A Cold Look at Transcription

Two single-particle reconstructions, one obtained by cryo-EM of yeast RNAPII polymerase and the other by EM of negatively stained molecules of yeast RNAPII holoenzyme, shine new light on the multistep transcription process in eukaryotes.

In both prokaryotes and eukaryotes, transcription (for reviews, see, for example [1]) involves a molecular machine formed by RNA polymerase II, several general helper proteins (transcription factors), and the target DNA. To get started, RNA polymerase II must first associate with five or more factors around the promoter in a process called preinitiation. This complex is the target of regulatory and activating factors. A decade ago it was discovered that regulation and activation in vitro require the presence of a cofactor called Mediator [2]. Mediator, a large protein, apparently associates with the preinitiation complex, and the complex forms with RNAPII is known as the RNAPII holoenzyme. In controlling this association, the phosphorylation of the C-terminal domain (in turn dependent on the action of one of the transcription factors) of RNAPII plays a crucial role. Upon dissociation, Mediator is recycled, and possibly forms a scaffold at the promoter for reinitiation.

The multiple stages of assembly and processing in transcription present a new familiar quandary in the structural biology of macromolecular assemblies: high-resolution X-ray structures can be obtained only for some but not all components, and these are often in undefined states or with peripheral loops chopped off to aid in crystallization. The biggest coup so far in the struggle for high-resolution structural data on the transcription machinery has been the successful X-ray study of a transcribing RNAPII/DNA/RNA complex from yeast by Roger Kornberg’s group at Stanford [3]. The structure of RNAPII has been solved by the same group [4]. Nevertheless, functional interpretations require the integration of information from X-ray crystallography with data gleaned from other sources. Increasingly, 3D electron microscopy of single particles is being used to provide this information [5–7]—this technique yields the structure of entire complexes, sometimes in defined processing states, albeit at lower resolution. In particular, the solution structure, obtained by cryo-EM of single molecules embedded in vitreous ice, can answer the critical question of what the molecule looks like in its active form. Mediator is among the molecules important in transcription that were first visualized by single-particle 3D EM [7].

Now, two articles from the group of Francisco Asturias, in the August issues of Structure and Molecular Cell [8, 9], describe EM reconstructions that shine new light on the conformation of yeast RNAPII in solution and on the yeast Mediator-RNAPII interaction in the holoenzyme. One question addressed by the cryo-EM study is the position of the clamp designed to hold the template DNA. Interestingly, the cryo-EM projections showed evidence that the molecules exist in a mixture of conformations, distinguished mainly by different clamp positions, and the challenge was to extract homogeneous data sets for meaningful reconstructions. There is no general way of achieving such a separation, except when credible reference structures exist for all conformations present. Applying image processing with virtuosity, the authors used reference structures based on the X-ray data but with different clamp positions, and were able to show that 25% of all molecules in solution had a collapsed clamp—a configuration prohibiting DNA access—while the majority exhibited the competent, DNA-accessible conformation, very similar to the X-ray structure of the transcribing complex [3]. Apparently, the two states are separated only by a small energy barrier.

Davis and coworkers [9] describe a reconstruction based on images of negatively stained specimens of the holoenzyme. To achieve complete staining of the large complex, the authors used a double-carbon layer, sandwich technique. Considering the use of negative staining, now frowned upon in the new age of frozen-hydrated techniques, the reconstruction shows a surprising amount of detail, allowing the orientation of the RNAPII molecule within the holoenzyme to be established with a high degree of certainty. The resulting model of the holoenzyme reveals the multiple contact sites between RNAPII and Mediator. It is particularly interesting that the template DNA binding cleft of RNAPII remains accessible in the holoenzyme, consistent with the idea that Mediator might remain at the promoter site, acting as a platform for reinitiation. There is no doubt that an extension of these studies with cryo-EM will bring further clarification of the matter.

To top it off, an X-ray structure of the prokaryotic RNA polymerase holoenzyme in complex with DNA has just appeared [10] that agrees with the results of Asturias’s group in all essential features. Thanks to the increasing number of structural studies of the modules of the transcriptional complex from several species, we can see a unified picture of transcription emerge.

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