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RNA polymerase II structure: from core to functional complexes

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New structural studies of RNA polymerase II (Pol II) complexes mark the beginning of a detailed mechanistic analysis of the eukaryotic mRNA transcription cycle. Crystallographic models of the complete Pol II, together with new biochemical and electron microscopic data, give insights into transcription initiation. The first X-ray analysis of a Pol II complex with a transcription factor, the elongation factor TFIIS, supports the idea that the polymerase has a 'tunable' active site that switches between mRNA synthesis and cleavage. The new studies also show that domains of transcription factors can enter polymerase openings, to modulate function during transcription.

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Abbreviations

CTD	carboxy-terminal repeat domain
NMR	nuclear magnetic resonance
NTP	nucleoside triphosphate
Pol II	RNA polymerase II
RNP	ribonucleoprotein
Rpb4/7	Rpb4–Rpb7 heterodimer
TBP	TATA-box-binding protein
TF	transcription factor

Introduction

The synthesis of eukaryotic mRNA is carried out by RNA polymerase II (Pol II). During the mRNA transcription cycle, Pol II associates transiently with many different factors, including the general transcription factors TFIIB, -D, -E, -F, and -H, which mediate promoter recognition and opening during initiation, coactivators, which transmit regulatory signals to Pol II, elongation factors, which enable efficient production of long RNAs, and multiprotein factors for RNA 3′-processing and transcription termination [1–3]. The elaborate regulation of the Pol II transcription cycle may, to a large extent, underlie organismal complexity and animal diversity [4].

Three years ago, three-dimensional structures of the 10-subunit core of budding yeast Pol II were reported [5–7], which gave insight into the basic aspects of transcription [8,9]. A mechanistic understanding of the transcription cycle, however, requires structural information on Pol II in complex with associated factors. The large size and transient nature of such complexes makes their preparation and analysis very difficult; but over the past year, crystallographic models could be obtained of the complete Pol II — a complex of the polymerase core with two additional subunits — and of a first Pol II complex with a transcription factor. Following a summary of previous structural studies of the Pol II core, I review here the new structures and recent biochemical and electron microscopic data on Pol II complexes.

Pol II core enzyme

Pol II from the yeast *Saccharomyces cerevisiae* has a molecular weight of 0.5 MDa and comprises 12 subunits that are highly conserved among eukaryotes. Yeast Pol II can dissociate in a 10-subunit catalytic core and a heterodimer of subunits Rpb4 and Rpb7 (Rpb4/7 complex) [10]. Structures of the Pol II core were determined in two forms and showed that the two large subunits, Rpb1 and Rpb2, form the central mass of the enzyme and a positively charged 'cleft' [5,6] (Table 1). One side of the cleft is formed by a mobile 'clamp' that adopts open states in both structures. The active center is formed between the clamp, a 'bridge helix' that spans the cleft, and a 'wall' that blocks the end of the cleft. A 'pore' beneath the active center widens towards the outside, creating an inverted 'funnel'. The rim of the pore includes a loop of Rpb1 that binds a Mg^{2+} ion ('metal A') with three aspartate residues. A second metal ion, 'metal B', can bind weakly further in the pore. In a structure of a minimal Pol II core elongation complex, DNA and RNA form a nine base pair hybrid duplex above metal A and the pore, and the clamp adopts a closed state [7]. From the outer side of the clamp base protrudes the beginning of a 100-residue 'linker' that connects to the C-terminal repeat domain (CTD) of Rpb1, which is disordered in all structures.

Complete Pol II

The Pol II core enzyme is catalytically active but requires the Rpb4/7 complex and the general transcription factors for initiation from promoter DNA [10]. The structure of an isolated archaeal Rpb4/7 counterpart revealed that the Rpb4 homolog binds between two Rpb7 domains, an N-terminal ribonucleoprotein (RNP)-like domain, and a C-terminal oligosaccharide-binding fold [11]. Cryo-electron microscopy of the 12-subunit yeast Pol II revealed that Rpb4/7 is located on the core surface below the closed clamp [12•]. This location coincided with that of the Rpb4/7 counterpart in Pol I, also revealed by electron microscopy [13•].

Table 1

X-ray crystallographic studies of RNA polymerase II.

Structure	Resolution [Å]	PDB code	References
Pol II core[a] form 1	3.1	1i3q	[5,6]
Pol II core[a] form 2	2.8	1i50	[6]
Pol II core[a]-DNA/RNA	3.3	1i6h	[7]
Pol II core[a]-α-amanitin	2.8	1k83	[66•]
Pol II	4.2	1nt9	[14••]
Pol II	4.1	1nik	[15••]
Pol II-TFIIS	3.8	1pqv	[44••]

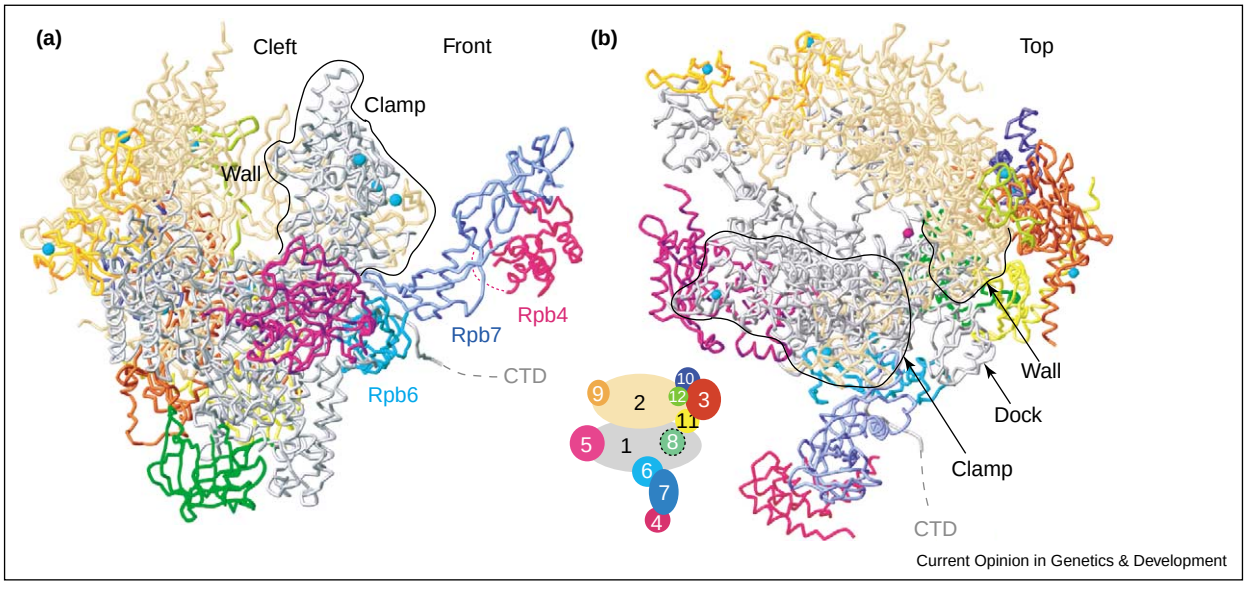
[a]Pol II core lacks the Rpb4/7 complex.

Crystallographic backbone models of the complete Pol II, including the Rpb4/7 complex, were now derived independently by two groups [14••,15••] (Figure 1). Crystallographic studies of the complete Pol II had previously been hampered by substoichiometric amounts of Rpb4/7. To overcome this obstacle, one group reconstituted the complete Pol II from endogenous yeast core and recombinant Rpb4/7 [14••], whereas the other purified the complete Pol II from a yeast strain carrying a tag on Rpb4 [15••]. The resulting crystal forms and backbone models are essentially identical. The models confirm that Rpb4/7 protrudes from the polymerase surface near the base of the clamp, and that the clamp is in a closed state, resembling that in the elongation complex [7]. Rpb4/7 binds to the Pol II core with the N-terminal domain of Rpb7, termed the 'tip'. Most of the Rpb4/7 surface is exposed and accessible for interactions with proteins or nucleic acids, and a potential nucleic-acid-binding surface on Rpb7 [11,16] faces the presumed RNA exit site. The Rpb4/7 complex can apparently recruit factors to exiting RNA or to the CTD. For example, the RNA-binding factor Nrd1 and the CTD phosphatase Fcp1 interact directly with the Rpb4/7 complex [17,18].

Initiation: pieces of a puzzle

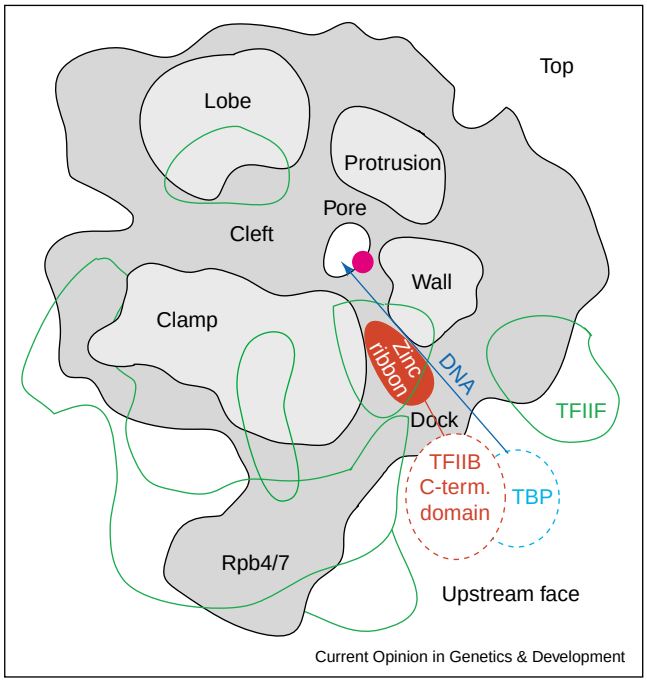
In the complete Pol II models, the clamp is in a closed position and its movement is severely restricted by the Rpb4/7 complex that forms a wedge below the clamp. This suggests that the clamp is closed during initiation and that the promoter duplex is initially bound outside the cleft. After DNA melting, the template single strand could slip inside the cleft, and would bind in the active center to initiate RNA synthesis. Wide opening of the clamp for passage of a DNA duplex [6] would require dissociation of Rpb4/7, and now appears unlikely. In addition to the complete Pol II, initiation requires TFIIB, -D, -E, -F, and -H. The additional factor TFIIA can contribute stabilizing interactions [19], as observed in refined structures of two TFIIA–TBP–DNA complexes [20]. The TFIID subunit TATA-box-binding protein (TBP) and the C-terminal domain of TFIIB assemble around the TATA box, a frequent promoter element located 25–30 base pairs upstream of the transcription start site. The TFIIB N-terminal zinc-ribbon domain binds Pol II and is essential for its recruitment [21–23], but also has post-recruitment functions.

Figure 1



Ribbon model of the complete Pol II [14••] (an essentially identical model has been reported in reference [15••]). The views are from **(a)** the front and from **(b)** the top, respectively [5,6]. Cyan spheres and a pink sphere depict eight zinc ions and an active site magnesium ion, respectively. The linker to the CTD is indicated as a dashed line. A key to subunit color is shown in the bottom left corner of (b), with subunits Rpb1–Rpb12 numbered 1–12.

Figure 2



Model of a minimal Pol II initiation complex. The Pol II structure in Figure 1b is shown schematically in gray with the locations of general transcription factors TBP, TFIIB, and TFIIF indicated in cyan, red, and green, respectively. The zinc ribbon domain of TFIIB is placed according to biochemical studies [24•]. The location of TFIIF domains is according to the electron microscopic study of the Pol II–TFIIF complex [30••]. The TFIIB C-terminal domain and TBP were placed assuming a topological analogy to the bacterial polymerase-σ-DNA complex [35••] and with the use of the TBP–DNA–TFIIB C-terminal domain complex structure [65]. A blue arrow indicates the presumed direction of upstream promoter DNA towards the active site (magenta sphere) but well above it.

With the use of photocrosslinking, radical probing, and mutational analysis, Hahn and coworkers showed that the TFIIB zinc ribbon binds to the Pol II 'dock' domain [24•], suggested previously to bind initiation factors [6] (Figure 2). The zinc ribbon is not in close contact with promoter DNA [24•], arguing for DNA loading above the cleft. After DNA melting, however, TFIIB seems to contact both DNA strands within the transcription bubble, as suggested by protein–DNA crosslinking studies of a related archaeal polymerase complex [25•]. Crystallography confirms that the zinc ribbon binds the dock domain and further shows that a protrusion from TFIIB reaches into the hybrid-binding site (D Bushnell, R Kornberg, personal communication). The zinc ribbon could thus stabilize an early transcribing complex until a complete nine-base pair DNA–RNA hybrid is formed. Indeed, TFIIB stabilizes a polymerase complex comprising a short RNA pentamer (D Bushnell, R Kornberg, personal communication). Exiting RNA beyond the hybrid could release TFIIB and facilitate promoter clearance because the TFIIB-binding site overlaps the proposed RNA exit site [24•]. Resembling the TFIIB protrusion, the bacterial transcription initiation factor σ penetrates the polymerase hybrid binding site with its region 3.2 [26••,27••], suggesting that parts of σ and TFIIB perform analogous functions.

TBP and a TFIIB homologue suffice for promoter loading onto archaeal polymerase. Promoter loading onto Pol II additionally requires TFIIF [28,29], which forms a tight complex with Pol II. A recent electron microscopic study of a Pol II–TFIIF complex showed that the second largest TFIIF subunit extends above and along the Pol II cleft, resembling the interaction of its sequence homologue σ with bacterial polymerase [30••]. Parts of TFIIF attributed to the largest subunit bind to the Rpb4/7 complex [30••]. The counterpart of the Rpb4/7 complex in Pol I also interacts with an initiation factor [31,32], and in Pol III, the Rpb4 homolog binds the TFIIB-related factor Brf [33], which in turn binds TBP with its C-terminal region [34•]. Thus Rpb4/7 and its counterparts bridge between the core polymerases and initiation factors, and should contribute to promoter specificity.

A comparison of the Pol II complexes with the structure of bacterial RNA polymerase bound to σ and promoter DNA [35••] suggests a general topological similarity of promoter loading onto Pol II and onto the bacterial polymerase. Parts of TFIIF and TFIIB bind to polymerase in regions that are also bound by the σ factor and apparently fulfil analogous functions. TBP, TFIIB, and parts of TFIIF apparently assemble at the Pol II 'upstream face', where promoter DNA is located in the bacterial complex. Some regulatory mechanisms may also be similar in Pol II and the bacterial polymerase. Electron microscopy shows that the coactivator complex Mediator binds near the Rpb3–Rpb11 subunit heterodimer of Pol II, and a corresponding surface on bacterial polymerase is important for transcription regulation [36•].

Following assembly of the Pol II–TFIIF–TFIIB–TBP–DNA complex, promoter DNA is melted with the help of TFIIE and TFIIH. The underlying mechanisms are poorly understood but may involve large structural changes, given that the single-subunit T7 RNA polymerase undergoes dramatic refolding during the transition from initiation to elongation [37••]. A recent structural study showed that the conserved and functionally essential N-terminal region of the large TFIIE subunit forms an unusual winged helix-turn-helix (winged helix) domain with distinct protein interaction surfaces that could recruit TFIIE to the polymerase complex [38•]. Although the winged helix fold is often found in DNA-binding proteins, this domain shows an atypical DNA-binding face and lacks DNA-binding activity [38•]. Another winged helix domain in the small TFIIE subunit, however, binds DNA [39]. TFIIF contains two

winged helix domains: one that binds DNA [40], and one that binds the helical C terminus of the phosphatase Fcp1 [41•,42•].

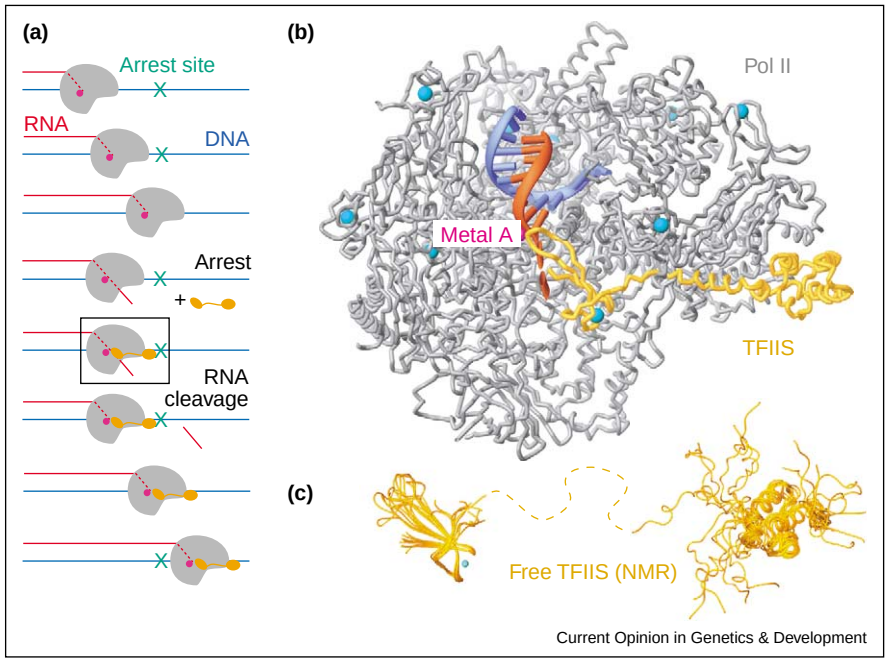
Pol II–TFIIS complex and mRNA cleavage

During mRNA elongation, Pol II can encounter DNA sequences that cause reverse movement of the enzyme. Such 'backtracking' apparently involves extrusion of the RNA 3′-end into the pore, and can lead to transcriptional arrest (Figure 3a). Escape from arrest requires cleavage of the extruded RNA with the help of the elongation factor TFIIS (or SII), the first Pol II transcription factor that was isolated. TFIIS strongly enhances a weak intrinsic nuclease activity of Pol II [43]. TFIIS consists of an N-terminal domain I, which is weakly conserved and not required for activity, a central domain II, and a C-terminal domain III. Domain II and the linker between domains II and III are required for Pol II binding. Domain III is a zinc ribbon domain essential for RNA cleavage.

Recently a backbone model for the Pol II–TFIIS complex was obtained from X-ray analysis of a Pol II crystal soaked with a recombinant TFIIS variant lacking domain I [44••] (Figure 3b). Assembly of this 13-polypeptide complex was possible within the crystals because large solvent channels leave the TFIIS-binding site accessible, and because the crystal packing accommodates TFIIS-induced structural changes. In the structure, TFIIS extends from the outermost jaws to the internal active site. TFIIS binds with domain II to one of the jaws, extends into the funnel, and inserts domain III into the pore, to reach the polymerase active site from below. The pore is wide enough to accommodate both TFIIS domain III and extruded RNA during rescue of arrested complexes. It is currently unclear whether TFIIS is only recruited to arrested complexes and, if so, what the trigger for such recruitment would be.

From TFIIS domain III protrudes a β-hairpin that complements the Pol II active site with two invariant and functionally essential acidic residues. This complementation supports the idea that Pol II contains a single active site for both RNA polymerization and cleavage [44••,45–47]. A model of a Pol II–TFIIS–DNA–RNA complex shows that the TFIIS hairpin approaches the RNA backbone near metal A, and that it could bind a metal ion B adjacently [44••]. During hydrolytic RNA cleavage, metal B could activate a water molecule for an S_N2-type nucleophilic attack of a phosphorous atom, in-line with the scissile RNA phosphodiester bond [44••], analogous to

Figure 3



Pol II–TFIIS complex and polymerase rescue by mRNA cleavage. **(a)** Model for rescue of arrested Pol II by TFIIS-induced mRNA cleavage. When transcribing Pol II (silver) approaches an arrest site (turquoise cross) on DNA (blue), it may stop, reverse direction (backtracking), and extrude RNA (red), leading to transcriptional arrest. TFIIS can rescue arrested Pol II by inducing cleavage of the extruded RNA fragment. Transcription is then resumed and continued past the arrest site. It is unknown when TFIIS enters and leaves the Pol II complex. **(b)** Cut-away view of the crystallographic model of the Pol II–TFIIS complex [44••]. Pol II is in silver, TFIIS in orange. The view is related to the one in Figure 1a by a 90° rotation around the vertical axis. DNA (blue) and RNA (red) have been modeled according to the Pol II core elongation complex [7]. The active site metal ion A is shown as a pink sphere. The model corresponds to the state indicated by a black box in (a). **(c)** Ensembles of NMR solution structures of free TFIIS domains III (left) and II (right) [62,64], demonstrating the flexibility of the unbound factor.

DNA cleavage by the Klenow DNA polymerase exonuclease domain [48,49]. Although this mechanism remains speculative, it is consistent with the requirement for divalent metal ions [50,51], and with evidence for a nucleophilic water molecule [50]. Indeed, biochemical analysis of the exonuclease activity of bacterial RNA polymerase led to the same proposal for the cleavage mechanism [52•].

Other nuclear RNA polymerases apparently use the same mechanism. RNA cleavage by Pol III requires its C11 subunit, which comprises a TFIIS-like zinc ribbon with two acidic residues that are essential for yeast viability [53]. The archaeal transcript cleavage factor TFS is a C11 homologue [54]. Several lines of evidence suggested that the bacterial transcript cleavage factors GreA and GreB also function essentially like TFIIS despite differences in structure [55–57]. This idea is strongly supported by an electron microscopic reconstruction of a bacterial polymerase–GreB complex [58••] (Figure 4). GreB binds with its coiled-coil protrusion in the secondary channel of the polymerase, which corresponds to the Pol II pore, and approaches the active site with two acidic residues that are critical for function [58••]. These findings demonstrate the conserved strategies employed for RNA cleavage stimulation by the structurally unrelated bacterial and eukaryotic transcript cleavage factors.

Tunable active site and proofreading

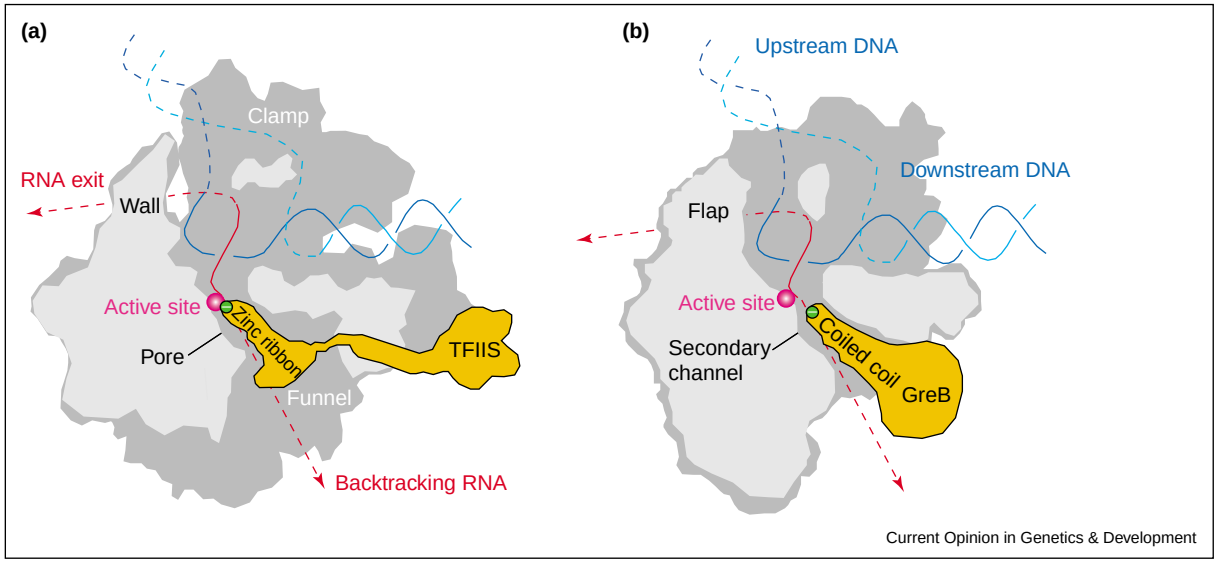
Biochemical and structural work led to the appealing model of a unified two-metal-ion mechanism for both RNA polymerization and cleavage at a single tunable active site in all nuclear RNA polymerases [44••,52•,58••]. In this model, RNA polymerization and cleavage both require metal A, but differential coordination of a metal B switches Pol II activity from polymerization to cleavage (Figure 5). For RNA polymerization, metal B would bind the phosphates of the substrate NTP. For stimulated RNA cleavage, the TFIIS acidic hairpin would position metal B and a nucleophilic water molecule. For cleavage in the absence of TFIIS, metal B could be bound by an additional unpaired nucleotide located in the pore [52•]. It is important to remember that the suggested mechanisms await verification by additional studies such as high-resolution structures.

The tunable polymerase active site could allow for efficient mRNA proofreading because RNA cleavage creates a new RNA 3′-end at metal A, from which polymerization can continue. Two types of proofreading reactions may occur, removal of a misincorporated nucleotide directly after its addition, or cleavage of a dinucleotide after misincorporation and backtracking by one nucleotide. There seems to be a substantial difference in proofreading strategies by DNA and RNA polymerases. In the Klenow DNA polymerase, the growing DNA shuttles between widely separated active sites for DNA synthesis and cleavage [59]. In Pol II, however, the growing RNA remains at a single active site that switches between RNA synthesis and cleavage.

Conformational regulation

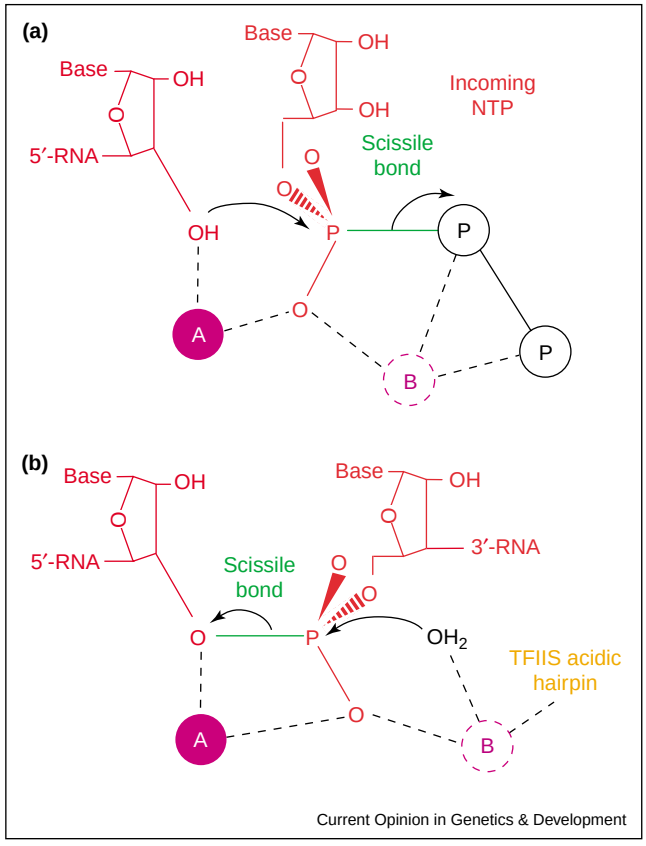
Comparison of the available Pol II structures begins to unravel the manifold conformational regulation of the

Figure 4



Conserved topology of RNA polymerase complexes with transcript cleavage factors. **(a)** Schematic cut-away drawing of the eukaryotic Pol II–TFIIS complex [44••]. **(b)** A corresponding drawing of the bacterial RNA polymerase–GreB complex, based on electron microscopy [58••]. The view is as in Figure 3b. The direction of RNA during synthesis (RNA exit) and during backtracking is indicated with dashed red arrows.

Figure 5



Model of the tunable RNA polymerase active site. **(a)** Presumed mechanism of nucleotide incorporation during RNA polymerization. **(b)** Presumed mechanism of TFIIS-stimulated hydrolytic RNA cleavage. A key feature of the model is that a metal ion B interacts either with the phosphates of the incoming NTP (RNA polymerization) or with the TFIIS acidic hairpin and a nucleophilic water molecule (RNA cleavage). Model according to references [6,44••,52•,58••].

enzyme. The core Pol II structures showed three different states of the mobile clamp, with the closed state adopted by the elongating enzyme. The complete Pol II model also showed a closed clamp, restricted by the wedge-like Rpb4/7 complex. Thus the clamp adopts several conformational states, which are influenced by crystal packing in case of the free Pol II core structures. In the Pol II core elongation complex, the clamp is, however, not involved in crystal contacts and its closed state may thus be a consequence of binding the DNA–RNA hybrid. By contrast, packing interactions remain essentially unaltered when TFIIS is soaked into preformed Pol II crystals, and observed conformational changes in the Pol II–TFIIS complex may thus be attributed to TFIIS binding. TFIIS induces structural changes in the active center, which may reposition nucleic acid substrates, and triggers a movement of about one-third of the polymerase mass. Several biochemical studies suggested different functional states of the polymerase with distinct conformations [60,61], but it is unclear how these are related to the crystallographically defined Pol II conformations.

Comparison of the TFIIS structure in its Pol II-bound state with the NMR structure of free TFIIS in solution [62–64] shows that the factor undergoes a dramatic folding transition upon polymerase binding (Figure 3c). In the free TFIIS fragment, 25% of the residues are disordered, but nearly all residues adopt a defined structure upon interaction with the Pol II target surface. Three short helices are added to domain II, the interdomain linker forms a helix, and the intrinsically flexible acidic hairpin becomes fixed in the context of Pol II. It can be expected that other modular and flexible transcription factors, including TFIIB, -E, and -F, also undergo structural transitions and induced folding upon their assembly within Pol II complexes.

Conclusions

Recent structural studies of Pol II complexes mark the beginning of a detailed structure–function analysis of the transcription cycle. The crystallographic Pol II structure was extended from the 10-subunit core to the complete 12-subunit enzyme. A first complex of Pol II with a transcription factor, TFIIS, was also resolved by X-ray analysis. These structural data and recent biochemical and electron-microscopic data on Pol II–TFIIB and Pol II–TFIIF complexes explain functions of Pol II-binding factors during initiation and mRNA cleavage. Small protein domains, such as zinc ribbon domains (TFIIS, TFIIB) and winged helix domains (TFIIE, TFIIF), and presumably flexible regions linking such domains, can apparently enter the enzyme's openings and modulate function by influencing polymerase–nucleic acid interactions. In the future, additional Pol II complex structures will continue to provide unexpected insights into transcription and to explain transcription factor function. Structural work will be complemented with a more and more elaborate biochemical and genetic dissection of the mechanisms underlying eukaryotic mRNA synthesis and its regulation.

Update

Shortly before this paper went to press, two biochemical studies appeared that provide support for the discussed general mode of action of transcription cleavage factors, and for a single tunable active site in RNA polymerases. One study [67] mapped the interaction interface of the bacterial transcript cleavage factor GreB with bacterial RNA polymerase and identified the two functionally essential acidic residues. The other study [68] used RNA–protein crosslinking, site-directed mutagenesis and biochemical assays to also show that the Gre factor functions by donating catalytic residues to the bacterial RNA polymerase active center, and possibly by coordinating a second metal ion. Both studies result in a model for the bacterial RNA polymerase–Gre complex similar to

the one described in [58••] and are in agreement with the structural study of the corresponding Pol II–TFIIS complex and the conclusions drawn from it [44••].

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- •• of outstanding interest

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The authors have used photocrosslinking, radical probing and site-directed mutagenesis to map the binding site of the N-terminal zinc ribbon domain of the general transcription factor TFIIB on RNA Pol II. The TFIIB domain is shown to bind to the Pol II dock domain. The authors note that this surface is best conserved in polymerases that use a TFIIB-like factor and that it overlaps the proposed RNA exit site of the polymerase. Consequences for understanding the function of the TFIIB zinc ribbon domain are discussed.

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The authors have carried out site-specific protein–DNA crosslinking studies to characterize factor–DNA interactions within an archaeal transcription initiation complex containing RNA polymerase, TBP, and the TFIIB homolog TFB. The detected crosslinking patterns generally agree with those observed previously for analogous eukaryotic complexes. However, additional contacts of TFB with DNA in the region of the transcription bubble near the start site are observed. This finding lets the authors suggest that the TFB N-terminal domain performs functions analogous to that of the bacterial σ factor region 3.2 that penetrates the hybrid binding site.

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The authors report a crystallographic model of the *Thermus aquaticus* RNA polymerase holoenzyme, consisting of the core polymerase and the σ factor. The model shows that two C-terminal domains of σ are separated on the surface of the polymerase and connected by a linker that winds near the polymerase active site, where it may help initiating RNA synthesis. The linker binds in the RNA exit channel. Advancing RNA is proposed to displace the linker, leading to release of σ and contributing to the transition to elongation.

27. Vassylyev DG, Sekine S, Laptenko O, Lee J, Vassylyeva MN,
•• Borukhov S, Yokoyama S: **Crystal structure of a bacterial RNA polymerase holoenzyme at 2.6 A resolution**. *Nature* 2002, **417**:712-719.

The authors report an atomic crystallographic model of a *Thermus thermophilus* RNA polymerase holoenzyme. The C-terminal domain of the σ factor is seen near the RNA exit channel and a linker between this domain and the N-terminal region passes through the RNA exit channel and protrudes into the active center of the enzyme. The structure gives important and detailed insights into core polymerase–σ interactions and the mechanism of transcription initiation. Two adjacent metal ions are observed at the active site, as well as a 'trigger loop' that binds the polymerase bridge helix.

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•• Bareket-Samish A, Kornberg RD, Asturias FJ: **RNA polymerase II/TFIIF structure and conserved organization of the initiation complex**. *Mol Cell* 2003, **12**:1003-1013.

The authors report a cryo-electron microscopic analysis of the RNA Pol II–TFIIF complex. The work shows that TFIIF density is distributed over the surface of Pol II. The largest TFIIF subunit seems to interact with the Rpb4/7 complex, whereas densities assigned to the second largest subunit are seen distributed above the polymerase cleft. The authors suggest that their finding reflects a general similarity of the mechanism of transcription initiation in eukaryotes and bacteria, in particular because the second largest TFIIF subunit shows clear sequence homology to the bacterial σ factor and appears to bind to corresponding locations on the polymerase surface.

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33. Ferri ML, Peyroche G, Siaut M, Lefebvre O, Carles C, Conesa C, Sentenac A: **A novel subunit of yeast RNA polymerase III interacts with the TFIIB- related domain of TFIIIB70**. *Mol Cell Biol* 2000, **20**:488-495.

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• **Crystal structure of a transcription factor IIIB core interface ternary complex**. *Nature* 2003, **422**:534-539.

The RNA Pol III-specific transcription factor IIIB consists of a TFIIB-like N-terminal region and a specific C-terminal region. The authors present the crystal structure of a complex of the TFIIIB C-terminal region with TBP and DNA. The TFIIB region is seen extending over the surface of TBP with several helices. The observed interactions are important for RNA Pol III-specific promoter recognition and initiation.

35. Murakami KS, Masuda S, Campbell EA, Muzzin O, Darst SA:
•• **Structural basis of transcription initiation: an RNA polymerase holoenzyme-DNA complex**. *Science* 2002, **296**:1285-1290.

The authors report a model of the bacterial RNA polymerase holoenzyme in complex with upstream promoter DNA. The DNA is seen lying across one face of the complex, way above the active site cleft of the enzyme. All sequence-specific DNA contacts are made by the σ factor. In addition to an understanding of promoter loading onto the bacterial polymerase, the model provides insight into possible mechanisms of promoter melting, and suggests how variably spaced DNA elements can be recognized.

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• **yeast RNA polymerase II holoenzyme: mediator conformation and polymerase interaction**. *Mol Cell* 2002, **10**:409-415.

The authors have generated a three-dimensional envelope of the yeast RNA Pol II–Mediator complex from electron microscopic images of single complex particles. The X-ray crystallographic structure of core RNA Pol II could be placed into the envelope, revealing that Mediator binds to polymerase near subunits Rpb3 and Rpb11, to a surface that is used as a regulatory target in bacterial RNA polymerase.

37. Yin YW, Steitz TA: **Structural basis for the transition from**
•• **initiation to elongation transcription in T7 RNA polymerase**. *Science* 2002, **298**:1387-1395.

The authors report the high-resolution structure of a phage T7 single subunit RNA polymerase elongation complex with a synthetic transcription bubble. Comparison with previous structure of an initiating complex reveals major structural changes in the polymerase that accompany the transition from initiation to elongation. The changes include a loss of the promoter binding site, partial creation of a binding site for a seven-base pair DNA–RNA hybrid, and the formation of an RNA exit tunnel.

38. Meinhart A, Blobel J, Cramer P: **An extended winged helix**
• **domain in general transcription factor E/IIEα**. *J Biol Chem* 2003, **278**:48267-48274.

The functionally essential N-terminal region of the largest subunit of the general transcription factor TFIIE could to date not be characterized structurally. We present the crystal structure of the related region of the archaeal homologue of the TFIIE largest subunit, called TFE. The structure reveals a winged helix-turn-helix domain that is frequently found in nucleic-acid-binding proteins. Several features suggest, however, that the domain is involved in specific interactions with RNA polymerase and/or general transcription factors. In particular, the domain shows a specific additional N-terminal helix, a negatively charged groove, an elongated wing structure, conserved hydrophobic surface patches, and it lacks DNA-binding activity *in vitro*.

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• **of recruitment of TFIIF- associating RNA polymerase C-terminal domain phosphatase (FCP1) by transcription factor IIF**. *Proc Natl Acad Sci USA* 2003, **100**:2296-2299.

The authors present a cocrystal structure of the TFIIF large subunit winged helix-turn-helix domain bound to a C-terminal α-helix of the CTD phosphatase Fcp1. The structure reveals details of the interaction between Fcp1 and TFIIF that are used to recruit the phosphatase for polymerase recycling and CTD dephosphorylation.

42. Nguyen BD, Abbott KL, Potempa K, Kobor MS, Archambault J,
• Greenblatt J, Legault P, Omichinski JG: **NMR structure of a complex containing the TFIIF subunit RAP74 and the RNA polymerase II carboxyl-terminal domain phosphatase FCP1**. *Proc Natl Acad Sci USA* 2003, **100**:5688-5693.

The authors present the solution NMR structure of the TFIIF large subunit winged helix-turn-helix domain bound to a C-terminal α-helix of the CTD phosphatase Fcp1. The structure reveals details of the interaction between Fcp1 and TFIIF. The authors suggest that the mode of interaction defines a general role for both hydrophobic and acidic residues in protein–protein interactions involving acidic domains in transcription factors.

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•• **polymerase II-TFIIS complex and implications for mRNA cleavage**. *Cell* 2003, **114**:347-357.

We present the first X-ray analysis of a Pol II-transcription factor complex. A 13-polypeptide 536 kDa RNA Pol II–TFIIS complex could be assembled by soaking a recombinant TFIIS variant into preformed polymerase crystals. Crystallographic analysis resulted in a backbone model that reveals that TFIIS extends over 100 Å along the polymerase's surface, reaching from the outermost jaws to the internal active site. TFIIS complements the polymerase active site with two invariant functionally essential acidic residues. The authors suggest that TFIIS stimulates RNA

cleavage activity by coordinating a metal ion, which in turn activates a nucleophilic water molecule. The authors suggest a single tunable active site that can switch from RNA polymerization to cleavage without major movement of the nucleic acid substrates. A similar mode of action is proposed for transcript cleavage in other nuclear RNA polymerases.

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On the basis of biochemical analysis of the exonuclease activity of bacterial RNA polymerase and on structural modeling, the authors suggest a unified two-metal ion mechanism that can explain all know enzymatic activities of RNA polymerases. A key aspect of the mechanism is that the second metal ion is coordinated differently depending on the type of reaction. The authors also propose a new non-specific NTP-binding site in the polymerase pore.

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54. Hausner W, Lange U, Musfeldt M: **Transcription factor S, a cleavage induction factor of the archaeal RNA polymerase**. *J Biol Chem* 2000, **275**:12393-12399.

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The authors have obtained a three-dimensional model of the bacterial RNA polymerase–GreB complex by cryo-electron microscopy and image processing of helical crystals. In the model, the N-terminal coiled–coil extension of the GreB transcription cleavage factor extends through the secondary channel of the polymerase, corresponding to the Pol II pore, until the active site of the enzyme. Mutational studies show that two key acidic residues at the tip of the coiled-coil are essential for function, highlighting the conserved mechanism of action of transcript cleavage factors.

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The authors have soaked the mushroom toxin α-amanitin, a small cyclic peptide, into preformed crystals of the polymerase core, and have used difference Fourier analysis to locate the amanitin-binding site. The toxin binds in a pocket in the polymerase funnel, where it cannot interfere directly with binding of nucleic acids to the active center cleft. Amanitin, instead, appears to block conformational changes that are important for polymerase function.

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