PERSPECTIVES: TRANSCRIPTION

Shifting RNA Polymerase into Overdrive

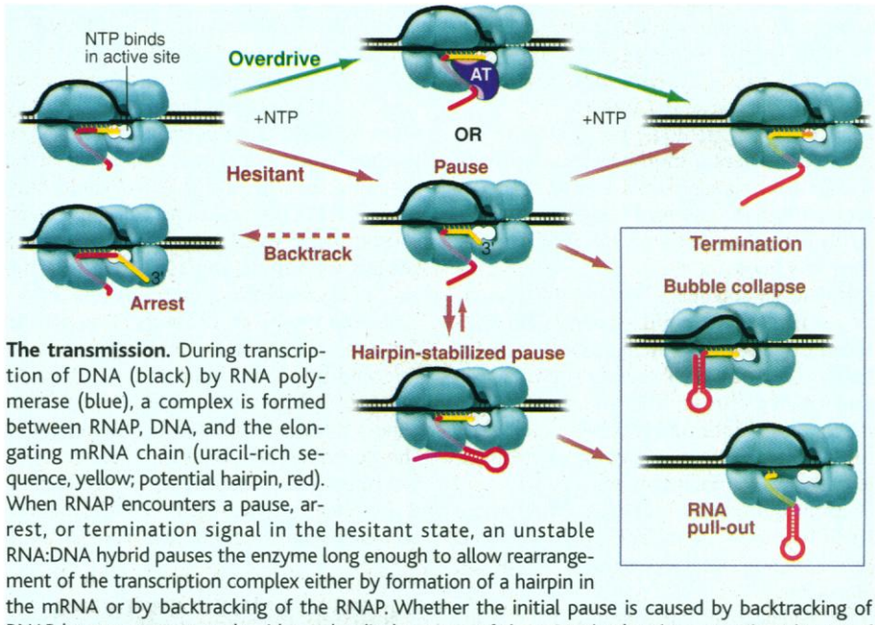
Robert Landick

To direct expression of a gene, RNA polymerase (RNAP) must efficiently transcribe (copy) DNA into an mRNA molecule that is thousands to millions of nucleotides long. Organisms from bacteria to humans regulate gene expression by making RNAPs that readily hesitate, stop, or fall off the DNA template when they encounter certain sequences (called pause, arrest, or termination signals). There also exist auxiliary proteins that switch RNAP from a state that is susceptible to pausing, arrest, and termination (defined here as hesitant) into a state that is resistant to these signals (defined here as overdrive). In a Research Article on page 611 of this issue, Yarnell and Roberts (*1*) explain how the bacteriophage λ protein Q makes the RNAP of *Escherichia coli* resistant to termination sequences in the DNA. Their study offers important insights not only into bacterial mechanisms of termination and overdrive (called antitermination in bacteria), but also into an intriguing but poorly understood mechanism of human gene regulation in which RNAPII switches between inefficient (hesitant) and efficient (overdrive) modes of mRNA chain elongation.

To understand the implications of this study, one must appreciate the extraordinary conservation of RNAPs and their contacts to RNA and DNA (*2*). All cellular RNAPs contain two large, highly conserved subunits that hold ~30 base pairs of DNA, melt (denature) ~18 base pairs into a bubble to unmask the template DNA strand, keep the first approximately eight nucleotides of RNA paired to the template in the bubble's downstream half, and then extrude the elongating mRNA chain through an upstream channel (see the figure; other less conserved subunits help to assemble RNAPs or play auxiliary roles). To work efficiently, RNAP must move freely along the DNA without losing its grip because, once released, mRNA chains cannot be extended any further. Recent findings suggest that RNAP retains its grip on the DNA by allowing the RNA:DNA hybrid, the duplex DNA in front of the RNAP's active site, and the exiting mRNA transcript to slide relatively freely though clamplike grips (*3, 4*). The sizes of the bubble and hybrid remain constant as RNAP moves along the DNA because base pairs at their front and back edges form and melt in synchrony; the free energies of the bubble and hybrid most likely control both translocation of RNAP along the DNA and alignment of the RNA 3′ OH in the active site.

Pause, arrest, and termination signals all appear to slow RNAP because unstable base pairs in the hybrid displace the RNA 3′ end from the active site, and may allow backwards sliding (backtracking) of RNAP into a more stable complex (see the figure). Yarnell and Roberts used termination sequences in which a particularly unstable RNA:DNA hybrid is produced by a seven- to nine-nucleotide uracil-rich RNA sequence preceded by an RNA secondary structure called a terminator hairpin. The unstable rU·dA base pairs induce RNAP to pause (*1, 5*), which gives the terminator hairpin time to form close enough to RNAP to trigger release of the mRNA transcript and the DNA from RNAP. Release occurs because the hairpin prevents RNAP from backtracking to form a more stable hybrid and then either breaks critical RNA contacts in the exit channel or pulls the mRNA out through the exit channel. If a hairpin forms three to four nucleotides further away from the mRNA 3′ end, it appears to contact RNAP and stabilize the pause by pulling the 3′ end away from the site where nucleotides are added to the elongating mRNA chain (*6, 7*). This hairpin contact also could help terminator hairpins to form. If RNAP is not blocked by a hairpin, uracil-rich mRNA may cause RNAP to backtrack into an arrested conformation from which it can resume elongation only by cutting off the protruding 3′ segment of mRNA.

Yarnell and Roberts study transcription termination and the shift of RNAP into termination-resistant overdrive using antisense oligonucleotides (that mimic the disrupting effects of the terminator hairpin) and mutant RNAPs with altered sensitivity to termination signals. Oligonucleotides that pair with a stretch of mRNA that includes a segment between nucleotides 18 and 10 from the 3′ end induce release of RNAPs that are paused by uracil-rich RNA. The authors argue for the pull-out model of termination because the bacteriophage Q protein shifts these annealing boundaries further from RNAP but does not block oligonucleotide-



The transmission. During transcription of DNA (black) by RNA polymerase (blue), a complex is formed between RNAP, DNA, and the elongating mRNA chain (uracil-rich sequence, yellow; potential hairpin, red). When RNAP encounters a pause, arrest, or termination signal in the hesitant state, an unstable RNA:DNA hybrid pauses the enzyme long enough to allow rearrangement of the transcription complex either by formation of a hairpin in the mRNA or by backtracking of the RNAP. Whether the initial pause is caused by backtracking of RNAP by one or more nucleotides or by displacement of the mRNA's 3′ end is currently unknown. If the hairpin reaches within seven to nine nucleotides of the mRNA's 3′ end, it dissociates the transcription complex through either the collapse or pull-out pathways. However, when switched into transcription overdrive by binding of an antiterminator protein (AT, purple), RNAP ignores pause, arrest, or termination signals. AT may prevent hairpin formation or stabilize the RNA:DNA hybrid (pink areas within AT) either directly or by inducing a conformation change in RNAP. NTP, nucleoside triphosphate.

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stimulated termination completely. They suggest that Q may act in part by blocking hairpin formation close to RNAP (see the figure). In other words, Q could block oligonucleotide access close to RNAP but allow some pull-out if pairing occurred further upstream; thus, Q would inhibit hairpin formation until after RNAP passes the uracil-rich sequence required for termination. However, a recent report showing that RNAP does not move forward when a termination sequence breaks mRNA's contacts with the exit channel and the DNA template (at low ionic strength) favors the alternative collapse model (*5*) (see the figure).

Mutant RNAPs that increase or decrease termination and that may affect contacts to the RNA:DNA hybrid also shift the "oligonucleotide-release" window downstream or upstream, respectively. Thus, like the Q protein, hybrid stability affects where the oligonucleotides can pair to trigger termination. This leads Yarnell and Roberts to suggest that Q could act by stabilizing the hybrid (see the figure). Stabilization would shift the "oligonucleotide-release" window because more base pairs are required to compete with the stabilized hybrid. This hypothesis is attractive because it explains how Q could block both hairpin formation and backtracking, and thus simultaneously inhibit pausing, arrest, and termination. It also is consistent with the finding that antisense oligonucleotides must pair to mRNA within eight nucleotides of its 3′ end to dissociate a paused, hairpin-stabilized RNAP containing a more stable RNA:DNA hybrid (*7*).

How can these ideas be applied to understanding human gene regulation? RNAPII must switch from hesitant to overdrive transcription to transcribe through the pause signals, arrest signals, and nucleosomes in human genes (typically 10^4 to 10^6 base pairs in length) that would otherwise halt RNAPII completely (*8*). Abnormal regulation of this efficiency switch has been implicated in several human diseases including myeloid leukemia, malignant transformation of several types of human cells, and growth of HIV (*9*). Flipping the efficiency switch to "on" requires phosphorylation of multiple sites on RNAPII's largest subunit. Phosphorylated RNAPII may recruit factors that stimulate mRNA elongation and transcription through nucleosomes (perhaps in a similar fashion to Q). Once RNAPII passes the polyadenylation site in DNA, dephosphorylation of the large subunit releases the transcription factors, restores RNAPII's intrinsic sensitivity to pause and termination signals, and quickly stops transcription (*10*).

The central idea that instability of the RNA:DNA hybrid leads to rearrangement of the transcription complex (see the figure) appears to explain how RNAPII pauses and arrests (*11*), and probably how it becomes a target for termination factors. Like the proposed antitermination action of Q, RNAPII's switch to efficient transcription could involve stabilization of the RNA:DNA hybrid (and possibly, inhibition of RNA hairpin formation) so that it never becomes susceptible to pausing, arrest, or termination.

From one perspective then, pausing, arrest, termination, and antitermination can all be explained by protein or nucleic acid interactions that affect both positioning of the RNA 3′ end in the active site and lateral sliding by a relatively rigid RNAP (*4*). However, strong evidence exists that the actual switch between inefficient and efficient transcription must involve a conformational change in RNAP. Removing a small subunit or promoting the rapid elongation of mRNA with high concentrations of nucleotides in vitro causes RNAP to switch to a state that resists pausing or termination (*12*). The mutant RNAPs of Yarnell and Roberts behave as if this switch were sticky, locking the enzyme in either the overdrive or hesitant state. Perhaps proteins like Q stabilize RNAP in an overdrive conformation that optimizes RNA contacts with the DNA template and active site; the change from this conformation to one that tolerates misplacement of RNA could be a feature built into the RNAP itself. The next hurdles to be overcome are determining whether a rigid RNAP or a conformational change best explains RNAP's overdrive switch and whether termination occurs by pull-out or collapse.

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PERSPECTIVES: PLANT BIOLOGY

Leaves in the Dark See the Light

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Photosynthesis—the process by which plants harness the sun's energy to generate simple carbon compounds—supports all life on Earth. It is a complex process of successive reduction-oxidation (redox) reactions that use up carbon dioxide and water and produce energy-rich carbohydrate and oxygen as the end products. The evolution of oxygen-giving photosynthesis radically altered Earth's atmosphere and enabled the development of aerobic life. Oxygen-consuming organisms, although able to exploit the powerful oxidizing properties of oxygen, are also condemned to exist in the unstable tinderbox atmosphere of 21% O_2.

Although photosynthesis cannot proceed in the absence of light, excess light is potentially dangerous to the plant because it can cause persistent decreases in the rates of photosynthesis (photoinhibition) (*1*). Leaves have evolved various mechanisms to deal with excess light energy, enabling plants to function optimally over a relatively broad window of light intensities. At low irradiance, harvesting of light predominates, but as the light intensity increases, effective dissipation of energy becomes progressively more important in preventing photoinhibition and is essential for plant survival (*2*). If the protective processes are overwhelmed, photoinhibition will decrease the efficiency and capacity of photosynthesis and cause leaf damage that is comparable to human sunburn. Now on page 654, Karpinski and colleagues report the intriguing finding that exposure of plants to high-intensity light activates a systemic signaling system that "warns" regions of the plant not exposed to bright light of an impending dangerous stimulus (*3*). The investigators exposed one-third of *Arabidopsis* leaves to high-intensity light—which is believed to result in the production of damag-

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