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Snapshots of transcription initiation

Steven Hahn and

Fred Hutchinson Cancer Research Center, Seattle, Washington 98109, USA

Stephen Buratowski

Department of Biochemical Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, Massachusetts 02115, USA

Abstract

The enzyme RNA polymerase II, along with several transcription factors, initiates DNA transcription. Analyses reveal the structures involved in this process in human and yeast cells at high-resolution.

The initiation of DNA transcription involves a fascinating interplay between RNA-synthesizing RNA polymerase (Pol) enzymes, transcription factors and DNA. The Pol II complex is of particular interest because it synthesizes all messenger RNA in eukaryotic (nucleus-bearing) cells. The size and flexibility of Pol II complexes present huge challenges for structural biologists, but two papers online in *Nature*, by He et al. (1) and Plaschka et al. (2), exploit advances in cryo-electron microscopy to produce near-atomic-resolution snapshots of the Pol II machinery.

The bacterial Pol machinery is a streamlined system containing only four Pol subunits and a single transcription factor, sigma (3). Because Pol active sites are highly evolutionarily conserved (4), bacterial Pol has been used to establish a general model of Pol action. This model suggests that Pols and their transcription factors first associate with the promoter region of double-stranded DNA, which lies immediately upstream of the sequences to be transcribed, to form a structure called the closed complex.

Next, around 10–13 base pairs of the promoter unwind, positioning the DNA strand to be transcribed at the Pol active site in an open complex (open and closed refer to the state of the DNA). Pol subunits form channels for incoming nucleotides and the exiting mRNA, and create a deep cleft for the template strand. A mobile clamp domain traps DNA in the active site during the transition from the closed to the open complex. Finally, the structure contorts into an initial transcribing complex, maintaining contacts with promoter DNA while downstream DNA is pulled into the active site as RNA starts to be synthesized.

In comparison with bacteria, the archaeal and eukaryotic transcription machineries are complex, with 12 to 17 Pol subunits and up to 6 transcription factors. In the Pol II system, the transcription factors TBP, TFIIA, TFIIB, TFIIE, TFIIIF and TFIIH are all required and, between them, perform the same functions as bacterial sigma (5,6). Years of biochemical, molecular and structural studies have probed the roles of each transcription factor to piece together a model of eukaryotic transcription initiation (7).

Many features of this model are brought to life in the current work. Both groups assembled purified transcription factors and Pol II on nucleic-acid scaffolds — on double-stranded promoter DNA for the closed complex, double-stranded DNA containing an unwound ‘bubble’ for the open complex, and a bubble with a short annealed RNA to resemble the initial transcribing complex. He *et al.* used a complete set of human factors and all three scaffolds, whereas Plaschka *et al.* used all the yeast (*Saccharomyces cerevisiae*) factors except TFIIH to visualize the closed and open complexes. Despite these differences, the positions of transcription factors and the trajectory of the nucleic acids show excellent agreement between the studies. Moreover, the structures are consistent with a range of previous analyses (8–12).

TBP recognizes the TATA element — a common DNA sequence in eukaryotic Pol II promoters. A subcomplex of TATA DNA, along with TBP, TFIIA and the cyclin domains of TFIIB interact with the Pol II wall, positioning downstream promoter DNA over the cleft. A long, flexible region of TFIIB snakes through the Pol II exit channel into the active site, helping to position DNA correctly. In a major advance, the studies reveal how both structured and flexible regions of the transcription factors TFIIE and TFIF are positioned on either side of the cleft, interacting with each other, Pol II and a TBP–TFIIB–DNA subcomplex to promote and stabilize structural transitions during open-complex formation.

How does the complex transition into an initiation-competent state? Evidence indicates (13, 14) that one subunit of TFIIH is a translocase enzyme that drives DNA opening by threading downstream DNA into the Pol II cleft. He and colleagues’ structures for the closed and initial transcribing complexes support this model, showing that the translocase maintains contact with DNA in both states, and that an extra 12 bases are threaded into the cleft by the time that the latter has formed.

The two groups present slightly different models for the transition from the closed to the open complex. He *et al.* propose that, during the transition, closure of the clamp — driven by translocase-generated torsional strain — traps single-stranded DNA. Clamp-associated TFIIE also shifts, and a flexible linker region in TFIIB is fixed to stabilize single-stranded DNA (Fig. 1). By contrast, Plaschka *et al.* propose that, in yeast, structural changes mediated by TFIIE–TFIIB contacts clear the cleft of flexible TFIIB segments, allowing entry of the template strand, followed by closure of the clamp. In this model, TFIIE, and probably TFIIB, stabilize single-stranded DNA in the open complex.

Discrepancies between the models could reflect species differences in transcription factors or promoter sequences, differences in the size of the DNA bubble, or the absence of TFIIH in the yeast system. Both models involve small changes in flexible segments of TFIIB, TFIIE and TFIF, so, despite the remarkable resolution achieved, the structures might still have insufficient resolution to identify subtle differences. Moreover, these static structures capture only single states of a dynamic, multi-step mechanism. Perhaps the torsional strain that drives these transitions affects the structural state of the complexes — an area for future study.

The current research highlights similarities and differences in initiation mechanisms between all multi-subunit Pols. For instance, bacterial-DNA unwinding is initiated when a wedge structure in sigma flips out a base, leading to the opening of the DNA bubble (15, 16). TFIIB, which has a completely different amino-acid sequence from sigma, interacts with the same segment of promoter DNA. Both factors stabilize the open state and must be ejected from the active site before transcription progresses into elongation mode. Furthermore, eukaryotic Pols I, II and III all use TBP, a TFIIB-like factor and subunits related to TFIIE and TFIIIF (ref. 17), but Pol II is the only one to require a translocase. The reason for this is unknown, but probably involves greater stability of the Pol I and III open complexes, analogous to the stable bacterial open complex.

These fantastic new structures provide a framework for interpreting many past results, and are a harbinger of future investigations. For instance, a recent structure (18) of a TBP-containing complex called TFIID, which helps to activate transcription, revealed that some TFIID subunits interact with promoter DNA. This large structure can be docked into the current studies' closed-complex structures — suggesting unexpected interactions between the basal transcription factors and TFIID subunits that may be important in transcriptional regulation.

Eventually, it should be possible to directly visualize how multi-subunit coactivator complexes, such as TFIID, or another coactivator, Mediator, interact with the transcription machinery. Mediator is missing from the authors' structures, but is required for nearly all Pol II transcription (19). The carboxy-terminal domain of Pol II's largest subunit, a key regulatory target (20), is also missing. Rapidly advancing technologies for structural biology hold great promise for many more interesting insights into the regulation of gene expression.

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Figure 1. Preparation for transcription

He et al. (1) and Plashka et al. (2) solved structures of the enzyme RNA polymerase II (Pol II) in complex with transcription factors (including TFIIB, TFIIE and TFIIF) and DNA for humans and yeast. They solved closed and open complexes, which form in the lead up to transcription initiation. **a**, The human closed complex reveals the trajectory of double-stranded DNA across a cleft in Pol II. DNA is enclosed by TFIIE and a subunit of TFIIF. Arrows show the direction in which DNA is moved by a translocase enzyme. A flexible 'B-linker' region of TFIIB is disordered in this state. **b**, In the open complex, about 12 bases of DNA have been threaded into active site of Pol II, leading to formation of a single-stranded bubble from which the messenger RNA (blue strand) will be transcribed. Single-stranded DNA is stabilized by interactions with TFIIB. For simplicity, several transcription factors have been removed from this schematic.