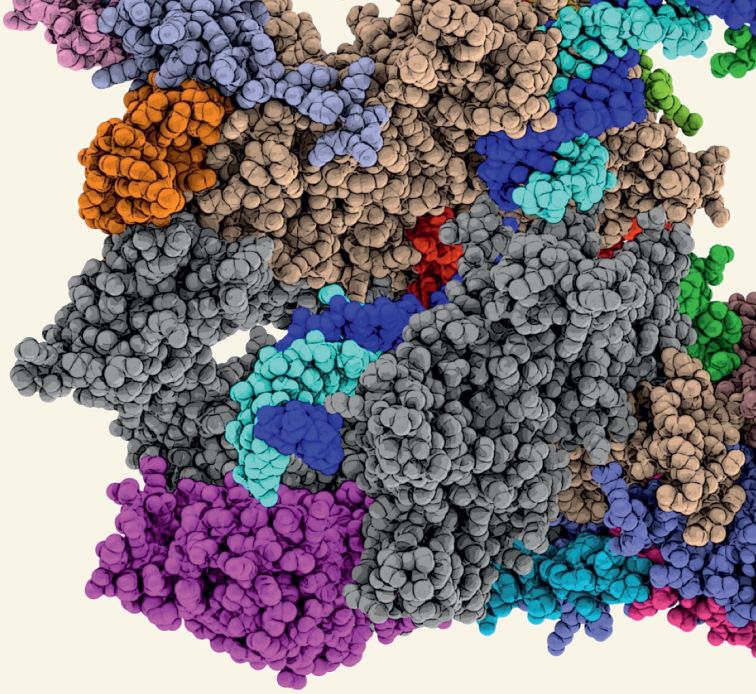

Taking the first steps: How ribosome biogenesis starts

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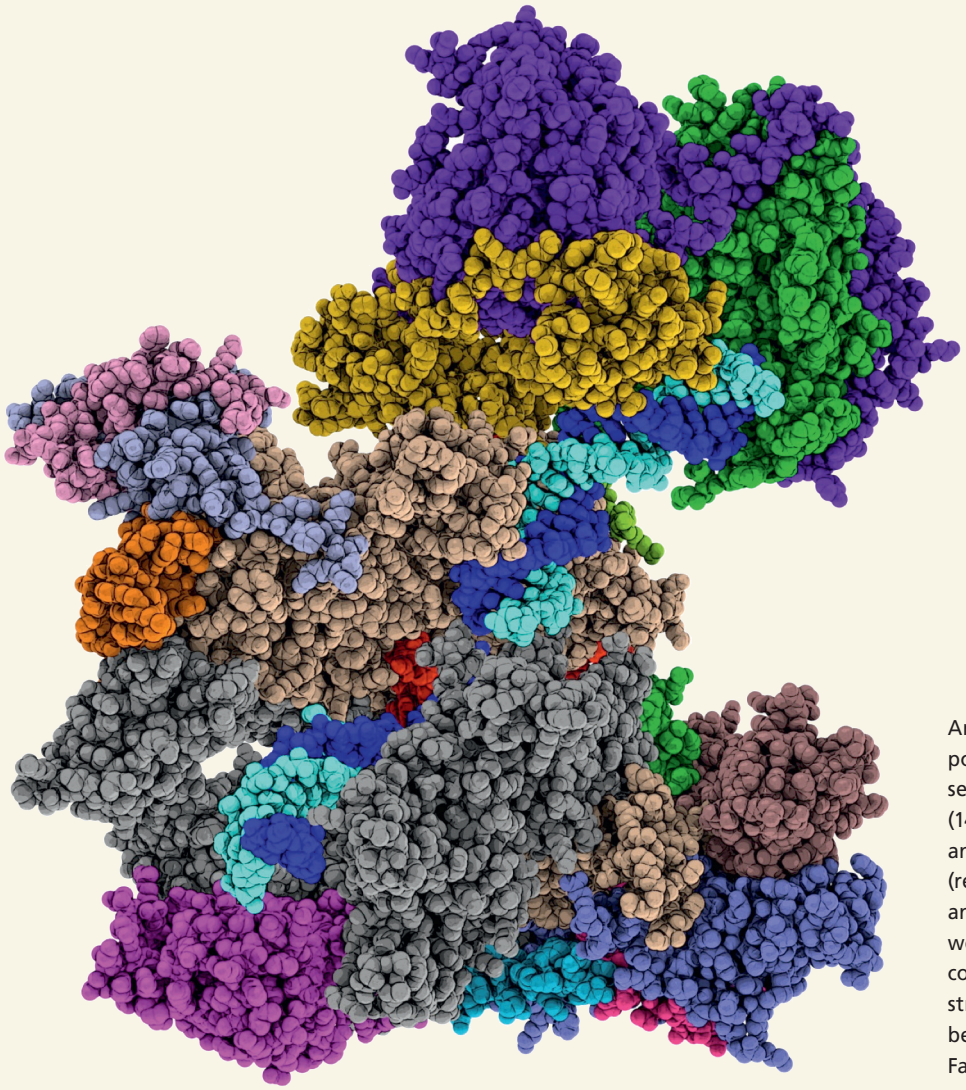
We have recently visualized how transcription of ribosomal RNA initiates and have suggested why selected DNA regions can serve as promoters for RNA polymerase I (*Cell,* March 23, 2017)

In growing eukaryotic cells, a large amount of ribosomal RNA (rRNA) is synthesized by RNA polymerase (Pol) I. The 14-subunit Pol I enzyme produces a long RNA from its DNA template, thereby supplying the basic building blocks for ribosome biogenesis. Due to the importance of this process for protein synthesis and cell growth, Pol I transcription is tightly regulated, and this is mainly achieved at the level of initiation. To start transcription, Pol I first binds the factor Rrn3 and is then recruited to its DNA promoter with the help of the core factor (CF). Although the involved proteins are generally conserved throughout evolution, details of their mechanism of action remain poorly understood and the ribosomal DNA (rDNA) promoter sequences themselves show no apparent similarity between organisms.

To elucidate the initiation of rRNA transcription, we set out to determine what defines the Pol I promoter, how it is recognized, and what the molecular mechanisms of Pol I initiation are. We began studying the interplay of Pol I with its transcription factors Rrn3 and CF, and with promoter DNA using an integrated structural biology approach. Over the course of several years, our team solved transcription factor structures using X-ray crystallography at atomic resolution and then analyzed complexes by cryo-electron microscopy (EM). In addition, biochemical assays allowed to test the hypotheses that were formulated on the basis of our structural insights. This culminated in a recent article in which we report the atomic structure of CF, analyze its interaction with Pol I and Rrn3, and describe the architecture of an initially transcribing complex that contains all the components required for transcription initiation [1].

Pioneering work on the messenger RNA (mRNA) producing Pol II enzyme has shaped our understanding of transcription mechanisms [2]. Compared to Pol II, however, a mechanistic understanding of Pol I transcription was lagging behind due to many technical challenges. The X-ray crystal structure of Pol I was elucidated in a *tour de force* and revealed that the enzyme can adopt an inactive expanded state [3]. Cryo-EM of transcribing Pol I later showed that the cleft contracts upon binding DNA and RNA to adopt a catalytically competent conformation [4], as predicted [3]. These studies showed that free Pol I was inactive and contained an expanded active center cleft, whereas transcribing Pol I showed a contracted cleft with bound DNA template and RNA transcript.

Despite these major advances, the mechanisms underlying initiation of rRNA transcription remained unknown. We could show that the initiation factor Rrn3 binds Pol I when it is dephosphorylated [5], which leads to partial contraction of the active center cleft, and stabilizes the monomeric form of the enzyme that is prone to initiation [6]. However, the key to understanding Pol I initiation was the essential CF, which bridges between promoter DNA and the



Architecture of an initially transcribing RNA polymerase (Pol) I complex. A surface representation of the molecular structure of Pol I (14 subunits) bound to a DNA scaffold (blue and cyan) with initially transcribed RNA (red). The initiation factors Rrn3 (brown) and Core Factor (purple, green and yellow) were positioned in a cryo-electron microscopy reconstruction of the complex. Upstream promoter DNA is characteristically bent between contacts with Pol I and Core Factor.

Pol I-Rrn3 complex. CF consists of the three protein subunits Rrn6, Rrn7, and Rrn11. Together with only Rrn3 and Pol I, CF is sufficient to promote initial transcription from a native DNA-template *in vitro*, defining a basal initiation system. In a novel bottom-up approach we co-expressed CF subunits in recombinant form and were able to crystallize the 220-kilodalton complex. Solving the CF structure using X-ray crystallography was hampered by intrinsic flexibility and limited diffraction, but eventually revealed a bi-modular architecture of the factor and undisclosed a unique, intimately interwoven network of three-dimensional folds. The structure showed that Rrn6 and Rrn11 adopt novel, characteristic folds. The Rrn7 polypeptide, although homologous to the general Pol II initiation factor TFIIB, showed unexpected differences in structure when compared to TFIIB.

We next asked how CF interacts with Pol I. *In vitro*, the CF-Pol I interaction requires the presence of Rrn3. Therefore, we formed a complex of all three factors, including a total of 18 polypeptide chains with a molecular weight of almost 900 kilodaltons. Electron microscopic data were collected with Jürgen Plitzko in the Department of *Molecular Structural Biology* at the MPI of Biochemistry, and allowed us to determine the architecture of the Pol I-Rrn3-CF complex. In the resulting reconstruction, CF adopted a position on top of the upstream end of the polymerase cleft. The structure revealed three interfaces between Pol I and CF and showed that the location of Rrn7 differs from that of TFIIB in the Pol II system. Furthermore, the observed CF position was not compatible with loading of promoter DNA into the cleft, suggesting that CF must reposition upon DNA binding.

We then added a DNA/RNA hybrid bubble-scaffold to the Pol I-CF-Rrn3 complex that mimics an initial transcription construct of the native *Saccharomyces cerevisiae* rDNA promoter. We again solved the cryo-EM structure of the complex, this time obtaining data at the MPI of Molecular Physiology in Dortmund with the help of Oliver Hofnagel. The reconstruction at an overall resolution of 3.4 ångström indeed showed a repositioning of CF. DNA was also seen in this complex extending through the active center cleft. The trajectory of upstream promoter DNA strongly differs from its Pol II counterpart due to a characteristic bend between its contacts with CF and Pol I. A novel, minimal, *in vitro* initiation assay confirmed the importance of all three CF-Pol I interfaces, Rrn3 dephosphorylation, and promoter-specific DNA.

These results provide the first molecular view of Pol I initiation. CF and Rrn3 are distinct from initiation factors for other polymerases. The Pol I initiation complex has a unique architecture that is characterized by an upstream promoter DNA bend. Since this bend is necessary to satisfy all protein-protein and protein-DNA contacts, we analyzed rDNA promoter sequences from several evolutionary distant

Simon Neyer, Christoph Engel, Tobias Gubbey, and Patrick Cramer (from left) at work. *(Photo: ibg)*

organisms. This analysis suggested that a DNA region situated between CF and Pol I has a high "bendability". In addition, the promoter region where DNA opening occurs is likely easy to melt such that RNA synthesis can commence. In conclusion, the conserved features of the rDNA promoter may lie in the biophysical properties of its DNA rather than in a specific sequence.

Our results were obtained in an integrated structural biology study. We first solved the high-resolution crystal structures of the 14-subunit Pol I, Rrn3, and the three-subunit CF. We then assembled the Pol I initiation complex on promoter DNA, and determined its architecture by cryo-EM. Placing the known high-resolution structures into the medium-resolution cyro-EM maps enabled us to reveal the architecture of the Pol I initiation complex. We are now working on a movie of Pol I transcription that visualizes how Pol I first engages with promoter DNA, then unwinds it to start RNA synthesis, and finally displaces initiation factors to form an elongation complex. Progressive contraction of the Pol I cleft will be a key feature of this movie, which can be used to teach students the first step in ribosome biogenesis.

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