

# Transcription Termination: Pulling Out All the Stops

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In this issue, Larson et al. (2008) describe the use of optical traps to pull on the DNA template or RNA transcript and thereby explore the termination mechanism for *E. coli* RNA polymerase at intrinsic terminators. Their results imply that, depending on the nature of the terminator sequence, RNA polymerase uses either hypertranslocation or RNA:DNA shearing to destabilize the hybrid in the transcription bubble.

Intrinsic terminators for *E. coli* RNA polymerase, also known as simple or factor-independent terminators, were the first to be discovered and soon became prototypes for the most abundant class of terminators in Eubacteria. They are used both to signal transcription termination at the ends of operons and as control elements in the attenuators and riboswitches that are found downstream of the promoters of a variety of bacterial biosynthetic operons. Intrinsic terminators consist of a hairpin structure followed by a 7–9 nucleotide U-tract, sometimes interrupted by one or more other residues, at the 3' end of the RNA (see Figure 1A), and many experiments have shown that these features are essential for the functioning of these terminators (Nudler and Gottesman, 2002).

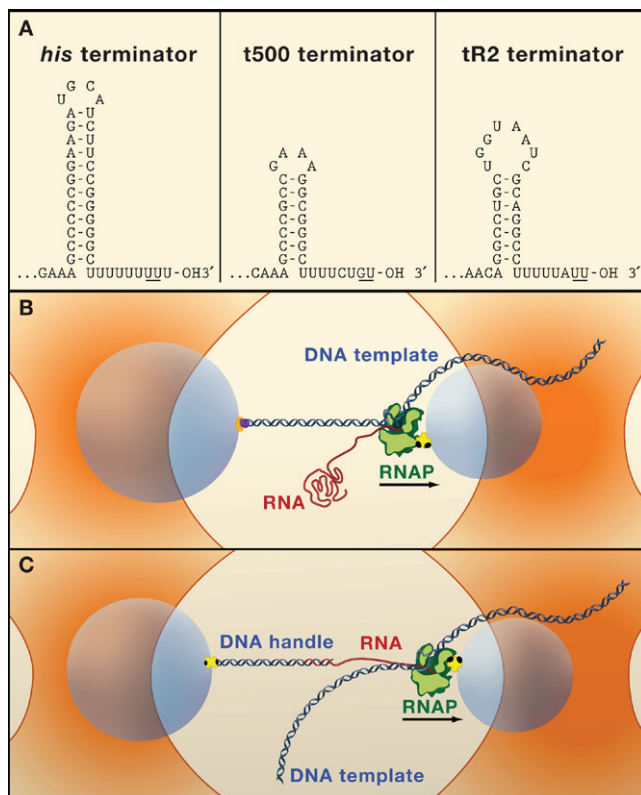
As their name suggests, intrinsic terminators are recognized directly by the multisubunit bacterial RNA polymerase core enzyme. Although the path of nucleic acids within the structure of an RNA polymerase elongation complex has been determined (Korzheva et al., 2000), it has not been easy to understand either the energetics or the mechanism of transcription termination. In this issue of *Cell*, Larson et al.

(2008) describe the use of single-molecule assays involving optical traps to monitor elongation through intrinsic terminators.

By attaching RNA polymerase and either one end of its DNA template (Figure 1B)

or the 5' end of its nascent RNA transcript (Figure 1C) to different beads, this assay allows force to be applied between the RNA polymerase molecule and either its template or its transcript. The results of this analysis have deepened our understanding of the functioning of these terminators.

Because the RNA:DNA hybrid in the transcription bubble is 9 bp long (Gnatt et al., 2001), it consists entirely (or almost entirely) of rU:dA base pairs at the termination site. The presence of the U-tract is sufficient to cause RNA polymerase to pause (Gusarov and Nudler, 1999; Yarnell and Roberts, 1999), thereby providing a kinetic window for termination. The rU:dA hybrid is also particularly weak, and weak hybrids have the potential to cause RNA polymerase to backtrack, moving the 3' end of the RNA away from the catalytic center of RNA polymerase and into its secondary channel (Nudler and Gottesman, 2002). However, backtracking should not promote dissociation of the elongation complex because the RNA:DNA hybrid would become stronger, not weaker. In any case, back-



**Figure 1. Single-Molecule Analysis of Intrinsic Terminators**  
 (A) Structures of three intrinsic terminators illustrating their characteristic RNA hairpins and U-tracts. The bases where termination occurs are underlined.  
 (B) Experimental set-up for the DNA-pulling assay (Larson et al., 2008). RNA polymerase (green) and one end of its DNA template (dark blue) are attached to different polystyrene beads (light blue) suspended in two separate optical traps (orange). The nascent RNA (red) is untethered. The arrow indicates the direction of transcription. In this orientation, the applied force assists translocation by RNA polymerase.  
 (C) Experimental set-up for the RNA-pulling assay. In this case, the nascent RNA is tethered to one of the beads via hybridization to a DNA handle.

tracking may be inhibited by the presence of the RNA hairpin. Consistent with this expectation, Larson et al. found that application to the RNA polymerase of a hindering force along the DNA to assist backtracking did not generally affect termination in their assay.

The RNA hairpin that precedes the U-tract has a key role in termination. There is evidence that formation of the base of the hairpin somehow leads to disruption of the upstream portion of the hybrid, weakening the hybrid and thereby facilitating termination (Gusarov and Nudler, 1999; Yarnell and Roberts, 1999; Komissarova et al., 2002). Indeed, interaction between the hairpin and RNA polymerase is stabilized by the elongation factor NusA, which increases RNA polymerase pause times at hairpin-dependent pause sites and increases the efficiencies of intrinsic terminators (Nudler and Gottesman, 2002). Consistent with expectations, Larson et al. found that termination was inhibited when sufficient force was exerted on the 5' end of the nascent RNA so as to begin disrupting the hairpin. However, one of the curiosities of their study is that exertion of a lesser force on the RNA increased the efficiency of termination. They used single-stranded DNA molecules complementary to the transcript to show that this is apparently caused by the formation in the upstream RNA of weak secondary structures that compete with the formation of the terminator hairpin.

In principle, a different way to shorten and weaken the RNA:DNA hybrid is the forward translocation by RNA polymerase in the absence of concomitant RNA synthesis. It has been shown previously that RNA polymerase hypertranslocation is an important component of the termination mechanism at the t500 intrinsic terminator (derived from bacteriophage  $\phi$ 82) (Santangelo and Roberts, 2004). When Larson et al. applied an assisting or hindering force to the RNA polymerase (as shown in Figure 1B), the kinetics of release were altered for t500 and the termination efficiency was altered for a hairpin mutant of t500. Moreover, the optical trap data for t500

predicted hypertranslocation by several nucleotides, consistent with the findings of Santangelo and Roberts (2004). Assisting or hindering forces did not, however, affect the efficiencies for two other intrinsic terminators, *his* (from the attenuator of the *E. coli his* operon) and tR2 (from bacteriophage  $\lambda$ ), indicating that these terminators, unlike t500, do not apparently use hypertranslocation to weaken the hybrid.

How then does release occur at these other terminators? Larson et al. found that applying tension between the RNA 5' end and the RNA polymerase (as shown in Figure 1C) led to sequence-dependent release at the U-tract. In this case, they propose that the event that leads to termination is shearing of the RNA:DNA hybrid by about 1 bp. Because shearing should be energetically more favorable for terminators containing pure U-tracts, like *his* (Figure 1A, left), or perhaps only one non-U residue, like tR2 (Figure 1A, right), and less favorable for t500 (Figure 1A, middle), which contains two non-U residues in its U-tract, this may explain why t500, unlike the other terminators in this study, requires hypertranslocation by RNA polymerase. Hypertranslocation requires melting of the DNA ahead of the termination site. Therefore, this hypothesis is also consistent with previous observations that the sequence of the DNA ahead of the U-tract specifically affects termination when the sequence downstream of the hairpin contains multiple non-U residues (Reynolds and Chamberlin, 1992).

Exerting force on the DNA or RNA is expected to alter the energy landscape for termination if the termination event requires motion of the RNA polymerase with respect to one or other nucleic acid. As a consequence, Larson et al. were able to use their data to derive a quantitative model that predicts termination efficiency as a function of the sequences and predicted stabilities of the RNA hairpin and RNA:DNA hybrid. Pulling on the RNA with sufficient force to dissociate the closing base pairs of the hairpin reduced the termination efficiency. They postulate that it is the closing of these

base pairs that generates the signal that leads to forward translocation or shearing of the hybrid (or both), as well as disruption of the upstream portion of the hybrid, and the energy that makes them possible. A short rU:dA hybrid would dissociate spontaneously if not for its stabilization by contact with RNA polymerase (Gnatt et al., 2001), implying that RNA polymerase would have a direct role in the shearing mechanism. Given that the base and loop of the hairpin are located far from the catalytic center of RNA polymerase and most of the hybrid, transmission of this signal must involve allosteric movements within RNA polymerase that ultimately alter its contacts with the hybrid and lead to melting or shearing of the hybrid or hypertranslocation. It is known that a specific contact between the hairpin and the flap domain of RNA polymerase is required for the functioning of hairpin pause sites (Touloukhonov et al., 2001). Exactly how the hairpin of an intrinsic terminator causes an allosteric signal to be transmitted within RNA polymerase to promote termination is an important subject for future investigation.

## REFERENCES

- Gnatt, A.L., Cramer, P., Fu, J., Bushnell, D.A., and Kornberg, R.D. (2001). *Science* 292, 1876–1882.
- Gusarov, I., and Nudler, E. (1999). *Mol. Cell* 3, 495–504.
- Komissarova, N., Becker, J., Solter, S., Kireeva, M., and Kashlev, M. (2002). *Mol. Cell* 10, 1151–1162.
- Korzheva, N., Mustaev, A., Kozlov, M., Malhotra, A., Nikiforov, V., Goldfarb, A., and Darst, S.A. (2000). *Science* 289, 619–625.
- Larson, H., Greenleaf, W.J., Landick, R., and Block, S.M. (2008). *Cell*, this issue.
- Nudler, E., and Gottesman, M.E. (2002). *Genes Cells* 7, 755–768.
- Reynolds, R., and Chamberlin, M.J. (1992). *J. Mol. Biol.* 224, 53–63.
- Santangelo, T.J., and Roberts, J.W. (2004). *Mol. Cell* 14, 117–126.
- Touloukhonov, I., Artsimovitch, I., and Landick, R. (2001). *Science* 292, 730–733.
- Yarnell, W.S., and Roberts, J.W. (1999). *Science* 284, 611–615.