Cell, Vol. 116, 1–20, February 6, 2004, Copyright ©2004 by Cell Press

Previews

Active-Site Dynamics in RNA Polymerases

New crystal structures of transcription complexes formed by bacteriophage T7 RNA polymerase reveal a nucleotide-addition cycle driven by active-site conformational changes similar to those observed in DNA polymerases, and suggest provocative hypotheses for the more complex multisubunit RNA polymerases of free-living organisms.

DNA-dependent, RNA polymerases (RNAPs) catalyze synthesis of mRNAs, the primary step in gene expression. Nature has produced two classes of these enzymes, a single-subunit version in mitochondria and bacteriophage (T7-like RNAPs) and a multisubunit version in nuclei, chloroplasts, and free-living organisms (msRNAPs). The T7-like RNAPs resemble DNA polymerases (DNAPs) and reverse transcriptases in structure and mechanism, whereas msRNAPs are structurally distinct. msRNAPs typically contain two large subunits that form an ~25×40 Å cleft or main channel containing the active site and an 8–9 bp RNA:DNA hybrid, side channels that guide DNA, RNA, and reactants into or out of the active-site cleft, and variable numbers of peripheral subunits. Despite being formed by unrelated secondary structures, msRNAPs, and T7-like RNAPs create similarly sized nucleic-acid scaffolds, use a two-Mg^{2+} ion mechanism of catalysis common to most if not all nucleic-acid polymerases, and exhibit striking similarities in the biochemical mechanisms by which they initiate, elongate, and terminate RNA synthesis. However, the exact cycle of events by which RNAPs recognize correctly templated nucleotides and couple forward movement on the DNA template to nucleotide addition is unresolved. In this issue of *Cell*, the Steitz, McAllister, and Vassylyev groups report new crystal structures that go a long way to defining this cycle, at least for T7-like RNAPs, and suggest interesting conjectures for msRNAPs based on their biochemical similarities (Temiakov et al., 2004; Yin and Steitz, 2004).

A minimal catalytic cycle for RNAPs consists of NTP binding, catalysis, pyrophosphate (PPi) release, and translocation. In DNAPs, catalysis occurs in a closed conformation formed by rotation of a domain containing an "O helix" that ends up contacting the dNTP in the active site (Johnson et al., 2003, and references therein). Translocation is thought to accompany springing back of the O-helix domain to the open conformation upon PPi release. Previously, these groups used artificial nucleic-acid scaffolds to snare T7 RNAP in a posttranslocated transcription elongation complex (TEC) poised for NTP binding, analogous to the DNAP open conformation. Yin and Steitz (2004, this issue of *Cell*) now have confirmed and substantially extended this interpretation by catching T7 RNAP in the closed conformation in two different structures. One structure is poised for catalysis with NTP bound in a reconstituted TEC lacking the RNA 3′ OH (which normally reacts with the NTP α phosphate); the other is trapped after catalysis but before PPi release by incubating a reactive TEC with 3′ deoxyNTP and additional PPi (to prevent release). Using instead a nonreactive α,β methylene NTP, Temiakov et al. (2004, this issue of *Cell*) captured a fourth state of T7 RNAP in which NTP is bound to a "preinsertion" site along the O helix, ready to be delivered to the substrate binding site upon closure of the O-helix domain. These new structures allow the groups to postulate a four-state catalytic cycle in which RNAP oscillates between only two conformations: open for NTP binding and closed for catalysis (Figure 1A). This cycle is similar to that of DNAPs, but T7 RNAP allows NTP in the preinsertion site to base pair with template DNA, whereas DNAPs sequester the template base to prevent slippage. Further, the PPi cocrystal structure defines a state not yet observed in DNAPs.

The details of this deceptively simple cycle include two ingenious features that govern translocation and NTP selection. The translocation event appears to be hard-wired into the closed-to-open rotation of the O-helix domain by a tyrosine (Y639) at the end of the O-helix. During the rotation, Y639 moves 3.4 Å toward the RNA:DNA hybrid (one bp step along the hybrid), so that it occupies the position of the template base in the substrate site when the movement is complete. Thus, Y639 drives translocation of the new RNA nucleotide into the product site after which it both prevents entry of the next template base into the catalytic site until a new NTP arrives and prevents the hybrid from slipping back into the substrate site. When a new NTP arrives in the preinsertion site, Y639 plays another role. By interacting via a Mg^{2+}-bridge with the 2′ OH of an arriving NTP that can pair to the template base (Figure 1A), it ensures that the correct NTPs, and not dNTPs or mismatched NTPs, trigger closing of the O-helix and incorporate into the transcript (upon closure, Y639 conveniently swings back out of action to await duty in the next catalytic cycle).

msRNAPs may behave similarly. In msRNAPs, a long α-helix called the bridge helix spans the central cleft downstream of the active site and bears at least superficial resemblance to the O-helix of T7-like RNAPs (Figure 1B). The bridge helix is continuous in yeast RNAPII and stacks against the 3′ end of the RNA:DNA hybrid in a pretranslocated TEC crystal (Gnatt et al., 2001). In bacterial RNAP, however, the bridge helix is distorted at the point of potential hybrid contact by a 2 aa loop that would clash with a pretranslocated 3′ bp. Thus, Kornberg and coworkers hypothesized that alternation of the bridge-helix conformations drives translocation (Cramer et al., 2001; Gnatt et al., 2001); coupled movements could involve contacts to nucleic-acid phosphates and other RNAP segments and may also gate NTP entry into the active site (see citations in Temiakov et al., 2004, this issue of *Cell*).

Temiakov et al. (2004; this issue of *Cell*) extend this

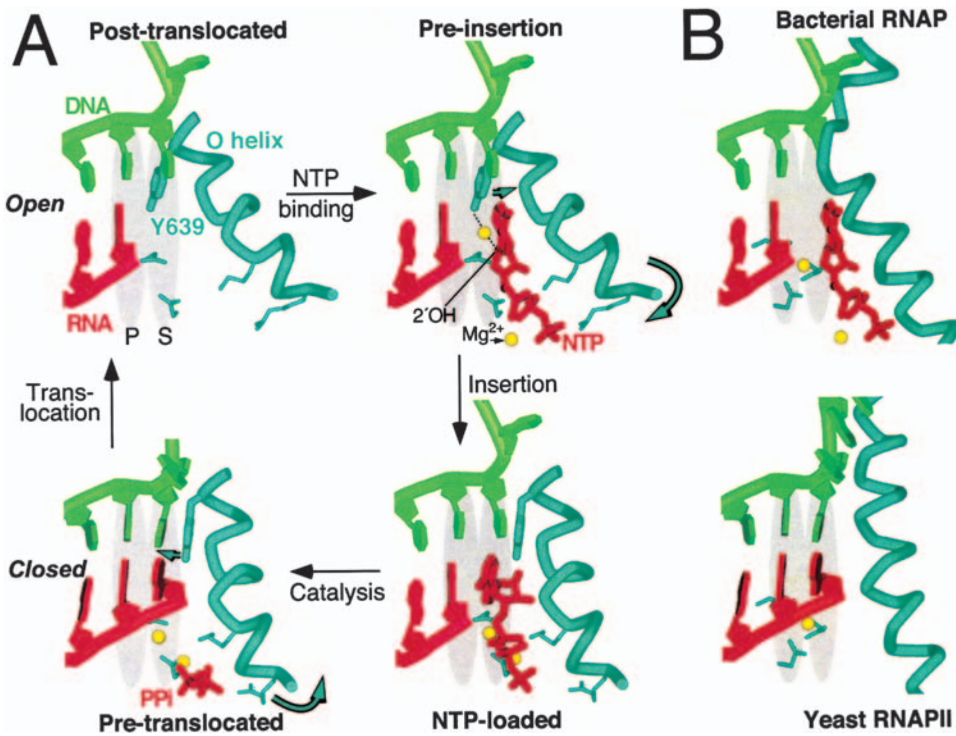

Figure 1. Active-Site Dynamics in RNAPs

(A) The four states of the T7 RNAP nucleotide addition cycle. These views are illustrative approximations of the crystal structures described by Yin and Steitz (2004, this issue of *Cell*) and Temiakov et al. (2004, this issue of *Cell*). P, product site (sometimes called *i* site in msRNAPs). S, substrate site (sometimes called *i*+*1* site in msRNAPs). Yellow spheres depict Mg^{2+} ions coordinated by phosphates, active-site residues, or (in the preinsertion state) Y639 and NTP 2′ OH groups.

(B) The bridge helix conformations of bacterial RNAP and yeast RNAPII, depicted as proposed for helix-distortion-driven translocation.

hypothesis by suggesting that msRNAPs also form an NTP preinsertion site in the distorted-helix conformation. Alignment (via RNA:DNA hybrids) of a T7 RNAP structure with a model msRNAP TEC positions the preinsertion NTP into msRNAP without major steric clash (Figure 1B). Thus, Temiakov et al. (2004, this issue of *Cell*) propose that NTP preinserts into the distorted-helix conformation, driving it into the straight helix upon delivery to the active site where, after catalysis, reformation of the distorted helix accompanying PPi release would drive translocation. This model attractively equates msRNAP bridge-helix distortion with T7 RNAP O-helix rotation, but several cautions are in order. So far, there is no direct evidence that the bridge helix of a given msRNAP can change conformation. A straight bridge helix is found in all yeast RNAPII structures and a distorted helix in all bacterial RNAP structures. A structure of an msRNAP TEC in a state predicted to contain a distorted bridge-helix conformation, such as the posttranslocated state, is not yet reported. Finally, two other locations for preinserted NTP in msRNAPs have been proposed recently (Holmes and Erie, 2003; Sosunov et al., 2003), but the three proposed preinsertion sites neither overlap nor are yet tested by site-directed mutation and biochemical assay. It seems a safe bet that the nucleotide addition cycle of msRNAPs involves alternating conformations around the active site, but it is currently difficult to handicap the competing models.

Yin and Steitz (2004, this issue of *Cell*) consider the implications for the energetics of translocation in both T7-like and msRNAPs. They propose that translocation in T7 RNAP is driven by tight coupling of PPi release to a once-per-nucleotide-addition-cycle movement of the O helix in which Y639 pushes the template base out of the substrate site. This is called a powerstroke mechanism (Wang and Oster, 2002) because the chemical energy derived from the phosphodiester bond cleavage directly moves RNAP along DNA. This proposal differs from recent translocation models that suppose RNAP can oscillate between pre- and posttranslocated registers and that NTP binding drives translocation by trapping the posttranslocated state. The latter mechanism is called a thermal ratchet (Wang and Oster, 2002) because thermal energy drives random RNAP movement, which is rectified when NTP binding acts similarly to the pawl of a ratchet. The thermal ratchet model has been favored because thermally driven translocation is known to occur on certain DNA sequences where backtracking of RNAP allows a more stable RNA:DNA hybrid to replace a weak one. It also can explain why the apparent binding constants for NTPs vary by orders of magnitude during elongation because the fraction of time RNAP is competent to bind NTP contributes to the apparent binding constant and depends on the sequence-dependent energetics of interactions among RNAP, RNA, and DNA (Guajardo and Sousa, 1997).

However, careful kinetic studies suggest that backtracking by msRNAP occurs only after the enzyme en-

ters an off-line, paused state (Palangat and Landick, 2001; Holmes and Erie, 2003; Nedialkov et al., 2003). Thus, powerstroke translocation could be the normal mode of RNAP movement on DNA. Indeed, an active-site rearrangement that disengages the powerstroke (e.g., by breaking hybrid-Y639 contact in T7 RNAP) suggests a possible structural basis for the off-line (paused) states that are the hallmark of RNAP elongation kinetics.

Much work remains to test these ideas. The distinction between a powerstroke and a thermal ratchet lies in details of the coupling of PPi release to the conformational change that are unknowable from the static pictures offered by crystallography. A true powerstroke requires tight coupling of the expenditure of chemical energy to a protein conformational change and motor proteins may exhibit fractional powerstroke/ratchet character (Wang and Oster, 2002). Demonstration of a powerstroke remains hotly debated even for the best studied motor proteins like myosin. The proposed coupling of translocation to a protein conformational change in both T7 RNAP and msRNAPs would remain consistent with the thermal ratchet mechanism if the pre- and post-translocation states interconvert prior to NTP binding irrespective of when PPi releases. Thus, aficionados of RNAP can rest assured of much remaining hard work, which will need to include both structures of msRNAP transcription complexes in additional states of the nucleotide addition cycle and many detailed biochemical studies, before we know the answers to the provocative hypotheses put forth in these two important reports.

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Selected Reading

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