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Proteins: how RNA polymerases work

Editorial overview

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Patrick Cramer is a professor of Biochemistry at the Ludwig Maximilians University of Munich, Germany, and head of the Gene Center Munich. He is a structural biologist who has contributed to our understanding of the structure and function of RNAP II from yeast and its associated factors. In a recent study, his laboratory described the structure of RNAP II in complex with the initiation factor TFIIB and proposed a mechanism for the initiation of gene transcription in eukaryotes.

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Eddy Arnold is a professor of Chemistry and Chemical Biology at Rutgers University and Resident Faculty Member at the Center for Biotechnology and Medicine. He is a structural biologist whose work has helped to define the structure and function of HIV-1 reverse transcriptase and of bacterial RNA polymerase. Structural studies from his laboratory have led to successful design of novel anti-AIDS drugs and have potential to facilitate the development of new antibiotics.

RNA polymerases (RNAPs) form a central class of enzymes responsible for the template-directed synthesis of RNA. The template can be either DNA or RNA. The RNA product may be structured, as for transfer or ribosomal RNAs, may be acting to direct protein synthesis, as for messenger RNAs, or it may be used as genetic material, as in RNA viruses. RNAPs show a great structural variety, from single-subunit enzymes to large complexes of up to 17 different polypeptide subunits and a molecular weight of around 700 kDa. Recent structure–function analyses of different RNAPs have dramatically improved our understanding of the mechanisms used by this class of proteins. In this issue of Current Opinion in Structural Biology, recent progress in our understanding of the structure and function of different RNAPs is reviewed. Underlying principles have emerged, but apparent are also diverse solutions to the evolutionary challenge of accurately copying a template nucleic acid into a complementary RNA chain.

Many early and fundamental insights into how RNAPs work have been obtained by studying the structure and function of the single-subunit RNAP from bacteriophage T7. To date, T7 RNAP is one of the best understood RNAPs. Tom Steitz, who received the Nobel Prize in Chemistry this year for his work on the structure and function of the ribosome, summarizes new insights into T7 RNAP function and in particular the structural basis for the transition of this enzyme from initiation to elongation modes. The polymerase undergoes very major structural rearrangements during this transition. During initiation, it binds upstream promoter DNA and places the template strand into the active site. During elongation, however, it accommodates a DNA–RNA hybrid and downstream DNA. This dramatic change is possible because the N-terminal domain of the enzyme entirely refolds, and this refolding destroys the promoter-binding site and creates a hybrid-binding site. A new structure of an intermediate of the transition helps to elucidate how these rearrangements may take place.

Structure and function of cellular RNA polymerases

In all cells, related multisubunit RNAPs carry out transcription of the genetic material. Bacterial RNAP has been studied in great detail both functionally and structurally. In his review, Dmitry Vassylyev summarizes insights into transcription mechanisms that were obtained with the bacterial enzyme. After providing an overview of the RNAP architecture, he reviews the structural basis for nucleotide incorporation into the growing RNA, and then describes conformational transitions and surfaces on the enzymes that are targeted by regulatory factors of transcription. Particular emphasis is given on the secondary channel, a pore beneath the active site of RNAP that accommodates protein factors involved in RNA cleavage and the regulation of transcription elongation.

One of the most dynamic and enigmatic aspects of the transcription mechanism is the translocation of nucleic acids after a nucleotide has been added to the growing RNA chain. Evgeny Nudler summarizes our current understanding of the translocation mechanism that has resulted from structural studies that trapped RNAPs in different conformational states, and from biochemical studies that used site-directed mutagenesis, kinetics, and site-specific crosslinking. Two elements in the RNAP active center, the bridge helix and the trigger loop, are involved in translocation and physically and functionally cooperate during the nucleotide addition cycle.

Additional aspects of the nucleotide addition cycle and the translocation mechanism of RNAPs are presented in the review of Dorothy Erie and Scott Kennedy. These authors summarize experimental evidence, mainly coming from kinetic studies, that suggests an extension of the model for nucleotide addition to include the binding of an additional, templated nucleoside triphosphate (NTP) to a noncatalytic site in the main channel of RNAP, the central cleft of the enzyme. The authors also discuss how NTPs can assist in translocation, and propose the site for binding a second NTP.

Understanding the details of the bacterial RNAP mechanism is highly relevant for understanding how antibiotics work that target this enzyme. In the review by Eddy Arnold, Richard Ebright, and coworkers, our current understanding of the structural basis of antibiotics action is summarized. Bacterial RNAP is a proven antibiotic target as the rifamycins are central drugs for the treatment of tuberculosis. New antibiotics are urgently needed as drug resistance has emerged against nearly all classes of the antibacterial drugs in current use. Crystal structures are described for four classes of antibiotics in complexes with bacterial RNAP: rifamycins, sorangicin, streptolydigin, and myxopyronin. Three distinct binding sites have been identified through these studies, and the structural, biochemical, and genetic data have elucidated mechanisms of inhibition. Myxopyronin and related inhibitors exhibit potent activity against *Mycobacterium tuberculosis* and structure-based design efforts are underway to develop new therapeutic agents targeting the novel allosteric site revealed by crystallographic and mutagenesis studies.

The catalytic core of bacterial RNAP is conserved in the archaeal RNAP and in the eukaryotic RNAPs, whereas the number of subunits is larger and the enzyme surfaces are specialized for interactions with large numbers of initiation and elongation factors that are generally not present or not conserved in bacteria. Recently structures of archaeal RNAPs were reported and these are presented in a review by Katsu Murakami. It was found that archaeal RNAPs resemble the eukaryotic RNAP II enzyme, but lack several domains and contain, in some cases, an additional iron–sulphur cluster not found in eukaryotic RNAPs. Since archaeal RNAPs can be obtained in recombinant form, can be mutated easily, and require only two additional factors for promoter-dependent transcription, the archaeal enzyme is a very good model system for the eukaryotic RNAP II, which is not readily available for promoter-dependent transcription analysis *in vitro*.

In addition to bacterial RNAP, the best studied cellular RNAP is RNAP II, which transcribes protein-coding genes to synthesize mRNAs in eukaryotes. Jasmin Sydow and Patrick Cramer review recent structural and functional studies that elucidate the mechanisms underlying the fidelity of RNAP II and cellular RNAPs in general. Two aspects are relevant for understanding fidelity: the ability of RNAPs to prevent the incorporation of incorrect nucleotides, and their ability to proofread the nascent transcript, and to thereby remove errors. A model for understanding proofreading by RNAPs is presented, which can now be further tested experimentally. A comparison with DNA polymerases reveals similar strategies to ensure fidelity during the nucleotide incorporation step during replication and transcription, but a different strategy for proofreading.

In addition to RNAP II, RNAPs I and III are present in eukaryotic cells. In his review, Michel Werner and colleagues summarize the recent advances in the structure–function analysis of RNAPs I and III, which transcribe mainly ribosomal and transfer RNAs, respectively. Thus far, no crystal structures are available for RNAPs I and III, but electron microscopy, crystallography of subunits, and modeling have elucidated their architecture. Surprising recent functional studies are also reviewed that indicated a role of an RNAP II-associated factor in RNAP I function. The role of polymerase-specific subunits and functions during initiation, elongation, and termination is also discussed. It is apparent that we begin to understand the basis for promoter-specific transcription and gene class-specific functions of RNAPs in eukaryotes.

The vagaries of viral RNA polymerases

Many RNA viruses encode RNA-dependent RNA polymerases (RdRps). These RdRps have dual roles of transcribing messages from RNA templates, and also copying the RNA genome in a replicase capacity. Common features of viral RdRps include the right-hand architecture with fingers, palm, and thumb subdomains (like most DNA polymerases and reverse transcriptases). Several experts describe elegant structural studies of viral RdRps and diverse and fascinating mechanistic implications. Viral polymerases, including RdRps, are attractive targets for new antiviral agents, as they are essential enzymes that catalyze reactions that are distinct from host cell processes. Success in structure-based design targeting the evolutionarily related HIV-1 reverse transcriptase, a DNA polymerase that can use either RNA or DNA templates,

gives encouragement that structural information about RdRps can be exploited to facilitate development of new agents targeting a variety of important human diseases caused by viruses.

Kay Choi and Michael Rossmann review the status of structural studies of RdRps from *Flaviviridae*. Structures of RdRps from the three genera of *Flaviviridae* (hepacivirus, pestivirus, and flavivirus) have been determined and these studies have provided complementary insights. A characteristic feature of the *Flaviviridae* RdRps is a close association of the fingers and thumb subdomains (via the 'fingertips'), which restricts the size of the template-binding channel to permit single-stranded but not double-stranded RNA binding. This article discusses conformational changes that likely occur with *Flaviviridae* RdRps and implications of the structures for *de novo* synthesis, in which the RdRp synthesizes a dinucleotide primer from nucleoside triphosphates. A structure of the pestivirus bovine viral diarrheal virus complexed with GTP together with knowledge from ϕ6 polymerase structural studies has permitted detailed modeling of the *de novo* initiation mechanism.

Nuria Verdaguer and colleagues describe a remarkable series of structures of the foot-and-mouth-disease virus (FMDV) RdRp. Like other picornaviruses, FMDV uses VPg, a small virally encoded protein, to prime replication. The RdRp, also known as the 3D polymerase (3Dpol), covalently links a UMP to a tyrosine hydroxyl of VPg, and then uses the uridylated VPg to prime replication. A series of seven FMDV RdRp structures have revealed critical insights into detailed mechanisms of replication initiation, nucleotide binding, and polymerase catalysis. A structure of the FMDV 3Dpol complexed with VPg-UMP in which the UMP is covalently attached to Tyr3 of VP3 shows the UMP bound at the active site, sharing chelation of two divalent metal cations shared with catalytic Asp residues. In addition to catalytic and elongation complexes with normal substrates, a structure with bound ribavirin triphosphate is described with its implications for the mechanism of the FMDV RdRp M296I drug-resistance mutation and for drug design opportunities.

Julien Lescar and Bruno Canard present a review of recent advances in structural studies of RdRps from flaviviruses and picornaviruses. Structures of the dengue virus, West Nile virus, and hepatitis C virus NS5 polymerases, which are potential antiviral targets, are described. Interactions of the polymerase domains with other nonstructural proteins from flaviviruses (especially the flaviviral NS5A methyltransferase) and picornaviruses (especially the 3C protease and VPg) are considered both before and after proteolytic processing of precursor polyproteins. Among the novel features of the dengue virus RdRp is the identification of zinc ions bound in zinc-finger motifs in the fingers and thumb subdomains. Structures of poliovirus and coxsackievirus 3Dpol are summarized. A structure of the coxsackievirus B3 3Dpol complexed with VPg shows the binding site expected from decades of biochemical and genetic studies, which is distinct from the second site described by Verdaguer and colleagues for the FMDV 3Dpol complexed with VPg.

Craig Cameron, Ibrahim Moustafa, and Jamie Arnold consider the problem of nucleotide incorporation fidelity from a structural perspective. Nucleotide incorporation fidelity of RdRps may have important implications on both antiviral agent and vaccine development. RNA viral polymerases, including RdRps and reverse transcriptases, are relatively error-prone largely because they lack proofreading exonuclease activities. Concentrating on viral RdRps, the authors conclude that existing experimental structures do not provide a complete understanding of fidelity. Kinetic and site-directed mutagenesis studies have indicated a function for a conserved basic residue in picornaviral RdRp and retroviral RTs to function as a general acid in catalysis, but this role has not been visualized in RdRp or RT structures determined to date. They suggest that further exploration of the problem, particularly probing the intrinsic dynamics of the system, through either computational molecular dynamics simulations and/or NMR studies, may provide some of the missing links required for a full understanding of fidelity.

Sarah McDonald, Yitzi Tao, and John Patton summarize structural studies of RdRps from *Reoviridae*. *Reoviridae* are double-stranded RNA (dsRNA) viruses that use unique mechanisms for packaging the correct complement of 10–12 dsRNA genomic segments. Ground-breaking studies of reovirus and rotavirus RdRps, known as λ3 and VP1 proteins, respectively, have revealed unique four-tunneled architectures of these polymerases. A series of relevant complexes of reovirus λ3 and rotavirus VP1 from the authors (in collaboration with Stephen C Harrison) have yielded critical insights into mechanisms by which *Reoviridae* RdRps access template RNA and synthesize RNA messages and dsRNA genomic segments in coordination with particle assembly. The four-tunnel topology neatly explains how substrates (template RNA, metal ions, and nucleotides) can efficiently access the enzymatic machinery and how RNA templates and products can exit following RNA synthesis.