Microsc. Microanal. 8 (Suppl. 2), 2002
10.1017.S1431927602100018

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Structure of the Eukaryotic Transcription Machinery: Insights into the Mechanism of Transcription Initiation and Regulation

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Precise regulation of gene expression underlies development, oncogenesis, and the constant reshaping of the cell in response to a variety of metabolic and environmental cues. Regulation is mediated by a large number of factors that control the action of RNA polymerase II (RNAPII), the enzyme responsible for synthesis of all mRNA in eukaryotes. Structural information about the complex molecular machinery responsible for transcription and its regulation is essential to advance our understanding of the mechanism of gene expression. Macromolecular single particle cryo-electron microscopy (cryo-EM) is ideally suited to the structural study of such large systems, and can provide moderate (10-50Å) resolution structural information. Combined with high-resolution structures of individual components obtained by x-ray crystallography or NMR spectroscopy, cryo-EM structures of large assemblies will lead to an understanding of the entire transcription apparatus at the atomic level. Single particle cryo-EM analysis does not require the use of crystalline samples. Comparatively small amounts of material are sufficient to study macromolecular structure under physiologically relevant conditions, and without the artificial constraints imposed on molecular conformation by the formation of crystals.

We have used single particle cryo-EM to study the conformation in solution of RNAPII, and its implications for interaction of the enzyme with promoter DNA. Our analysis indicates that the conformation of RNAPII under physiologically relevant conditions precludes it from interacting with double-stranded promoter DNA (Fig. 1). Our 18Å resolution structure of RNAPII also reveals the location of subunits Rpb4/Rpb7, which was not determined in previous x-ray studies of the polymerase [1]. The essential Rpb7 subunit contains two single-stranded nucleic acid binding sites [2], and our structure shows that it is ideally positioned to determine the path of the nascent RNA transcript exiting the active site cleft of RNAPII (Fig. 1). Transcription is closely coupled to processing of the RNA transcript, and the essential role of Rpb7 is most likely related to the efficient delivery of the nascent RNA to the RNA processing machinery.

RNAPII is unresponsive to regulation without the intervention of a coactivator complex, that functions as an essential interface between polymerase and the activator and repressor proteins that bind to enhancer and repressor DNA control sequences. We have pursued structural characterization of the holoenzyme formed by RNAPII and the Mediator coactivator complex. Mediator was first identified in the yeast *Sacharomyces cerevisiae*, but homologs of the complex have now been characterized in all eukaryotes, from yeast to man. A 35Å resolution structure of the RNAPII holoenzyme (>35 polypeptide components, and >1.5 MDa MW) shows Mediator organized in three distinct

https://doi.org/10.1017/S1431927602100018 Published online by Cambridge University Press

structural modules, wrapped around RNAPII (Fig. 2A). Although multiple contacts are established between Mediator and RNAPII, must of the polymerase surface remains accessible for interaction with additional components of the transcription machinery. Comparison with the x-ray structure of RNAPII has identified RNAPII subunits involved in contacts with Mediator. The interaction is centered on RNAPII subunits Rpb3 and Rpb11 (Fig. 2B). These subunits are located opposite to the entrance to the active site cleft of the enzyme, implying that interaction of RNAPII with the coactivator complex does not interfere with association of the enzyme with its promoter DNA substrate. Factors involved in transcription regulation in prokaryotic organisms have been shown to interact with the __’ homodimer, the bacterial homolog of the eukaryotic Rpb3/Rpb11 complex, and mutations in the Rpb3 subunit of RNAPII have been shown to interfere with activated transcription [3]. Therefore, it appears that a similar mechanism is involved in regulation of transcription from prokaryotes to man.

References

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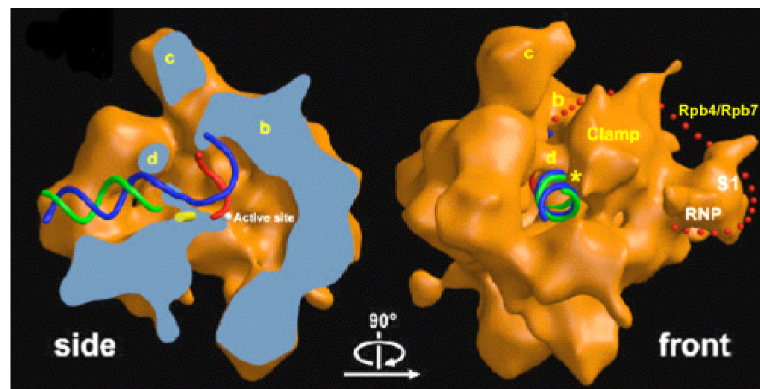

Fig. 1. Structure of wild-type RNAPII in solution and location of subunits Rpb4/Rpb7.

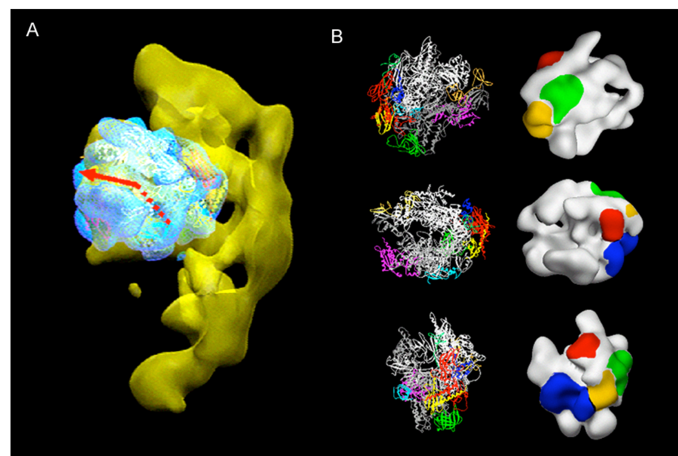

Fig. 2. Structure of the RNAPII holoenzyme and analysis of the RNAPII/Mediator interface.

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