8308883]
5. Bogenhagen DF, Brown DD. Nucleotide sequences in Xenopus 5S DNA required for transcription termination. Cell. Apr.1981 24:261. [PubMed: 6263489]

6. Orioli A, Pascali C, Pagano A, Teichmann M, Dieci G. RNA polymerase III transcription control elements: Themes and variations. Gene. Jun 25.2011
7. Braglia P, Percudani R, Dieci G. Sequence context effects on oligo(dT) termination signal recognition by Saccharomyces cerevisiae RNA polymerase III. J Biol Chem. May 20.2005 280:19551. [PubMed: 15788403]
8. Sidorenkov I, Komissarova N, Kashlev M. Crucial role of the RNA:DNA hybrid in the processivity of transcription. Mol Cell. Jul.1998 2:55. [PubMed: 9702191]
9. Zenkin N, Yuzenkova Y, Severinov K. Transcript-assisted transcriptional proofreading. Science. Jul 28.2006 313:518. [PubMed: 16873663]
10. Kuhn CD, et al. Functional architecture of RNA polymerase I. Cell. Dec 28.2007 131:1260. [PubMed: 18160037]
11. Wang D, et al. Structural basis of transcription: backtracked RNA polymerase II at 3.4 angstrom resolution. Science. May 29.2009 324:1203. [PubMed: 19478184]
12. Materials and methods are available as supplementary material on *Science* Online.
13. Zenkin N, Naryshkina T, Kuznedelov K, Severinov K. The mechanism of DNA replication primer synthesis by RNA polymerase. Nature. Feb 2.2006 439:617. [PubMed: 16452982]
14. Whitehall SK, Bardeleben C, Kassavetis GA. Hydrolytic cleavage of nascent RNA in RNA polymerase III ternary transcription complexes. J Biol Chem. Jan 21.1994 269:2299. [PubMed: 7507490]
15. Yager TD, von Hippel PH. A thermodynamic analysis of RNA transcript elongation and termination in Escherichia coli. Biochemistry. Jan 29.1991 30:1097. [PubMed: 1703438]
16. Yarnell WS, Roberts JW. Mechanism of intrinsic transcription termination and antitermination. Science. Apr 23.1999 284:611. [PubMed: 10213678]
17. Komissarova N, Becker J, Solter S, Kireeva M, Kashlev M. Shortening of RNA:DNA hybrid in the elongation complex of RNA polymerase is a prerequisite for transcription termination. Mol Cell. Nov.2002 10:1151. [PubMed: 12453422]
18. Zaychikov E, et al. Mapping of catalytic residues in the RNA polymerase active center. Science. Jul 5.1996 273:107. [PubMed: 8658176]
19. Westover KD, Bushnell DA, Kornberg RD. Structural basis of transcription: nucleotide selection by rotation in the RNA polymerase II active center. Cell. Nov 12.2004 119:481. [PubMed: 15537538]
20. Larson MH, Greenleaf WJ, Landick R, Block SM. Applied force reveals mechanistic and energetic details of transcription termination. Cell. Mar 21.2008 132:971. [PubMed: 18358810]
21. Santangelo TJ, Roberts JW. Forward translocation is the natural pathway of RNA release at an intrinsic terminator. Mol Cell. Apr 9.2004 14:117. [PubMed: 15068808]
22. Epshtein V, Cardinale CJ, Ruckenstein AE, Borukhov S, Nudler E. An allosteric path to transcription termination. Mol Cell. Dec 28.2007 28:991. [PubMed: 18158897]
23. Kassavetis GA, Han S, Naji S, Geiduschek EP. The role of transcription initiation factor IIIB subunits in promoter opening probed by photochemical cross-linking. J Biol Chem. May 16.2003 278:17912. [PubMed: 12637540]
24. Vannini A, et al. Molecular basis of RNA polymerase III transcription repression by Maf1. Cell. Oct 1.2010 143:59. [PubMed: 20887893]
25. Verdier JM, et al. Preparation and characterization of yeast nuclear extracts for efficient RNA polymerase B (II)-dependent transcription in vitro. Nucleic Acids Res. Dec 11.1990 18:7033. [PubMed: 2263463]
26. Roghanian M, Yuzenkova Y, Zenkin N. Controlled interplay between trigger loop and Gre factor in the RNA polymerase active centre. Nucleic Acids Res. May.2011 39:4352. [PubMed: 21266474]
27. Bochkareva A, Yuzenkova Y, Tadigotla VR, Zenkin N. Factor-independent transcription pausing caused by recognition of the RNA-DNA hybrid sequence. Embo J. Feb 1.2012 31:630. [PubMed: 22124324]

Europe PMC Funders Author Manuscripts

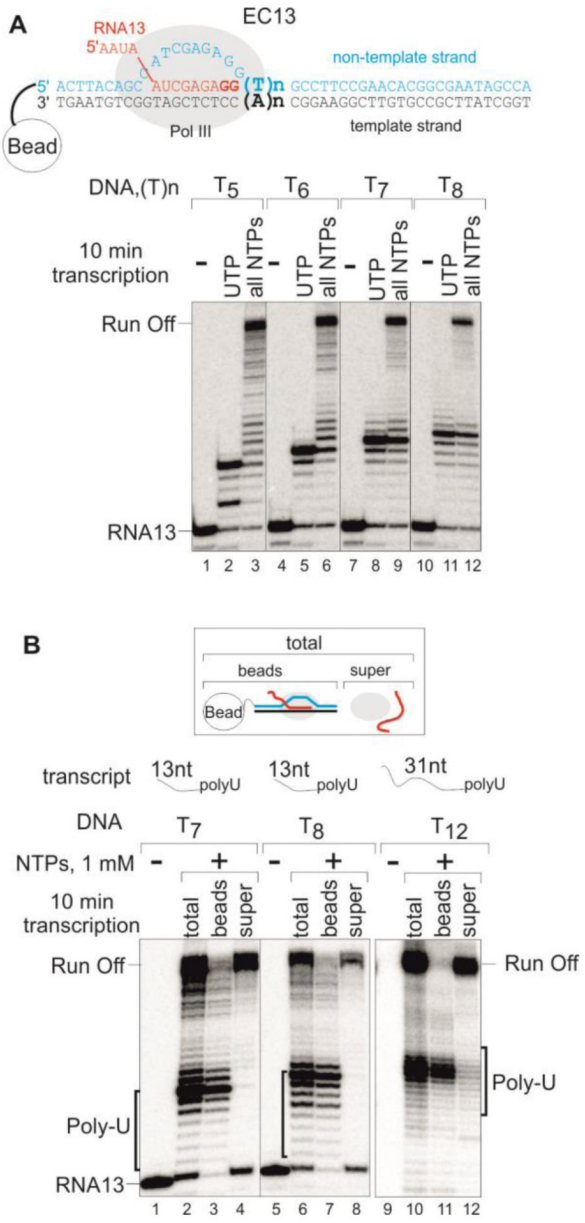

Fig. 1.

Pol III pauses on the poly-T signal but does not terminate. **A**. Scheme of assembled elongation complexes is shown at the top. RNA was radiolabelled at the 3′ end GMPs (bold) (12). Complexes were immobilized on beads via biotin on the 5′ end of the non-template strand. Transcription for 10 min on templates with poly-T signals of different lengths in the presence of 1 mM either UTP or all NTPs. Here and after black lines separate parts of one gel that were brought together. **B**. After 10 min transcription on the templates depicted above gels (12), released transcripts (“super”) were separated from transcripts that remained in the immobilized complexes (“beads”) (scheme in the frame above the gels). Length of RNA preceding poly-U tract is depicted above the gels.

Europe PMC Funders Author Manuscripts

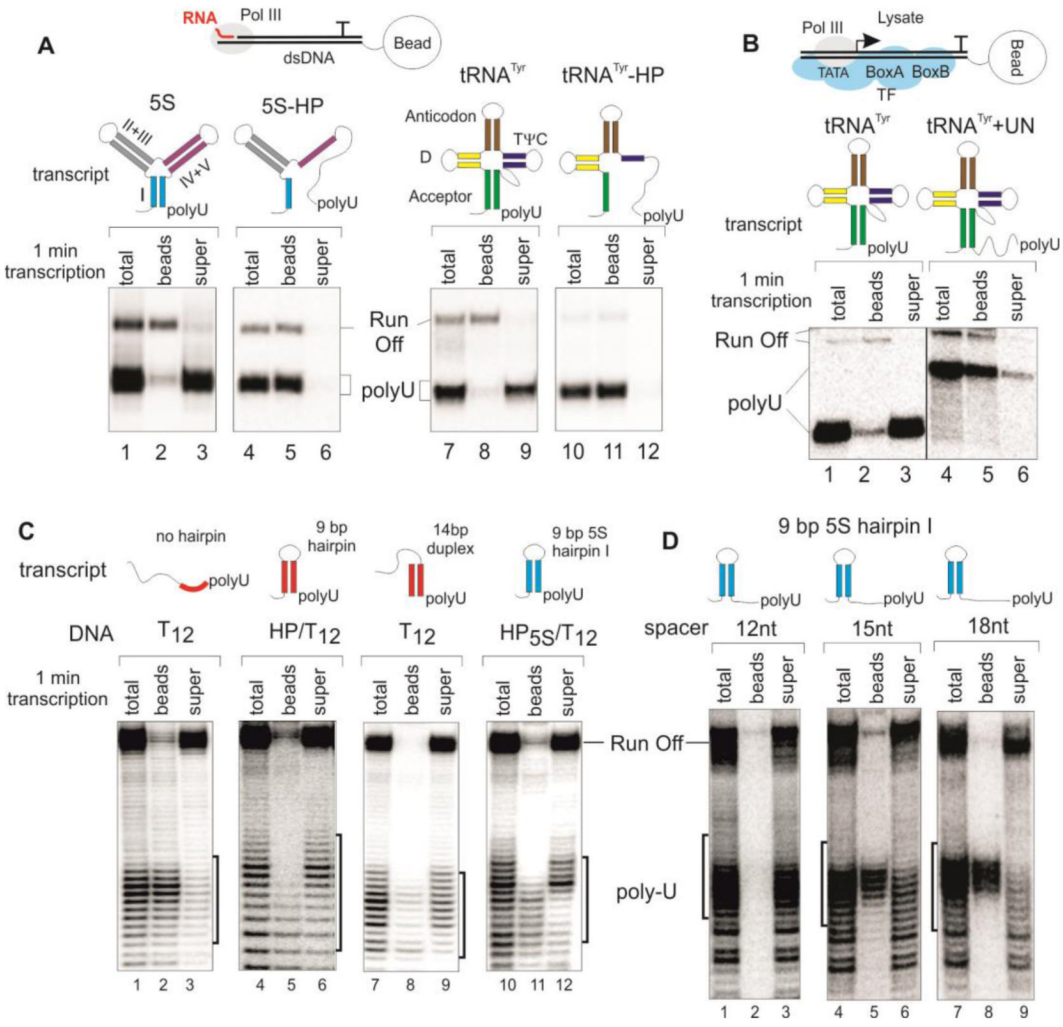

Fig. 2.

Termination by Pol III is facilitated by the secondary structure of the transcript. **A, B**. Termination by Pol III on full-length genes and their mutant variants lacking secondary structure before poly-U of the transcripts (5S-HP and $tRNA^{Tyr}$-HP, $tRNA^{Tyr}$+UN) (12). Release was analyzed after 1 min of transcription. **A.** Transcription was initiated by purified Pol III on the construct with the single-stranded overhang (scheme above the gels). **B.** Transcription was performed in yeast cell lysate on templates carrying promoter (promoter elements and transcription factors schematically shown above the gels). Secondary structure of $tRNA^{Tyr}$ transcript could not be altered as in panel A, because it would destroy the internal promoter of Pol III. **C.** Absence of release of transcript without hairpin (lanes 1-3), and release of transcripts containing arbitrary hairpin (lanes 4-6), duplex formed by externally added RNA oligonucleotide (lanes 7-9) or helix I of 5S RNA (lanes 10-12; see also scheme in panel A) before the poly-U tract (12). Release was analyzed after 1 min of transcription. **D**. Termination of transcripts (after 1 min of transcription) bearing spacers of different length between poly-U and termination hairpin (5S helix I) (12). See also Fig. S4.

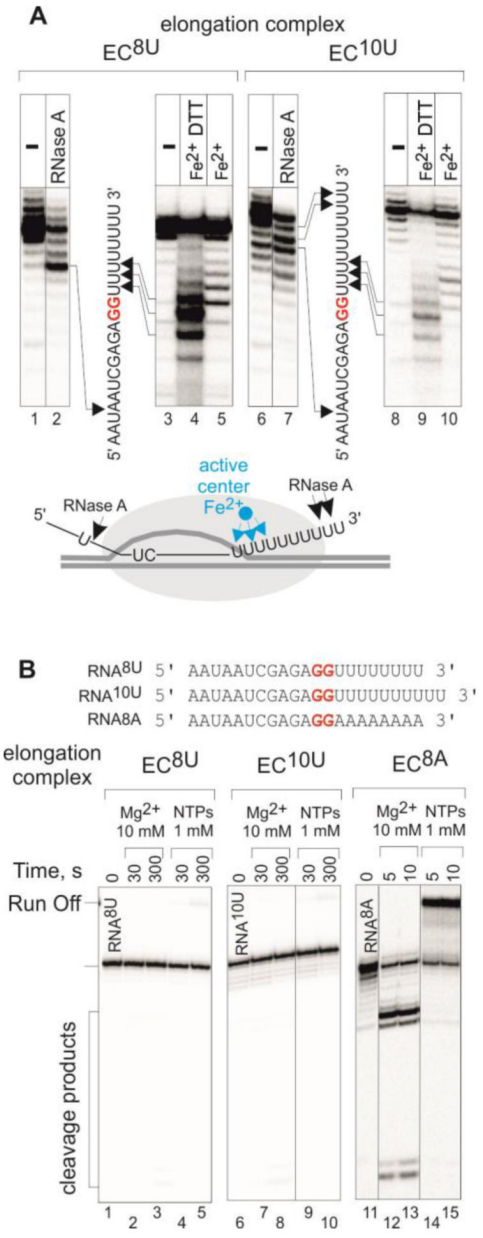

Fig. 3.

Complex paused on termination signal undergoes deep backtracking. **A**. Probing of paused complexes containing 8U and 10 U tracts at the 3′ ends of transcripts with RNase A and hydroxyl radicals generated by Fe^{2+} bound in the Pol III active center (scheme below the gels). Lanes 5 and 10 (without DTT) are controls for hydrolysis caused by Fe^{2+}. Radiolabels in transcripts are shown in red. Cleaved positions are shown with arrows. The identity of positions cleaved by RNase A was confirmed with 5′ end labeled RNA (Fig. S6). Interpretation of the probing results is shown schematically below the gels. **B**. RNA extension and hydrolysis in paused complexes containing 8U,10U and 8A tracks at the 3′ end of RNA (see also Fig. S6). Radiolabels in transcripts are shown in red.