Published in final edited form as:

Nature. 2013 October 31; 502(7473): 629–630. doi:10.1038/nature12700.

STRUCTURAL BIOLOGY:

Pivotal findings for a transcription machine

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Abstract

Crystal structures of the complete RNA Polymerase I complex are now revealed. These structures link opening and closing of the DNA binding cleft of this enzyme to control of transcription.

RNA polymerases (Pol's) are intricate molecular machines that transcribe DNA into RNA, combining RNA synthesis with the precise movement of a DNA template across their active site. Animal, plant and fungal (eukaryotic) cells have several Pol's, each dedicated to the production of specific RNAs. Pol I synthesizes the ribosomal RNA component of the cell's protein-producing factories and, therefore, is crucial for cell survival, growth and proliferation; malfunction of this machine can result in cell death or support the unrestrained proliferation characteristic of cancer cells¹. In two ground-breaking papers published on Nature's website today, Fernández-Tornero *et al.*² and Engel *et al.*³ present the first crystal structures of the complete 14-subunit Pol I complex of yeast at 3.0- and 2.8-Ångström resolutions, respectively, providing unprecedented insight into Pol I-specific features, potential transcription mechanisms and evolutionary conservation of structures and functions of Pol enzymes.

Crystal structure analyses of bacterial Pol, eukaryotic Pol II and archaeal Pol have detailed the architecture of these enzymes, the molecular interactions of their subunits and their inner workings in transcription⁴. Pol's -I and -III share overall architecture with the 12-subunit Pol II⁵. Each Pol incorporates specific sub-complexes and features influencing their ability to transcribe a specific subset of genes - whereas Pol I produces rRNAs, Pol II generates messenger RNAs and Pol III synthesizes small non-coding RNAs, including tRNAs and 5S rRNA⁶.

Amongst the eukaryotic Pols, Pol I is the most productive. To achieve high-throughput transcription, multiple Pol I complexes transcribe the rDNA, densely packed along each template and highly processive. The Pol I crystal structures reveal various distinguishing features with potential to influence the output of the enzyme, partly by facilitating its productive association with the DNA template.

The structures from both teams confirm that the zinc-ribbon domain at the carboxy-terminus of Pol I subunit A12.2 inserts into, and forms an integral part of, the active site region of the Pol I enzyme⁷ (Figure 1). (By contrast, TFIIS, the functional counterpart of A12.2 in Pol II, only transiently associates with the active site of paused Pol II). Positioned within the active site, the A12.2 zinc-ribbon can stimulate removal of faulty and redundant RNA sequences to

prevent Pol I arrest and consequential traffic jams along the template, thus increasing transcription efficiency. Stability of A12.2 in Pol I is influenced by its interaction with the (TFIIIF-like) dimerization domain of Pol I-specific sub-complex A49-A34.5⁸ (Figure 1). This can now be rationalized by the structural data, which reveals the contact points of A12.2 and the A49-A34.5 amino-terminal dimerization domain, as well as, the extensive interactions of the A34.5 carboxy-terminus as it wraps around the outer face of A135, helping to anchor the sub-complex to Pol I.

Procession of Pol's along a DNA template is facilitated by a closed clamp component. The structures of Pol I reveal that the A43-A14 sub-complex, comprising the fixed stalk (Figure 1), contributes to a permanently closed state of the clamp (Figure 1) and, therefore, to the high processivity of Pol I. By contrast, the clamps of other RNA polymerases are mobile elements. In Pol II, attachment of the Rpb4-Rpb7 stalk to the polymerase is required to lock the clamp in a closed state over the RNA-DNA produced during transcription, but the stalk is detachable^{9,10}.

Intriguingly, the crystals of both teams are dimers of Pol I. The A43 carboxy-terminal 'connector' domain of the stalk of each Pol I in the dimer inserts into the DNA-binding cleft of the other, making extensive contacts with the cleft and the clamp coiled coil. The Pol I dimers have an unusually wide or expanded cleft (Figure 1), perhaps partly due to the A43-connector insertion. The cleft is too wide to anchor the RNA-DNA, particularly near the active site. This widening contributes to further re-arrangements near the active site, for example: critical aspartate loop interactions are configured differently from those of Pol II, the bridge helix contributing to DNA movement through the active site is unfolded in the middle and kinked, and, there is partial blockage of the gate to the exit channel for newly synthesized RNA. In addition, the wide cleft is occupied by a Pol I-specific extended loop of A190, referred to as the 'expander'³ or the 'DNA mimicking loop'². Due to its location, this loop would interfere with DNA loading at the active site. In one of the three Pol I crystal structures presented by Fernández-Tornero *et al.*, no loop was detectable at the active site², hinting that the loop is unlikely to be essential for stabilization of the expanded cleft, though not excluding a role in its establishment.

The three crystals of Fernández-Tornero *et al.* display varying degrees of widening². Comparative structural modeling of Pol's suggests that the cleft of Pol I widens due to relative pivoting of the 'core' and 'shelf' modules (formed mainly by the largest subunits A135 and A190) at the cleft base, near the active site (Figure 1)^{2,3}. Engel *et al.*³ draw parallels to similar domain-pivoting in bacterial inhibitor-bound or paused Pol, where a pivoted or ratcheted state is associated with cleft opening and coupled rearrangements of domains near the active centre, making the polymerase inactive in transcription^{11, 12}.

As the Pol I structure implies that the DNA template must be loaded into Pol I with a closed clamp, perhaps the open or shut status of the cleft contributes to DNA-loading efficiency. It is possible that template DNA binding in the open cleft of a Pol I monomer could trigger cleft closure, potentially coupled with re-location of the expander loop, rendering the enzyme active. Cleft closure by pivoting of core and shelf modules is, presumably, concomitant with bridge helix re-folding, opening of the RNA exit gate and the approach of

A135 to anchor the DNA template in the active site. Understanding of the exact re-arrangements will hinge on structural analysis of Pol I engaged in transcript elongation and, therefore, in complex with DNA and RNA.

Engel *et al.* propose that factors binding at the core-shelf interface might facilitate cleft closure. They speculate that Rrn3 - a factor that tethers Pol I to proteins bound specifically to promoter DNA sequences - might trigger cleft closure by binding Pol I near the RNA-exit channel^{3,13}. This attractive possibility awaits confirmation, perhaps through analysis of a Pol I-Rrn3 co-crystal. Conversely, factors terminating Pol I transcription might induce cleft opening. In all probability, regulation of transcription by modulation of the core-shelf interface, as observed in bacterial Pols, is also a feature of eukaryotic Pols³.

Solving the crystal structure of the complete Pol I complex is a triumph, providing a wealth of information with which to build a picture of the specific mechanisms and regulation of transcription of the rRNA genes in eukaryotes and to explore the general mechanisms of transcription by all Pol's. Another major tour de force will be necessary to solve the structure of Pol I in elongation mode and, further, that of the complete Pol I pre-initiation complex, incorporating Rrn3, the core promoter-binding factors (Rrn6, Rrn7 and Rrn11 with TBP) and the rDNA promoter sequences. Such structures, together with those presented by Fernández-Tornero *et al.*² and Engel *et al.*³, will yield information vital to establish when and where crucial protein and DNA contacts are made, disrupted or rearranged as Pol I steps through the transcription cycle.

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Figure 1. RNA Polymerase I (Pol I)

Front view of the 14-subunit Pol I complex with approximate locations of the core (blue) and shelf (red) modules, which broadly overlap the cleft sides formed by subunits A135 and A190, respectively, as well as the DNA-binding cleft. The open and closed states of the cleft are determined by relative pivoting of the core and shelf modules at the cleft base near the active site (arrows). Also indicated are: the permanently closed clamp; the fixed stalk, comprised of the A43-A14 sub-complex, which contributes to the permanent closure of the clamp; the active site of the enzyme, where RNA synthesis occurs; the TFIIS-like carboxy-terminal domain of A12.2, integral to the active site; and, the TFIIF-like A49-A34.5 sub-complex, which stabilizes A12.2.