

# RNA Polymerase I: A Multifunctional Molecular Machine

Jeremy R. Haag<sup>1</sup> and Craig S. Pikaard<sup>1,\*</sup>

<sup>1</sup>Department of Biology, Washington University, 1 Brookings Drive, St. Louis, MO, USA

\*Correspondence: pikaard@biology2.wustl.edu

DOI 10.1016/j.cell.2007.12.005

In this issue, Kuhn et al. (2007) report the complete structure of the 14-subunit yeast RNA polymerase (Pol) I enzyme at 12 Å resolution using cryo-electron microscopy (cryo-EM). Their study reveals that three subunits of Pol I perform functions in transcription elongation that are outsourced to the transcription factors TFIIF and TFIIIS in the analogous Pol II transcription system.

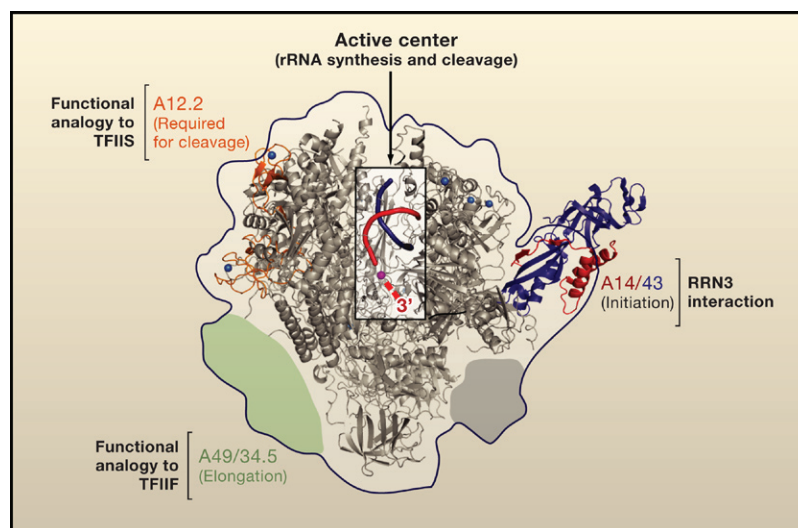
Bacteria and Archaea decode their genomes using a single DNA-dependent RNA polymerase, whereas eukaryotes have evolved at least three (Pol I, II, and III, plus IVa and IVb in plants). Furthermore, whereas the RNA polymerase of *Escherichia coli* is composed of only four different proteins, yeast RNA Pol I, II, and III are far more complicated, consisting of 14, 12, and 17 subunits, respectively (Werner, 2007). Among these are subunits that are orthologous to the bacterial polymerase subunits. Five additional subunits of Pol I, II, and III are identical and are encoded by the same genes. The remaining subunits are unique to Pol I, Pol II, or Pol III and are thought to mediate their distinct functions: Pol II mostly transcribes protein-coding genes and regulatory RNA genes (Hahn, 2004); Pol I transcribes genes encoding the 18S, 5.8S, and 25–28S rRNAs that form the catalytic core of ribosomes (White, 2005); Pol III primarily transcribes tRNA genes and 5S rRNA genes (White, 2005); and in plants, Pol IVa and Pol IVb function in a pathway generating short-interfering RNAs that direct DNA methylation (Pikaard, 2006).

Understanding the functions of the various eukaryotic polymerase subunits is a major challenge in which structural biology is playing a critical role. The high resolution (2.8–3.3 Å) crystal structures of bacterial RNA polymerase and yeast RNA Pol II (Cramer et al., 2001; Gnatt et al.,

2001; Zhang et al., 1999) revealed a remarkable conservation of structure at the core of these enzymes. Now, Kuhn et al. (2007) provide the most detailed and complete view of the Pol I enzyme to date. By combining structural analyses with manipulations of subunit compositions and biochemical assays, their study is a tour-de-force that reveals functions conserved among Pol I, II, and III as well as aspects of Pol I functional specialization.

As the starting point for their current work, Kuhn et al. (2007) derived a cryo-EM density map based on the analysis of ~40,000 purified Pol I

molecules and looked for correspondence between the density map and the Pol II crystal structure (Cramer et al., 2001). The Pol II structure fit perfectly onto the Pol I EM density map in the regions corresponding to the five subunits that are common to Pol I, II, and III. Highly conserved domains within paralogous catalytic subunits also fit nicely, including the active center and bridge helix that spans the template cleft. Interestingly, some domains of Pol II that lack obvious Pol I counterparts based on sequence comparisons, such as the jaw and lobe domains, are nonetheless apparent in the Pol I



**Figure 1. RNA Polymerase I**

Annotated overview of the 12 Å RNA polymerase I structure highlighting the positions of functional subdomains. Figure adapted from Kuhn et al. (2007).

structure and presumably carry out analogous functions—a hypothesis that can now be tested based on the structural insight.

Regions displaying distinct structural variation between Pol I and Pol II are candidates for polymerase-specific functions. One such region of Pol I includes the A14/A43 subunit heterodimer, which has weak homology to the Rpb4/Rpb7 and C17/C25 heterodimers of Pol II and Pol III, respectively, but insufficient similarity to allow homology modeling based on the Pol II crystal structure. Kuhn et al. (2007) determined the crystal structure of the A14/A43 heterodimer at 3.1 Å resolution and fit the structure unambiguously into the EM density map. A43, in turn, is known to interact with Rrn3 (TIF-IA in mammals), an essential transcription factor that regulates Pol I activity in response to growth status and the cellular need for ribosomes and protein synthesis (Peyroche et al., 2000). Collectively, the new structural data indicate that Rrn3 interacts with Pol I on an upstream surface relative to the direction of transcription (Figure 1), an important new piece of the puzzle for understanding Pol I transcriptional activation.

One of the most interesting aspects of the study by Kuhn et al. (2007) involves the function of the Pol I-specific subunits A49 and A34.5. By determining the cryo-EM structures of Pol I with or without these subunits, the precise position of the A49/34.5 subcomplex was defined. The authors recognized that the A49 and A34.5 subunits have weak sequence and structural homology to the RAP74 and RAP30 subunits of transcription factor TFIIF, a factor needed for Pol II promoter clearance and transcript elongation. Indeed, data from *in vitro* and *in vivo* assays indicate that Pol I lacking the A49 and A34.5 subunits has impaired

transcription elongation activity that can be rescued by exogenously supplied A49/34.5 heterodimers. Collectively, the data suggest that the A49/34.5 subcomplex fulfills an elongation function accomplished by TFIIF in the context of Pol II transcription (Figure 1). The authors further suggest that the weakly homologous C37/C53 subcomplex is likely to carry out this same function in Pol III. Interestingly, RAP30 and RAP74 got their names as RNA polymerase II-associating proteins (Sopta et al., 1985). The fact that these proteins do not stably associate with Pol II, unlike the functionally analogous Pol I and Pol III subunits, provides one potential explanation for why Pol II has fewer subunits than Pol I and Pol III.

An important biochemical insight provided by Kuhn et al. (2007) is that Pol I has a strong 3'-end RNA cleavage activity *in vitro*. A similar RNA cleavage activity for Pol III is attributable to the C11 subunit, which shares sequence similarity with the Pol I subunit A12.2 (Figure 1). Indeed, Pol I missing the C-terminal domain of A12.2 is unable to cleave RNA. This domain also shows homology to TFIIS, a Pol II elongation factor that works with the Rpb9 subunit to stimulate RNA cleavage when Pol II encounters a roadblock to elongation and backtracks to extricate itself, yielding a 3' end that can be elongated in a second attempt to read through the problematic region. Ribosomal RNA gene primary transcripts are approximately 5 kb, so a similar activity may be necessary for Pol I to maintain its processivity. Importantly, the A12.2 subunit is required for Pol I termination (Prescott et al., 2004), suggesting that RNA cleavage may be part of the Pol I termination process as is the case for Pol II termination following the cutting of nascent Pol II transcripts at Poly(A)

cleavage sites. A third potential role of the RNA cleavage activity is in the proofreading of nascent transcripts and correction of misincorporated nucleotides in order to prevent non-functional or potentially deleterious RNAs from being incorporated into ribosomes.

The paper by Kuhn et al. (2007) is yet another clear example of how structure can illuminate function, and no doubt numerous follow-up studies will be spurred by their observations and speculations. Breakthrough papers always provide food for thought, and Kuhn, Cramer, and their colleagues have served up a feast with this exciting new study.

## REFERENCES

- Cramer, P., Bushnell, D.A., and Kornberg, R.D. (2001). *Science* 292, 1863–1876.
- Gnatt, A.L., Cramer, P., Fu, J., Bushnell, D.A., and Kornberg, R.D. (2001). *Science* 292, 1876–1882.
- Hahn, S. (2004). *Nat. Struct. Mol. Biol.* 11, 394–403.
- Kuhn, C.-D., Geiger, S.R., Baumli, S., Gartmann, M., Gerber, J., Jennebach, S., Mielke, T., Tschochner, H., Beckmann, R., and Cramer, P. (2007). *Cell*, this issue.
- Peyroche, G., Milkereit, P., Bischler, N., Tschochner, H., Schultz, P., Sentenac, A., Carles, C., and Riva, M. (2000). *EMBO J.* 19, 5473–5482.
- Pikaard, C.S. (2006). *Cold Spring Harb. Symp. Quant. Biol.* 71, 473–480.
- Prescott, E.M., Osheim, Y.N., Jones, H.S., Alen, C.M., Roan, J.G., Reeder, R.H., Beyer, A.L., and Proudfoot, N.J. (2004). *Proc. Natl. Acad. Sci. USA* 101, 6068–6073.
- Sopta, M., Carthew, R.W., and Greenblatt, J. (1985). *J. Biol. Chem.* 260, 10353–10360.
- Werner, F. (2007). *Mol. Microbiol.* 65, 1395–1404.
- White, R.J. (2005). *Nat. Rev. Mol. Cell Biol.* 6, 69–78.
- Zhang, G., Campbell, E.A., Minakhin, L., Richter, C., Severinov, K., and Darst, S.A. (1999). *Cell* 98, 811–824.