Published in final edited form as:

Science. 2009 May 15; 324(5929): 927–928. doi:10.1126/science.1169237.

Direct Detection of Abortive RNA Transcripts in Vivo

Seth R. Goldman¹, Richard H. Ebricht², and Bryce E. Nickels^{1,*}

¹Department of Genetics and Waksman Institute, Rutgers University, Piscataway, NJ 08854, USA

²Department of Chemistry, Waksman Institute, and Howard Hughes, Medical Institute, Rutgers University, Piscataway, NJ 08854, USA

Abstract

During transcription initiation in vitro, RNA polymerase can engage in abortive initiation—the synthesis and release of short, 2 to 15 nucleotide, RNA transcripts—prior to productive initiation. It has not been known whether abortive initiation occurs in vivo. Using hybridization with locked nucleic acid probes, we directly detect abortive transcripts in vivo and thereby show that abortive initiation occurs in vivo. We further show that abortive initiation in vivo is a determinant of promoter strength, a determinant of RNA polymerase function, and a target of regulation by transcription factor GreA. Abortive transcripts may have functional, physiologically important roles in regulating gene expression in vivo.

During transcription, RNA polymerase (RNAP) synthesizes the first ~8–15 nucleotides (nt) of RNA as an RNAP-promoter initial transcribing complex (1–3) [using a “scrunching” mechanism (4)]. Upon synthesis of an RNA transcript of a threshold length of ~8–15 nt, RNAP breaks its interactions with promoter DNA, escapes the promoter, and enters into processive synthesis of RNA as an RNAP-DNA transcription elongation complex (1–3) [using a “stepping” mechanism (5)]. In transcription reactions in vitro, the RNAP-promoter initial transcribing complex can engage in tens to hundreds of cycles of synthesis and release of short RNA transcripts (“abortive initiation”) (1–3,6–8). Abortive initiation competes with productive initiation in vitro and, as such, is a critical determinant of promoter strength and a target of transcription regulation in vitro (1–3,7–13). It has been proposed that abortive initiation likewise occurs in vivo (6,8). In support of this proposal, factors that affect yields of full-length transcripts in vitro through effects on abortive initiation likewise affect yields of full-length transcripts in vivo (9–14). However, no direct evidence that abortive initiation occurs in vivo has been presented.

The bacteriophage T5 N25 promoter and its derivative N25anti are classic model systems for the study of abortive initiation and promoter escape (9,11–14). N25 and N25anti differ only in their initial transcribed sequences (positions +3 to +20) but exhibit radically different characteristics in vitro with respect to the abortive:productive ratio (APR; 40 for N25; ~300 for N25anti), the maximum size of abortive transcripts (10 nt for N25; 15 nt for N25anti), and the kinetics of promoter escape ($k_{\text{clear}} \sim 1.7$ per min for N25; ~0.06 per min for N25anti).

To determine whether abortive initiation occurs in vivo, we sought to detect abortive transcripts generated during transcription from a plasmid-borne copy of N25anti in *Escherichia coli*, using locked nucleic acid probes developed for hybridization-based detection of microRNAs (15, 16). We reasoned that the relatively high APR of N25anti would facilitate accumulation of detectable quantities of abortive transcripts and that the relatively high maximum size of

*To whom correspondence should be addressed. E-mail: E-mail: bnickels@waksman.rutgers.edu.

abortive transcripts of N25anti would facilitate efficient hybridization of the locked nucleic acid probes. To validate the method, we performed *in vitro* transcription reactions using *E. coli* RNAP and a DNA template carrying the N25anti promoter, a 100 nt transcription unit, and the tR2 terminator (N25anti-100-tR2). We performed parallel reactions using radioactive NTPs with analysis by autoradiography (Fig. 1A, left) and using non-radioactive NTPs with analysis by hybridization (Fig. 1A, right). Hybridization was able to detect abortive transcripts having lengths of 11–15 nt.

To detect abortive transcripts *in vivo*, we introduced a plasmid carrying N25anti-100-tR2 into cells, isolated RNA, electrophoresed RNA on urea-polyacrylamide gels, and visualized transcripts by hybridization (Fig. 1B). RNA samples from cells with the plasmid carrying N25anti-100-tR2 (but not from cells with a control plasmid lacking N25anti-100-tR2) exhibited transcripts corresponding in mobility to the 11–15 nt abortive transcripts observed *in vitro* (Fig. 1B). The 11–15 nt transcripts generated *in vivo* were observed with both N25anti-100-tR2 carried on a multi-copy plasmid (Fig. 1B) and N25anti-100-tR2 carried on a single-copy, F-plasmid-derived, plasmid (Fig. S1).

To show that the 11–15 nt transcripts detected *in vivo* are abortive transcripts, we demonstrated that they exhibit three hallmarks of abortive transcripts as defined *in vitro*: (1) altering interactions between RNAP and promoter DNA alters yields of the 11–15 nt transcripts (Figs. S2, S3) (see 1, 3, 10, 13, 17), (2) altering interactions between RNAP and transcription initiation factor σ alters yields of the 11–15 nt transcripts (Figs. S4, S5) (see 18, 19), and (3) transcription elongation factor GreA alters yields of the 11–15 nt transcripts (Fig. S6) (see 1, 3, 9).

We conclude that abortive initiation occurs *in vivo* (Figs. 1, S1–S7). We further conclude that abortive initiation is a determinant of promoter strength (Figs. S2, S3), a determinant of RNAP function (Figs. S4, S5), and a target of transcription regulation *in vivo* (Fig. S6). The results were obtained with bacterial RNAP. However, because abortive initiation occurs *in vitro* with all characterized RNAPs—bacterial, archaeal, eukaryotic, and bacteriophage—we consider it likely that abortive initiation occurs *in vivo* with all RNAPs. Analysis of initial-transcribed-region sequences of experimentally defined *E. coli* promoters indicates that as many as ~4,000 different 2–10 nt abortive transcripts may be generated *in vivo* (Table S1).

The finding that abortive transcripts are generated *in vivo*, and accumulate to detectable levels *in vivo*, raises the possibility that abortive transcripts may play functional roles. For example, an abortive transcript produced from a first promoter could function as a sequence-specific primer for transcription initiation at a second promoter or could function as an antisense effector against a specific RNA. Key priorities will be to define the full set of abortive transcripts produced *in vivo* (the “abortome”), and to define functional roles of abortive transcripts *in vivo*.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

References

1. Hsu LM. *Biochim Biophys Acta* 2002;1577:191. [PubMed: 12213652]
2. Murakami KS, Darst SA. *Curr Opin Struct Biol* 2003;13:31. [PubMed: 12581657]
3. Hsu LM. *Methods* 2009;47(1):25–36. [PubMed: 18948204]
4. Kapanidis AN, et al. *Science* 2006;314:1144. [PubMed: 17110578]
5. Abbondanzieri EA, Greenleaf WJ, Shaevitz JW, Landick R, Block SM. *Nature* 2005;438:460. [PubMed: 16284617]

6. McClure WR, Cech CL. *J Biol Chem* 1978;253:8949. [PubMed: 363713]
7. Carpousis AJ, Gralla JD. *Biochemistry* 1980;19:3245. [PubMed: 6996702]
8. Gralla JD, Carpousis AJ, Stefano JE. *Biochemistry* 1980;19:5864. [PubMed: 6450614]
9. Hsu LM, Vo NV, Chamberlin MJ. *Proc Natl Acad Sci USA* 1995;92:11588. [PubMed: 8524809]
10. Monsalve M, Mencia M, Rojo F, Salas M. *EMBO J* 1996;15:383. [PubMed: 8617213]
11. Chan CL, Gross CA. *J Biol Chem* 2001;276:38201. [PubMed: 11481327]
12. Hsu LM, Vo NV, Kane CM, Chamberlin MJ. *Biochemistry* 2003;42:3777. [PubMed: 12667069]
13. Vo NV, Hsu LM, Kane CM, Chamberlin MJ. *Biochemistry* 2003;42:3798. [PubMed: 12667071]
14. Kammerer W, Deuschle U, Gentz R, Bujard H. *EMBO J* 1986;5:2995. [PubMed: 3539590]
15. Information on materials and methods is available as supporting material
16. Valoczi A, et al. *Nucleic Acids Res* 2004;32(22):e175. [PubMed: 15598818]
17. Ellinger T, Behnke D, Bujard H, Gralla JD. *J Mol Biol* 1994;239:455. [PubMed: 8006961]
18. Ko DC, Marr MT, Guo J, Roberts JW. *Genes Dev* 1998;12:3276. [PubMed: 9784501]
19. Leibman M, Hochschild A. *EMBO J* 2007;26:1579. [PubMed: 17332752]
20. We thank A. Hochschild, A. Revyakin, and J. Roberts for materials, and A. Gann, S. Garrity, A. Hochschild, L. Hsu, W. McAllister, E. McGlinn, and R. Sousa for discussion. Work was supported by NIH grant GM41376 and a Howard Hughes Medical Institute Investigatorship to R.H.E. and by a Pew Scholars Award to B.E.N.

Figure 1. Abortive initiation occurs in vivo

A. Abortive initiation in vitro. Left, detection by autoradiography of transcripts generated in “hot” reactions. Right, detection by hybridization of transcripts generated in “cold” reactions.

B. Abortive initiation in vivo. Detection by hybridization of transcripts generated in vivo.