

RNA Polymerase as a Molecular Motor

Minireview

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To transcribe DNA, RNA polymerase (RNAP) rapidly moves along the DNA template, powered by free energy liberated by nucleotide polymerization and RNA folding reactions. This chemical-to-mechanical energy conversion by RNAPs is analogous to that of myosins, kinesins, dyneins, and the bacterial flagellar motors. However, RNAPs are more mechanically complex than the other molecular motors reviewed in this issue in that they perform a variety of different types of movements along DNA during transcription. RNAPs are also more chemically complex than other motors because they function as the primary targets for the regulation of gene expression. This review describes our current understanding of RNAP mechanical processes, focusing on recent studies that have observed directly the movement of single RNAP molecules along DNA. These studies already have yielded insights into the mechanics and mechanisms of RNAP movement; their further development and application promises to help resolve fundamental questions about how RNAP translocates along DNA and to help elucidate mechanisms of transcription regulation.

Overview of RNA Polymerase Movements during Transcription

All cellular RNAPs, whether bacterial or eukaryotic, are homologous multisubunit enzymes (Polyakov et al., 1995) that execute a remarkable series of choreographed movements while transcribing DNA (Uptain et al., 1997). Initially, RNAP can bind to DNA and then randomly slide along the duplex until a promoter is encountered. Engagement with a promoter requires protein initiation factors that make sequence-specific DNA contacts. The initiation of the RNA chain is accompanied by loss of strong promoter contacts and release of the initiation factors. Subsequent chain elongation in the polymerase-DNA-RNA transcription elongation complex (TEC; Figure 1, left) is accompanied by translocation of RNAP on the DNA. Elongation proceeds until a termination signal in the DNA sequence triggers TEC disruption. Both the elongation rate and the effectiveness of termination signals vary depending on the association of different elongation and termination factors with the TEC; the final RNA length can be up to 10^6 nucleotides (nt). RNAPs are *obligately* processive enzymes; once an RNA chain is released by one RNAP molecule, no other RNAP molecule can elongate it.

Mechanism of Polymerase Translocation during Transcript Elongation

The catalytic cycle of RNA chain elongation minimally involves (i) nucleoside triphosphate (NTP) binding, (ii) nucleophilic displacement of pyrophosphate from the NTP by the RNA 3' hydroxyl, (iii) pyrophosphate release, and (iv) translocation of the new RNA 3' nucleotide to vacate the NTP-binding site (Figure 1, right). Much of the available data are consistent with a simple "monotonic"

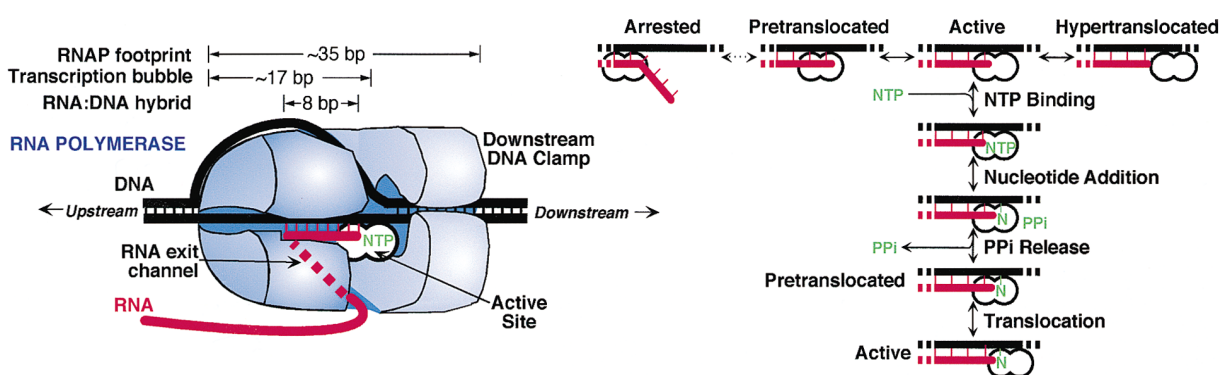


Figure 1. Structure of a Transcription Elongation Complex (TEC) and the TEC Nucleotide Addition Cycle

(Left panel) Hypothesized TEC structure. Electron microscopy studies of bacterial and yeast RNAPs reveal a groove surrounded by jaw-like protrusions that may clamp around downstream duplex DNA and a tunnel that may function as a channel through which RNA exits from the active site. Chemical experiments show that RNAP in a canonical TEC protects ~ 17 bp of DNA on either side of the active site position, keeps the upstream half of this DNA melted, and maintains an ~ 8 bp RNA:DNA hybrid upstream from the active site (Landick, 1997; Nudler et al., 1997; Uptain et al., 1997, and references therein). RNAP adds nucleotides to nascent RNA in a bipartite active site that coordinates the RNA 3' end and the nucleoside triphosphate (NTP) α phosphate via two Mg^{2+} ions.

(Right panel) States of the TEC active site. The vertical pathway shows a minimal RNA chain elongation cycle; the horizontal pathway depicts additional states of the TEC thought to arise when the RNAP active site (double circle) slides relative to the DNA:RNA heteroduplex. Arrested and hypertranslocated TECs are catalytically inactive because the RNA 3' end is displaced from the active site. The four steps of the chain elongation cycle are all required; however, the order of the last two steps and the relative rates of all 4 steps are unknown and may vary at different template positions.

model of the translocation step, in which RNAP structure does not change as the enzyme moves along the RNA and DNA chains, thus advancing the whole enzyme 1 bp and 1 nt with each nucleotide polymerized (see Landick, 1997; Komissarova and Kashlev, 1997; Nudler et al., 1997; and references therein). An alternative “discontinuous elongation” model, in which RNAP deforms so that one or more nucleotide can be added without complete translocation of the enzyme along the DNA and RNA, has also been considered (see Landick, 1997; Uptain et al., 1997, and references therein).

How do the chemical steps of RNA polymerization power the translocation step? One hypothesis is that RNAP operates by a “Brownian ratchet” mechanism, in which the enzyme rapidly oscillates between two adjacent template positions (n and $n+1$), with this backward and forward movement driven randomly by thermal energy (i.e., it is Brownian motion). At some instant when the enzyme is bound to position $n+1$, a chemical process within the enzyme causes a conformational change so that some structure (the “pawl” of the ratchet) blocks movement back to position n . The enzyme then begins the next cycle of movement by allowing thermally driven oscillation between positions $n+1$ and $n+2$, and the process repeats.

Although Brownian ratchet mechanisms have been proposed for other motors (e.g., see Córdova et al., 1992), such mechanisms are particularly easy to visualize for nucleic acid polymerases. In such enzymes, bound NTP at the active site could directly serve as the pawl by acting as a steric obstruction that prevents the 3' end of the transcript from reentering the downstream half of the active site; the binding of each successive NTP would then serve to block backward movement of enzyme in the “active” state to form the preceding “pretranslocated” state (see Figure 1, right). This simplest Brownian ratchet mechanism can be elaborated by allowing the enzyme to slide between >2 positions on the template during each stage of the cycle (Guajardo and Sousa, 1997); recent studies (reviewed in Landick, 1997) provide evidence for these multi-base pair sliding movements and demonstrate that they are associated with the slowing or arrest of transcription that occurs at certain DNA sequences (Figure 1, right).

The opposite extreme from a pure Brownian ratchet mechanism is a “power-stroke” mechanism in which an enzyme domain binds the DNA tightly and a subsequent conformational change pushes the remainder of the enzyme 1 bp along the template. Because such a step directly generates displacement rather than simply rectifying random Brownian motion, a power-stroke motor can in principle move more quickly than can a Brownian ratchet when the enzyme must operate against the high opposing mechanical loads that may arise in vivo from DNA supercoiling or from RNAP collision with DNA-binding proteins or nucleosomes.

Determining whether RNAPs move by a Brownian ratchet, a power-stroke, or by some intermediate mechanism and then elucidating the mechanism's reaction pathway and kinetics will require expanding our knowledge of RNAP function. We will need data not only on the chemical steps catalyzed by the enzyme but also on the movements generated in individual mechanical

steps, on the step rates, and on how the rates are affected by the application of external forces that oppose RNAP movement. Analysis of the movement mechanism will also require more complete information on TEC structure and mechanical properties (e.g., on the rigidity of the various protein domains).

Biochemical methods used to study transcription (e.g., analyzing the distribution of in vitro transcript lengths by gel electrophoresis) are limited in their ability to reveal the nature of RNAP movement along DNA and the mechanism of chemomechanical coupling. Populations of enzyme molecules that undergo a series of consecutive reactions with similar rates rapidly become unsynchronized; analyzing single-step kinetics from data averaged over such a population is nearly impossible. This loss of synchronization is unusually severe for RNAP due to branches in the elongation reaction at pause or arrest sites, even when elongation is begun from halted TECs (Erie et al., 1993). Also, biochemical methods cannot easily analyze heterogeneity in the elongation properties of different TECs in the population. Such heterogeneity is reported to occur even with highly purified RNAPs and is known to arise upon modification of TECs by antitermination factors. The single-molecule experimental techniques described below promise to help overcome these limitations and provide needed information about RNAP mechanisms.

Single-Molecule Techniques for Studying RNAP Translocation along DNA

In recent years, physical methods have been developed to analyze transcription mechanisms by directly visualizing DNA translocation by RNAP molecules. These techniques all isolate a single RNAP molecule by immobilizing it on a surface; each then observes DNA translocation by some form of microscopy (Figure 2). Each method has its own advantages: the tethered particle motion approach (Figure 2, top) is arguably the least perturbational; it neither exerts force on DNA nor significantly alters its conformation. Since bead diffusion is more rapid than the mean velocity of DNA translocation in a polymerase catalytic cycle, attachment of the bead should not significantly affect the translocation reaction provided the bead is attached further from the RNAP molecule than the DNA persistence length (~ 150 bp). (The persistence length is the characteristic distance over which the orientation of the double helical axis is changed by random bending of the duplex.) On the other hand, the laser tweezers method (Figure 2, middle) has high time resolution and is thus far the only one capable of measuring translocation forces as well as displacements. The surface force microscopy technique (Figure 2, bottom) is best able to observe features of TEC structure (for example, the bend angle of the DNA at the polymerase) while simultaneously detecting translocation.

Thus far, single-molecule transcription methods are in some cases able to measure DNA translocation with a precision of a few tens of nanometers—roughly 100 bp. Precision in future experiments should be substantially increased by improved instrumentation and modifications to experimental designs. Despite the fact that the experiments use preparations of surface-immobilized RNAPs, kinetic properties of transcription (e.g., steady-state elongation rates and transcription pause lifetimes)

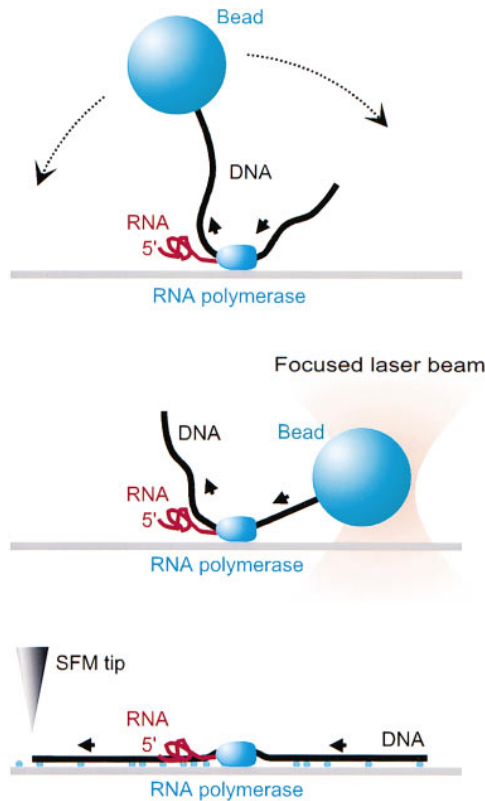


Figure 2. Techniques for Detecting DNA Translocation by Single Molecules of RNAP

RNAP bound to a glass or mica surface covered by an aqueous solution of NTPs moves the DNA template in the indicated direction (small arrows) during transcript elongation (not to scale).

(Top) Tethered particle motion method. A plastic bead or colloidal gold particle undergoes rapid Brownian motion (curved arrows) constrained by its attachment to the upstream end of the DNA template. The spatial extent of the Brownian motion is observed by light microscopy and used to determine how the length of the DNA segment linking the bead to the polymerase changes during transcript elongation (Yin et al., 1994, and references therein).

(Middle) Laser tweezers method. A focused laser beam pulls on a silica bead attached to the downstream end of the DNA template, keeping the segment of DNA between the bead and the polymerase under tension. Movement of the template by the polymerase pulls the bead away from the focus of the laser; this displacement is detected optically (Yin et al., 1995).

(Bottom) Surface force microscopy (SFM) method. Template DNA is loosely held against a mica surface by surface-bound metal cations (green circles). The DNA and the RNAP molecules are imaged by tapping-mode SFM, and the position of the enzyme along the DNA contour is measured in the images (Kasas et al., 1997).

are identical or nearly identical to those inferred from conventional solution studies. Thus, results from the two classes of studies can be combined to achieve a coherent picture of the chemical and mechanical aspects of RNAP function.

The most significant results from the initial round of single-molecule studies are that the enzyme is capable of translocating DNA against large opposing loads and that the deduced efficiency of chemical-to-mechanical energy conversion is high, similar to that of the canonical cytoskeletal motor enzymes. Indeed, the single-molecule stall force is at least twice that measured for myosin

and kinesin, showing that RNAP can legitimately be classed with those powerful cytoskeletal motors. These experiments demonstrate that RNAP molecules themselves, i.e., without accessory proteins, produce sufficient force to overcome some of the mechanical obstacles to translocation that are known to exist *in vivo*. In particular, the lower limit of 14 pN for the RNAP stall force greatly exceeds the forces opposing translocation that are calculated to arise at high levels of transcription-induced supercoiling (Yin et al., 1995). Other important mechanical impediments to transcription *in vivo* (e.g., the rearrangement of nucleosomes or the displacement of DNA-bound proteins during RNAP movement on the template) are also likely to require the exertion of substantial forces by RNAPs.

Prospects

The existing single-molecule studies of *E. coli* RNAP suggest a number of promising avenues for future research into transcription mechanisms in both prokaryotes and eukaryotes. First, if measurements can be made precisely enough to resolve 1 bp steps with high time resolution, the experiments could discriminate between alternative translocation mechanisms and reveal essential features of RNAP chemomechanical coupling. In particular, such studies could differentiate between Brownian ratchet and power stroke translocation mechanisms since these models make quantitatively different predictions about the way that translocation step durations vary with applied force. Visualizing RNAP movements with single-base pair precision would also settle the question of whether >1 bp sliding movements are characteristic features of the ordinary chain elongation cycle. Second, single-molecule approaches are ideally suited to examining the differences in reaction kinetics between TECs in the same population that are following different, parallel reaction pathways (Erie et al., 1993) or that have heterogeneous structures. Third, single-molecule methods can not only detect movement of RNAP along the DNA, but also directly visualize RNAP attachment to and release from DNA. Therefore, the techniques can visualize transcription initiation and termination, making them powerful tools to investigate mechanisms of transcription regulation and the control of gene expression. Fourth, these techniques can visualize the large-scale structural changes in DNA or chromatin associated with transcription or transcription regulation. These include DNA looping (e.g., Finzi and Gelles, 1995; Rippe et al., 1997), DNA bending (e.g., Rippe et al., 1997), and chromatin rearrangements (see Fritzsche et al., 1995, and references therein). Single-molecule techniques can potentially give a more detailed structural picture than biochemical (e.g., gel-shift) methodologies and can observe changes in structure with high (in some cases, millisecond) time resolution.

Perhaps the most important future applications of single-molecule microscopy techniques are analyses of biochemical pathways that involve assembly of large macromolecular complexes. Transcription and transcription regulatory systems involve the assembly of complex structures consisting of multiple protein molecules that interact with each other and with sites on the DNA and RNA. Despite the importance of these systems, in few cases has it been possible to define fully the kinetic

mechanism of assembly (that is, the complete pathway of assembly and the rates of all steps) and its temporal relationship to catalytic and regulatory events. Recently, technology has been developed to visualize by fluorescence microscopy single protein molecules tagged with small organic dyes or expressed as fusions with green fluorescent protein (Funatsu et al., 1995). This should permit observation of the assembly of multiple proteins (perhaps each tagged with a different color fluorophore) into single transcription or regulatory complexes while translocation is simultaneously observed using one of the techniques described above. The technology is thus particularly well-suited to ask questions about the relationship between elongation factors binding to transcription complexes and their effects on the rate and persistence of transcription, because both the factor binding and kinetic changes can be individually monitored. For example, such studies could answer fundamental questions about the mechanisms of antitermination in bacteria, and in eukaryotes could reveal the temporal relationships between transcription complex assembly, regulatory factor binding, promoter escape, and conversion to an elongation-proficient TEC. By using polarization optics, single-molecule fluorescence microscopy can also directly observe reorientation of single dye molecules relative to the excitation light (Sase et al., 1997). It thus has the potential to detect not only binding events but also structural reorganizations within single macromolecular complexes.

Analysis of RNAPs as molecular motors is still in its infancy. Single-molecule experiments should improve our knowledge of the fundamental mechanisms of transcription and its regulation, particularly as the techniques become more widely used and better instrumentation is developed. These methods should be of value to study other DNA-based motor enzymes, including DNA polymerases, *exo-* and *endonucleases*, *helicases*, and *topoisomerases*.

Selected Reading

- Córdova, N.J., Ermentrout, B., and Oster, G.F. (1992). *Proc. Natl. Acad. Sci. USA* 89, 339–343.
- Erie, D.A., Hajiseyedjavadi, O., Young, M.C., and von Hippel, P.H. (1993). *Science* 262, 867–873.
- Finzi, L., and Gelles, J. (1995). *Science* 267, 378–380.
- Fritzsche, W., Vesenka, J., and Henderson, E. (1995). *Scanning Microsc.* 9, 729–737.
- Funatsu, T., Harada, Y., Tokunaga, M., Saito, K., and Yanagida, T. (1995). *Nature* 374, 555–559.
- Guajardo, R., and Sousa, R. (1997). *J. Mol. Biol.* 265, 8–19.
- Kasas, S., Thomson, N.H., Smith, B.L., Hansma, H.G., Zhu, X., Guthold, M., Bustamante, C., Kool, E.T., Kashlev, M., and Hansma, P.K. (1997). *Biochemistry* 36, 461–468.
- Komissarova, N., and Kashlev, M. (1997). *J. Biol. Chem.* 272, 15329–15338.
- Landick, R. (1997). *Cell* 88, 741–744.
- Nudler, E., Mustaev, A., Lukhtanov, E., and Goldfarb, E. (1997). *Cell* 89, 33–41.
- Polyakov, A., Severinova, E., and Darst, S. (1995). *Cell* 83, 365–373.
- Rippe, K., Guthold, M., von Hippel, P.H., and Bustamante, C. (1997). *J. Mol. Biol.* 270, 125–138.
- Sase, I., Miyata, H., Ishiwata, S., and Kinosita, K., Jr. (1997). *Proc. Natl. Acad. Sci. USA* 94, 5646–5650.

Uptain, S., Kane, C., and Chamberlin, M. (1997). *Annu. Rev. Biochem.* 66, 117–172.

Yin, H., Landick, R., and Gelles, J. (1994). *Biophys. J.* 67, 2468–2478.

Yin, H., Wang, M.D., Svoboda, K., Landick, R., Block, S.M., and Gelles, J. (1995). *Science* 270, 1653–1657.