RNA Polymerase Marching Backward

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The DNA-dependent RNA polymerases catalyze the synthesis of RNA on DNA templates. In their forward march along DNA, these enzymes elongate RNA chains by forming 3'-5' phosphodiester linkages, by using ribonucleoside 5'-triphosphates as substrates and releasing pyrophosphate as reaction product. Since RNA chains can be prodigiously long, the processivity of RNA synthesis and the dynamic stability of ternary transcription complexes (DNA plus RNA plus polymerase) must also be very great.

Enzymes that catalyze the elongation of RNA chains must, a priori, be able to catalyze their retraction. In this reverse reaction, RNA polymerase marches backward, subjecting 3' ends of RNA in ternary transcription complexes to pyrophosphorolysis that yields back the substrates of RNA synthesis (*1*, *2*). Both the elongation of RNA chains and their pyrophosphorolytic retraction are far from smooth and steady (*2*, *3*).

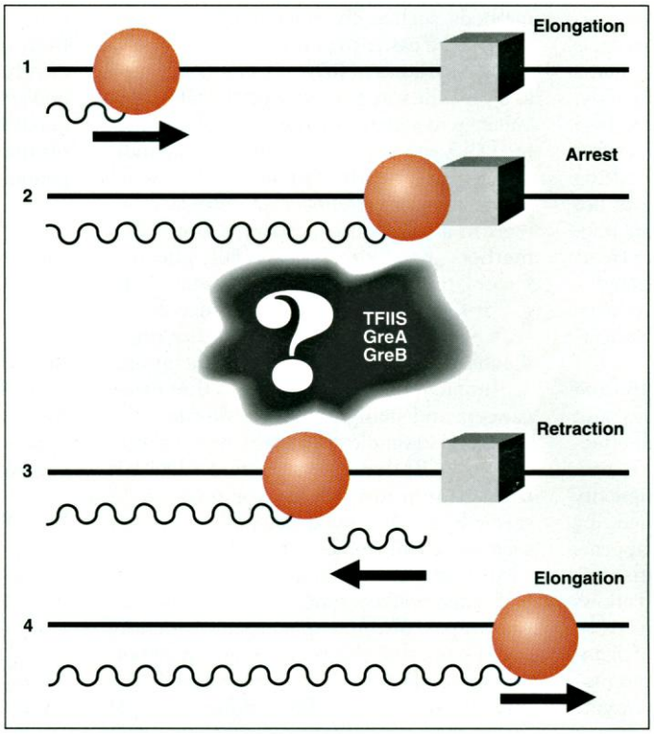
Of particular interest is the growing realization that RNA polymerases also march backward to the beat of another drummer. This second RNA chain-retracting process also takes place in ternary transcription complexes, but is hydrolytic and generates short chains of RNA. Experiments on this second reaction reveal previously unsuspected complexities of the process of RNA chain elongation and excite new speculations about mechanisms of gene regulation through chain elongation (*4*) and about transcriptional fidelity.

Certain accessory proteins of transcription, the elongation factors, enhance the overall activity of RNA polymerase by increasing the elongation rate and the completion of RNA chains. In concert with pol II, one class of these proteins, the transcription factors TFIIF, accelerates RNA chain growth relatively uniformly. A second group of proteins, the transcription factors TFIIS (also called SII), greatly mitigates transcriptional pausing. TFIIS-like proteins must be ubiquitous; they are now known to be encoded by various mammalian genomes, *Drosophila*, yeast, and vaccinia virus. TFIIS stimulates transcriptional elongation by pol II past intrinsic sites on DNA at which it otherwise tends to get stuck (*5*, *6*). TFIIS can also relieve the obstruction of RNA chain elongation caused by a *lac* repressor-operator complex (*7*). The human TFIIS contains two domains that are relevant to its function as the transcription elongation factor: one domain secures binding to pol II, another binds zinc and may participate in interactions with nucleic acid (*8*). Both human TFIIS and the yeast TFIIS homolog interact with the largest subunit of pol II (*9*).

TFIIS is also a determinant of the recently discovered hydrolytic cleavage that occurs at the 3' ends of RNA chains in arrested pol II ternary transcription complexes (*10*–*13*). The salient features of this reaction are that RNA cleavage is 3'→5' processive and that the resulting shortened nascent RNA chain continues to be held in its transcription complex, fully competent to resume elongation when supplied with nucleoside triphosphates, while hydrolytic products are released. The hydrolytic 3' end cleavage of RNA seems to be essential for transcription factor TFIIS-mediated readthrough past strong transcriptional pause sites and protein-DNA complexes: (i) Hydrolysis precedes readthrough in time. (ii) In the presence of nucleotide mixtures that include a chain-terminating nucleotide analog and should allow limited extension of the RNA chain, TFIIS instead can generate shortened chains exclusively; this is precisely what would be expected if hydrolytic RNA chain retraction preceded the incorporation of the chain-terminating nucleotide (*7*, *11*). Thus, RNA polymerase moves backwards under the direction of TFIIS before it moves forward through the block to elongation.

It is most likely, but not yet certain, that the 3'→5' processive RNA hydrolytic activity resides in pol II and is greatly enhanced by TFIIS: (i) The hydrolytic activity, like RNA polymerization, is α-amanitin–sensitive (*11*–*13*); α-amanitin is thought to block the translocation of RNA polymerase during transcription. (ii) A low level of hydrolytic activity is detected in the absence of exogenously added TFIIS (*10*, *12*, *13*). (iii) Mutant TFIIS that cannot bind to pol II also cannot generate the hydrolytic activity (*11*, *14*). (iv) The second largest subunit of pol II contains a region of significant homology to the catalytic site of bacterial ribonucleases (*15*).

Hydrolytic cleavage at the 3' end of nascent RNA also occurs in *Escherichia coli* RNA polymerase (*16*). Newly discovered elongation factors, GreA and GreB, mediate this RNA cleavage. GreA likely interacts directly with RNA polymerase because the *greA* gene at high copy number can suppress a temperature-sensitive mutation of the *E. coli* RNA polymerase β subunit (*17*) and, at a more empirical level, because GreA is persistently present as a substoichiometric passenger of the most highly purified preparations of *E. coli* RNA polymerase. Using recombinant RNA polymerase assembled in vitro, Borukhov and co-workers (*18*) have shown



RNA polymerase marches forward and backward. RNA (wiggly line) chain elongation (forward arrow) by RNA polymerase can be blocked by DNA (straight line) sequence or bound protein (box). Subsequent hydrolysis at the 3' end of nascent RNA requires accessory proteins, but the mechanism is unknown (black cloud). The catalytic site of RNA polymerase (ball) must retract simultaneously with RNA cleavage (backward arrow). It is not known if this involves internal motions within stationary RNA polymerase or if each step of RNA hydrolysis is accompanied by a precisely proportional backward motion of the entire RNA polymerase molecule along DNA (the title of this Perspective notwithstanding).

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that the 158–amino acid GreA and GreB proteins, like TFIIS, mediate RNA hydrolysis and relief of transcriptional arrest in "dead-end" (permanently paused) ternary transcription complexes. Whereas GreB is effective when added to dead-end complexes, GreA must be present before the polymerase reaches the arrest site. For both proteins, relief of arrest is accompanied by RNA cleavage. GreA and GreB are both effective in promoting RNA cleavage when added to transiently stalled complexes, and GreA recycles effectively between, and therefore acts catalytically upon, these complexes. The presence of a residual, weak RNA hydrolytic activity in *E. coli* RNA polymerase assembled from individual subunits argues in favor of (and provides a means for testing) the notion that the hydrolytic activity resides in the polymerase and is activated by GreA and GreB. There is evidence for comparable RNA hydrolytic activities associated with other RNA polymerase systems, including vaccinia virus RNA polymerase, one of whose subunits displays significant homology to TFIIS (*19*), and yeast RNA polymerase III.

The view that accessory factor–controlled RNA hydrolytic activities may be universally associated with all DNA-dependent RNA polymerases has rapidly gained acceptance. In contrast, there is a surprising diversity of evidence regarding the products of RNA hydrolysis; in arrested pol II transcription complexes, TFIIS has been reported to generate exclusively 5'-mononucleotides, dinucleotides and some trinucleotides, and larger products between 8 and 14 nucleotides long (*13*, *20*). The various experiments differ in the sites at which transcription is arrested, in the manner of forming arrested complexes, and, quite possibly, in the composition of the complexes. When *E. coli* RNA polymerase transcription complexes are stalled by withholding a required nucleotide or arrested at an intrinsic block to RNA chain elongation, cleavage products of two to ten nucleotides have been found (*16*,*18*). Of particular interest is the fact that GreA and GreB can generate different cleavage products: GreA yields dinucleotides and trinucleotides, while GreB can yield longer oligonucleotides (*18*).

The diversity of nucleolytic products may reflect a diversity of topographies within ternary transcription complexes. The ability of RNA polymerase to reextend an RNA chain that has had one to ten nucleotides removed from its 3' end in a single cleavage event is consistent with recent studies indicating that ternary transcription complexes arrested at different positions along the DNA template are conformationally distinct (*21*). Further analysis of diversity in the RNA cleavage reaction is likely to provide important insights into the structure of the DNA template and the nascent transcript within ternary transcription complexes.

It is not yet known whether hydrolysis and pyrophosphorolysis of RNA chains take place at the same site within RNA polymerase. That pyrophosphorolysis and hydrolysis of RNA 3' ends are in competition (*13*) and that α-amanitin blocks both reactions are inconclusive in this regard, being compatible with a single site of catalytic action or with a requirement for a translocation mechanism to shuttle the nascent RNA chain between two spatially separated active sites. Such translocation would contribute to the polymorphism of transcription complexes.

Two interesting groups of ideas about the broader role of hydrolytic RNA retraction are currently under consideration: (i) RNA 3'→5' hydrolysis may play the error-correcting role in transcription that DNA 3'→5' hydrolysis plays in replication. Whether error correction can be effected in this way on the time scale of RNA chain elongation in vivo (with average elongation rates of 1.5 to 2.5×10^3 nucleotides per minute) remains to be shown; the hydrolysis that has been demonstrated in vitro thus far is notably slower. (ii) Hydrolytic RNA cleavage may mitigate problems that are associated with the high processivity of RNA polymerase. There are sites in transcription units at which RNA polymerases have substantial probabilities of getting stuck, sometimes permanently freezing in place. The ability to back up frozen or stuck complexes, such as the dead-end complexes of *E. coli* RNA polymerase, allows transcriptional barriers to be approached repetitively. At each approach, there is a chance to breach the transcriptional barrier. Eventually, all transcribing chains get through (see figure).

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