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The Structures of Eukaryotic Transcription Pre-initiation Complexes and Their Functional Implications

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Abstract

Transcription is a highly regulated process that supplies living cells with coding and non-coding RNA molecules. Failure to properly regulate transcription is associated with human pathologies, including cancers. RNA polymerase II is the enzyme complex that synthesizes messenger RNAs that are then translated into proteins. In spite of its complexity, RNA polymerase requires a plethora of general transcription factors to be recruited to the transcription start site as part of a large transcription pre-initiation complex, and to help it gain access to the transcribed strand of the DNA. This chapter reviews the structure and function of these eukaryotic transcription pre-initiation complexes, with a particular emphasis on two of its constituents, the multisubunit complexes TFIID and TFIIF. We also compare the overall architecture of the RNA polymerase II pre-initiation complex with those of RNA polymerases I and III, involved in transcription of ribosomal RNA and non-coding RNAs such as tRNAs and snRNAs, and discuss the general, conserved features that are applicable to all eukaryotic RNA polymerase systems.

Keywords

Transcription; Initiation; General transcription factors; TFIIF; TFIID; RNA polymerase; Gene expression; Structural biology; Cryo-electron microscopy

Introduction to Transcription in Eukaryotes

Transcription is the synthesis of RNA based on a DNA template. While phage, viral, and organellar gene expression systems generally use simple single-subunit RNA polymerase enzymes, bacterial, archaeal, and eukaryotic RNA polymerases are increasingly complex multisubunit enzymes. A 5-subunit core is conserved from bacteria to humans, though archaeal and eukaryotic RNA polymerases harbor up to 12 additional subunits (Fig. 5.1a–e). Eukaryotes use three structurally and functionally distinct RNA polymerases (Roeder and Rutter 1969, 1970; Sklar et al. 1975), abbreviated Pol I, Pol II, and Pol III, each of them specialized for the synthesis of certain classes of RNAs. Pol I synthesizes long ribosomal RNA (rRNA) precursors in the nucleolus that are later processed into 25S/28S, 18S, and 5.8S rRNAs, Pol II synthesizes mostly messenger RNAs (mRNAs) that are later translated into proteins, and Pol III synthesizes tRNAs, 5S rRNA, and other small non-coding RNAs (Roeder and Rutter 1970; Weinmann and Roeder 1974).

The DNA core promoters that control expression of their corresponding genes also differ substantially between the three eukaryotic polymerase systems. In the Pol II system, several core promoter elements have been identified (Burke and Kadonaga 1996, 1997; Chen and Struhl 1985; Deng and Roberts 2005; Gannon et al. 1979; Juven-Gershon and Kadonaga 2010; Lagrange et al. 1998; Lenhard et al. 2012; Lim et al. 2004; Roy and Singer 2015), and a synthetic promoter combining a TATA-box, initiator (Inr), downstream promoter element (DPE), and motif ten element (MTE), which provides a highly specific and high-affinity binding site for the transcription machinery (Juven-Gershon et al. 2006) has been used in structural analysis of promoter-bound human Pol II pre-initiation complexes. However, not all of these elements are typically present in any natural promoter. For example, most Pol II promoters lack a TATA box (Carninci et al. 2006; Sandelin et al. 2007), the canonical binding site of TATA-box binding protein (TBP), which is a subunit of the general transcription factor IID (TFIID) and also ubiquitously used in the Pol I and Pol III systems (see below). Nevertheless, TBP still participates in assembly of the transcription machinery on Pol II promoters lacking TATA boxes because the recognition of the other core promoter motifs by TFIID allows TBP loading on the DNA (Burke and Kadonaga 1997; Kutach and Kadonaga 2000; Lim et al. 2004; Pugh and Tjian 1991). Panels f–h of Fig. 5.1 provide examples of the promoter elements typically found in eukaryotic genes.

Transcription is commonly subdivided into three phases (Fig. 5.1i). After the step-wise assembly of a pre-initiation complex (PIC) on the DNA (Buratowski et al. 1989), whereby general transcription factors and RNA polymerase are recruited to the promoter, the transcription bubble opens, allowing the synthesis of the first phosphodiester bond in the active site of the RNA polymerase. This step is referred to as *initiation* of transcription. Subsequently, RNA polymerase needs to clear the promoter and, in the case of Pol II, overcome a promoter-proximal pause, allowing it to enter the *elongation* phase. After synthesis of the product RNA, transcription needs to be terminated during the *termination* phase of the transcription reaction.

The initiation of transcription is a highly regulated process. Together with the subsequent regulation of RNA processing, RNA stability, and regulated translation of mRNAs into

proteins, transcription plays a major role in the control of gene expression as a whole. Deregulation of transcription is implicated in human pathologies, and elevated transcription levels by each of the three eukaryotic RNA polymerases have been associated with cancer (Cabarcas and Schramm 2011; Johnson et al. 2008; Lockwood et al. 2010).

The Pol II Pre-initiation Complex

Pol II and the General Transcription Factors

The enzymatic core of Pol II shows extensive structural homology to the 5-subunit $\alpha_2\beta\beta'\omega$ core of bacterial RNA polymerases (Cramer et al. 2000; Zhang et al. 1999) (Fig. 5.1a, d) and the fundamental mechanism of phosphodiester bond synthesis is highly conserved (Cramer et al. 2001; Gnatt et al. 2001; Kaplan 2013; Svetlov and Nudler 2013; Wang et al. 2006; Westover et al. 2004). However, Pol II includes 7 additional subunits to form a 12-subunit complex (Figs. 5.1a, d and 5.2a, b). Structural landmarks of Pol II (Fig. 5.2c, d) include the stalk, two jaws that interact with the incoming DNA substrate, the flexible clamp domain that closes over the bound nucleic acids in initiation and elongating complexes, and two pores that allow substrates to enter and product mRNAs to exit the catalytically active complex (Fig. 5.2c, d) (Cramer et al. 2000, 2001; Gnatt et al. 2001). Behind the unwound transcription bubble in the active site, a protein wall forces the DNA to adopt a bend before exiting the polymerase (Fig. 5.2c). In the transcribing complex (Fig. 5.2e, f), two fork loops from the Rpb2 subunit maintain the open transcription bubble (Cramer et al. 2001; Gnatt et al. 2001). Motions of the bridge helix that contacts the DNA-RNA hybrid in the active site are coupled to substrate translocation, while opening and closing of the trigger loop is associated with catalysis, which is mediated by two metal ions bound in the active site (Fig. 5.2f) (Gnatt et al. 2001; Wang et al. 2006; Westover et al. 2004).

The initiation of mRNA synthesis requires Pol II and the general transcription factors TFIIA, TFIIB, TFIID, TFIIE, TFIIIF, and TFIIH (Sainsbury et al. 2015) to form a pre-initiation complex (PIC) on the promoter. Activated transcription additionally depends on Mediator, a 1.2 MDa multiprotein complex, and the activity of a number of cofactors and gene-specific activators and repressors. In contrast to the smaller, oligomeric general transcription factors TFIIA, TFIIB, TFIIE, and TFIIIF, two general transcription factors are large multiprotein assemblies: TFIIH is a heterodecameric protein complex with a molecular weight of 0.5 MDa, and TFIID is a 1.3 MDa multiprotein assembly of TATA-box binding protein (TBP) and 13 different TBP-associated factors (TAFs) in humans. We will discuss the structures and functional mechanisms of these two general transcription factors in more detail in the following sections, while the remaining general transcription factors will be discussed in the context of the Pol II-PIC.

TFIID

Function of TFIID—The assembly of the PIC begins with the recognition of the core promoter DNA by TFIID, with the help of TFIIA (Burke and Kadonaga 1997; Chalkley and Verrijzer 1999; Lee et al. 2005; Verrijzer et al. 1995). TBP then interacts with TFIIB, which in turn interacts with Pol II, thereby nucleating the assembly of the full PIC (Buratowski et al. 1989). In addition to TBP, direct interactions between TFIID TAFs and other components

of the general transcription machinery have also been proposed (Dubrovskaya et al. 1996; Ruppert and Tjian 1995). Furthermore, TFIID, together with Mediator and the multifunctional SAGA complex, integrates signals from transcriptional activators and has been shown to be required for activated transcription (Baek et al. 2002; Chen et al. 1993, 1994; Horikoshi et al. 1988; Pugh and Tjian 1990; Stringer et al. 1990; Wu and Chiang 2001). Additionally, a number of domains within the complex recognize specific histone modifications associated with active genes (Jacobson et al. 2000; Vermeulen et al. 2007). Thus, TFIID is important for nucleating PIC assembly at the promoter and also serves as a hub for the integration of regulatory cues that are important for regulated transcription.

Conformational Complexity of TFIID—Due to the size and complexity of TFIID, structural efforts to characterize the full complex have involved electron microscopy (EM) of natively purified samples. Early studies of negatively stained human and budding yeast TFIID showed it to have an overall horseshoe-shaped architecture of three major lobes, termed lobes A, B and C (Andel et al. 1999; Brand et al. 1999; Leurent et al. 2002). Improving the resolution by cryo-EM proved to be extremely challenging due to conformational heterogeneity (Elmlund et al. 2009; Grob et al. 2006; Papai et al. 2009). It was ultimately shown that lobes B and C form a relatively stable core (the BC core), while lobe A is flexibly attached and can undergo conformational rearrangements on the scale of 100 Å, from interacting with lobe C to interacting with lobe B (Fig. 5.3a) (Cianfrocco et al. 2013). Free TFIID was found to occupy two preferred conformational states, termed the canonical and rearranged states, with addition of TFIIA and promoter DNA shifting this equilibrium towards the rearranged state (Fig. 5.3a). In this conformation, lobe A interacts with lobe B and is positioned near the TATA box, suggesting the presence of TBP within lobe A (Cianfrocco et al. 2013) (Fig. 5.3a).

The Structure of TFIID—Subsequent structural studies of human TFIID aimed to provide higher-resolution analysis of both free and DNA-bound TFIID (Louder et al. 2016; Patel et al. 2018) (Fig. 5.3b, c). While we will focus on the structure of human TFIID below, similar work in yeast has also been reported recently, although promoter DNA could not be clearly visualized in the cryo-EM structure of promoter-bound yeast TFIID (Kolesnikova et al. 2018).

The structure of stably DNA-bound human TFIID (Fig. 5.3b), using the super core promoter, and in complex with TFIIA, showed that lobe C includes TAFs 1, 2, and 7, with a TAF1-TAF7 module binding the DPE and MTE downstream promoter elements, while a small domain within TAF1 contacts the Inr motif (indicated in Fig. 5.3b) that includes the transcription start site (TSS) (Louder et al. 2016). Lobe C is connected to lobe B via a long helix in TAF8 that also interacts with a centrally positioned dimer of HEAT repeats from the two copies of TAF6. The TAF6 dimer connects to lobe B, which in turn contacts the TBP-TFIIA module, as well as promoter sequences near the TATA box (Louder et al. 2016; Nogales et al. 2017a; Nogales et al. 2017c). The TATA box DNA in this structure is bent (Fig. 5.3b, bottom), mirroring the structure of the isolated TBP-DNA complex (Nikolov et al. 1996). By virtue of this arrangement, lobes B and C act as a molecular ruler around the

TSS, providing a platform for transcription pre-initiation complex assembly (Louder et al. 2016; Nogales et al. 2017b).

The complete molecular architecture of all three lobes of human TFIID was derived from the cryo-EM analysis of free TFIID (Patel et al. 2018) (Fig. 5.3c). Due to the scarcity, structural heterogeneity, fragility, and preferred orientation of this specimen on the cryo-EM grid, determination of this structure was a tour-de-force. A striking structural feature of TFIID is that lobes A and B share a common core architecture (Fig. 5.3d), with six TAFs being present in two copies, one in each lobe (Bieniossek et al. 2013; Leurent et al. 2002; Patel et al. 2018; Sanders et al. 2002). In this common module, TAF5, which contains a helical N-terminal domain and a WD40 domain, binds to three dimers of TAFs that contain histone-fold domains (HFDs) and themselves form a hexameric assembly that resembles the overall architecture of the nucleosome core particle (Patel et al. 2018), in agreement with earlier biochemical and structural data (Hoffmann et al. 1996; Xie et al. 1996). TAF5 and five of the histone fold-containing TAFs, TAF10, and the pairs TAF4-TAF12 and TAF6-TAF9, are present in both lobes A and B (Fig. 5.3d). The TAF6 HFDs present in both lobes, A and B, are flexibly connected to their corresponding C-terminal HEAT repeat domains that exist as a dimer at the center of the complex, within lobe C (Fig. 5.3b). In spite of their shared core architecture, lobes A and B are functionally and structurally unique because their common TAF10 subunit associates with the HFD of TAF3 in lobe A but with the HFD of TAF8 in lobe B (Fig. 5.3d). TAF8 contributes to the lobe B-lobe C interaction, while TAF3 recruits the TAF11-TAF13 HFD dimer to lobe A. Together with N-terminal regions of TAF1 (Anandapadamanaban et al. 2013; Kokubo et al. 1993, 1994), the TAF11-TAF13 pair binds TBP, which consequently hangs peripherally on this mobile lobe A within TFIID.

Mechanism of TBP Loading onto the Promoter—The extreme mobility of lobe A, along with its ability to carry TBP, suggests a role for the dynamics of this lobe in the deposition of TBP onto the promoter (Fig. 5.4a). An exhaustive classification of a cryo-EM dataset where TFIID, TFIIA and core promoter DNA were mixed together revealed five distinct states, including previously observed as well as new conformational and functional states of lobe A. These states were termed canonical, extended, scanning, rearranged and engaged states (Fig. 5.4a), with the first corresponding to lobe A positioned proximal to lobe C, and consequent states being further from lobe C and ultimately contacting lobe B (Patel et al. 2018). The model derived from the co-existence of these states proposes that in the canonical state, where lobe A is far removed from the DNA, TBP is bound by the TAF11-TAF13 HFD dimer, as well as the TAF1 TAND1 and TAND2 motifs (Figs. 5.3d, 5.4b), which have been implicated in inhibition of DNA and TFIIA binding by TBP (Fig. 5.4c) (Anandapadamanaban et al. 2013; Kokubo et al. 1993, 1994). These inhibitory interactions are sequentially broken in the scanning and rearranged states as they are replaced by interactions of TBP with DNA and TFIIA (Fig. 5.4d), facilitated by the scaffolding role of TFIID and by lobe A-lobe B interactions. Specifically, we have proposed that in the scanning state TAND1 is displaced as TBP loosely associates with the DNA, while in the rearranged state TAND2 is displaced as TBP binds to TFIIA that also interacts with lobe B. In the final, engaged state, TBP binds tightly and bends the DNA, and it releases from

TAF11-TAF13. Displacement of TAF11-TAF13 opens up the binding site for TFIIB, enabling PIC assembly (Patel et al. 2018).

Notably, current data indicate that the overall architecture of TFIID is conserved between promoters that do and do not contain TATA boxes (Cianfrocco et al. 2013; Patel et al. 2018), in agreement with the observation that TBP also participates in initiation on TATA-less promoters (Pugh and Tjian 1991). The intricate mechanism of TBP loading onto the upstream promoter may ensure that even in the absence of a TATA box, TBP engages with DNA in a defined, controlled, and productive fashion, rather than binding DNA nonspecifically. Completion of TBP loading onto the core promoter in such cases (reaching the engaged state) may depend on chromatin marks, transcriptional activators, or co-activator complexes, because both structural and biochemical analysis shows that TBP only weakly binds TATA-free promoters in reconstituted systems that contain TFIID and TFIIA (Cianfrocco et al. 2013) even though transcription assays show transcription from such promoters (Juven-Gershon et al. 2006; Lim et al. 2004).

TFIIH

Functional Roles of TFIIH in Transcription—TFIIH is a protein complex of ten different subunits organized into a core and a CdK activating kinase (CAK) subcomplex (Fig. 5.5a). Both the core and the CAK are required for TFIIH to function in transcription initiation, while only the TFIIH core complex is required in DNA repair (Svejstrup et al. 1995). The TFIIH core complex includes seven protein subunits: XPB, XPD, p62, p52, p44, p34, and p8 (Fig. 5.5a). Two of these, XPB and XPD, are SF2-family DNA-dependent ATPases/helicases (Guzder et al. 1994; Sung et al. 1993; Weeda et al. 1990). While the enzymatic function of XPD is dedicated to DNA repair (Kuper et al. 2014), only XPB activity is required to help promoter opening during transcription initiation (Fishburn et al. 2015; Grünberg et al. 2012; Kim et al. 2000). Originally thought to act like a wrench to introduce torsion into downstream promoter DNA to enable opening of the transcription bubble (Kim et al. 2000), it was later proposed that XPB acts as a DNA translocase that promotes the movement of downstream promoter DNA towards the Pol II active site, thereby facilitating bubble opening (Fig. 5.5b) (Fishburn et al. 2015; Grünberg et al. 2012; He et al. 2013).

The CAK subcomplex includes the CDK7, Cyclin H, and MAT1 subunits (Devault et al. 1995; Fisher et al. 1995; Fisher and Morgan 1994; Shiekhattar et al. 1995) (Fig. 5.5a). CDK7 is a cyclin dependent kinase that requires the presence of Cyclin H for full activity, while MAT1 aids formation of the CDK-cyclin complex (Devault et al. 1995; Fisher et al. 1995) and anchors the CAK subcomplex to the core complex via interactions with XPD and XPB (Abdulrahman et al. 2013; Busso et al. 2000; Greber et al. 2017; Rossignol et al. 1997). The role of XPD in recruitment of the CAK subcomplex (which is required only for transcription) explains why its presence in TFIIH is important for transcription initiation, while its enzymatic activity is not (Dubaele et al. 2003; Kuper et al. 2014). The CAK is able to phosphorylate a number of targets, which include the C-terminal low-complexity region of the Pol II subunit Rpb1, referred to as the Pol II CTD here, as well as transcriptional

activators, general transcription factors, and, in humans, cell-cycle CDKs (Fisher and Morgan 1994; Shiekhattar et al. 1995).

TFIIH as a DNA Repair Complex—In addition to its role as a Pol II general transcription factor, TFIIH also serves as a helicase complex in the nucleotide excision DNA repair pathway. This pathway is responsible for removing a large number of bulky base alterations that result in distortion of the DNA backbone, including 6–4 photoproducts, cyclobutane pyrimidine dimers, and other large base adducts (Koch et al. 2016). In these pathways, TFIIH serves to open a DNA bubble around damaged nucleotides, which can then be excised by the structure-specific endonucleases XPF-ERCC1 and XPG (Marteijn et al. 2014). Initial recognition of the damage occurs independently of TFIIH, either by a global genome surveillance pathway that depends on the DDB1-DDB2 and XPC complexes, or via transcription-coupled repair (TCR), in which Pol II stalled by DNA lesions acts as the initial damage sensor, leading to the recruitment of specialized TCR factors such as CSA and CSB, and ultimately, TFIIH (Compe and Egly 2012). Unlike in transcription initiation, the enzymatic activity of both XPD and XPB is required during NER. XPD acts as a bona-fide helicase to unwind the DNA double helix and scan for the DNA lesion (Kuper et al. 2014; Li et al. 2015), while the role of XPB has been described either as an ATP-dependent conformational switch that anchors TFIIH to DNA (Coin et al. 2007), or as a weak helicase and DNA translocase that supports scanning of the DNA for the lesion that originally triggered TFIIH recruitment (Li et al. 2015; Sugawara et al. 2009). Upon lesion recognition in a binding pocket in XPD (Buechner et al. 2014; Mathieu et al. 2013), both helicase motors stall (Li et al. 2015; Naegeli et al. 1993), enabling assembly of the repair bubble, double incision, and ultimately repair of the DNA lesion by DNA polymerases Pol δ and Pol κ (Compe and Egly 2012; Marteijn et al. 2014).

The Structure of TFIIH—Due to its importance for two critical cellular processes, the structure of TFIIH has been intensively investigated for almost two decades. Initial studies resulted in low-resolution EM reconstructions that showed a ring- or horseshoe-shaped complex (Chang and Kornberg 2000; Schultz et al. 2000) and allowed the approximate assignment of the XPB and XPD ATPase subunits to the open ends of a horseshoe (Gibbons et al. 2012). An integrative modelling approach provided a tentative overall architecture for the complex, which was, however, complicated by the horseshoe-shaped overall architecture of the complex that puts many subunits within crosslinking distance of each other, and the high flexibility of the CAK subcomplex (Luo et al. 2015). Sub-nanometer resolution structures of TFIIH in the context of both human and yeast PICs (Fig. 5.5c) confirmed the assignment of the ATPase subunits to density at the open end of the horseshoe-shaped TFIIH complex and revealed the locations of p44 near XPD and of the p52-p8 dimer near XPB (Fig. 5.5c) (He et al. 2013, 2016; Murakami et al. 2015), in agreement with regulatory roles of these subunits on the two ATPase subunits (Coin et al. 2007; Dubaele et al. 2003; Jawhari et al. 2002; Kim et al. 2015). The interpretation of these cryo-EM maps relied heavily on docking of crystal structures or homology models. Structures of bacterial and archaeal homologs of XPB (Fan et al. 2006) and XPD (Bienstock et al. 2003; Fan et al. 2008; Liu et al. 2008; Wolski et al. 2008) provided structural templates for interpretation of the low-resolution cryo-EM maps, and for modelling of human disease mutations.

The TFIID-containing PIC structures (He et al. 2013, 2016; Tsai et al. 2017) also suggested that in the absence of Mediator, the CAK subcomplex localizes close to XPD and the C-terminal region of Rpb1, thus in proximity to its major substrate (Greber et al. 2017; He et al. 2013; Tsai et al. 2017). However, later Mediator-bound structures showed that in this context, which likely better resembles the *in vivo* situation, CAK is found in a different location (Robinson et al. 2016; Schilbach et al. 2017) (Fig. 5.5d, e). Both in the absence and in the presence of Mediator, the CAK subcomplex is poorly resolved, and in spite of the availability of high-resolution crystal structures for both CDK7 and Cyclin H (Andersen et al. 1997; Kim et al. 1996; Lolli et al. 2004), the interaction with the third CAK subunit, MAT1, and the mechanism of CAK regulation remain unresolved.

A more detailed and complete molecular model of the TFIID core complex resulted from the improved cryo-EM reconstructions of apo-TFIID (Greber et al. 2017) and of the yeast Pol II-PIC (Schilbach et al. 2017). These studies revealed the overall structure of additional TFIID subunits, including p62, and the positioning of the zinc-binding domains of p34, p44, and the CAK subunit MAT1. The latest breakthrough came from the 3.7 Å-resolution cryo-EM structure of human apo-TFIID (Greber et al. 2019). This structure revealed the complete network of interactions that governs the assembly of the TFIID core complex (Fig. 5.5f, g), allowed the mapping of numerous disease mutations in XPD and XPB (Fig. 5.5h and see below), and showed that XPD is probably tightly regulated, not only by MAT1 and p44, as demonstrated by previous biochemical and mutagenesis experiments (Abdulrahman et al. 2013; Dubaele et al. 2003; Kim et al. 2015; Sandroch and Egly 2001), but also by XPB and p62, which block access to known XPD DNA- and ATP-binding sites (Fig. 5.5g) (Greber et al. 2019).

TFIID in Health and Disease—Defects in TFIID subunits are associated with xeroderma pigmentosum, Cockayne syndrome, and trichothiodystrophy (Fig. 5.5h), human disease syndromes characterized by high incidence of cancers, premature ageing, or transcriptional defects (Cleaver et al. 1999; Rapin 2013). Mutations that cause xeroderma pigmentosum, characterized by extreme sensitivity to sunlight and a high incidence of cancers, interfere with the global genome NER pathway by impairing (i) detection of lesions by the specialized damage recognition sensors (e.g. XPC), (ii) repair bubble opening by interfering with the function of the TFIID helicases XPB and XPD, or (iii) excision of the damaged nucleotide by the XPF-ERCC1 or XPG endonucleases. Cockayne syndrome is a premature ageing condition, which in the case of mutations in the XPD subunit of TFIID, occurs in combination with xeroderma pigmentosum (Cleaver et al. 1999; Rapin 2013). Cockayne syndrome is thought to be a disease induced by defects in TCR. However, recent data suggest that CS mutations in XPD may lead to stalling of the helicase after initiation of the repair process, which may interfere with completion of the repair reaction and with the resumption of transcription (Herrera-Moyano et al. 2014; Moriel-Carretero et al. 2015; Theron et al. 2005). Mapping of XP and XP-CS mutations onto the structure of human XPD (Greber et al. 2017, 2019) (Fig. 5.5h) or its archaeal and bacterial homologs (Bienstock et al. 2003; Fan et al. 2008; Liu et al. 2008; Wolski et al. 2008) shows that they localize primarily to the enzymatic core of the helicase, affecting either nucleotide binding and hydrolysis, or

DNA binding and translocation, in agreement with the enzymatic function of XPD in NER (Kuper et al. 2014), and the observation that these mutations primarily lead to NER defects.

In contrast, trichothiodystrophy (TTD) mutations in TFIIH mostly affect peripheral regions of XPD (Fig. 5.5h) (Bienstock et al. 2003; Fan et al. 2008; Greber et al. 2017; Liu et al. 2008; Wolski et al. 2008), where they structurally destabilize the protein or its interactions within TFIIH, thereby disrupting TFIIH assembly and impairing proper placement of the CAK during transcription initiation (Coin et al. 1998; Dubaele et al. 2003). Additional TTD mutations in XPB and p8 have been shown to destabilize these proteins and reduce the overall levels of properly assembled TFIIH (Botta et al. 2002; Dubaele et al. 2003; Gigliamari et al. 2004; Kainov et al. 2008). This lack of structurally intact TFIIH, either due to reduced protein levels or disrupted protein-protein interfaces, is what leads to a general transcription defect in addition to DNA repair defects.

In addition to its association with these inherited disorders, TFIIH has also been implicated in promoting cancer cell growth due to the transcription-promoting activity of its CAK module and the requirement for elevated transcription in cancer cells. Therefore, TFIIH is a possible drug target in cancer chemotherapy (Berico and Coin 2017; Fisher 2018; Gervais et al. 2018).

Structural Insight into the Pol II-PIC

Visualization of the Step-Wise Assembly of the Human Pol II-PIC—Early crystallographic studies revealed the structures of promoter-bound TBP, alone and in complex with TFIIA and TFIIB (Fig. 5.6a, b), as well as TFIIB bound to Pol II (Pol II-ITC with bound TFIIB shown in (Fig. 5.6c). These studies showed that TBP binding to the TATA-box induces an almost 90° bend in the overall trajectory of the upstream promoter DNA (Fig. 5.6a), and how TFIIB interacts with TBP and with the BRE promoter elements near the TATA-box (Fig. 5.6b) (Bleichenbacher et al. 2003; Nikolov et al. 1992, 1995, 1996; Tan et al. 1996; Tsai and Sigler 2000). Structures of TFIIB bound to Pol II (Bushnell et al. 2004; Kostrewa et al. 2009; Liu et al. 2010; Sainsbury et al. 2013) showed how the cyclin domains of TFIIB bind above the Pol II wall, while the N-terminal B-ribbon binds to the Pol II dock (Fig. 5.6c). The segment between the B-ribbon and the cyclin folds is seen inserted into the Pol II cleft, approaching the active site, which led to the suggestion that this B-reader aids in start site selection (Kostrewa et al. 2009; Liu et al. 2010). A combination of these structures with the previously mentioned structure of the TPB-TFIIB-DNA complex (Nikolov et al. 1995; Tsai and Sigler 2000) and the structure of the Pol II elongation complex (Gnatt et al. 2001) suggested approximate models for the closed and open Pol II-PIC, and the subsequent structure of the TFIIB-bound Pol II-ITC (Fig. 5.6c), complete with substrate DNA and an RNA product mimic, revealed that the TFIIB B-reader contacts the single stranded DNA in the polymerase active site, leads to binding of one of the catalytic metals, and stimulates RNA synthesis (Sainsbury et al. 2013).

The architecture of the complete Pol II-PIC and a structural characterization of its assembly pathway have remained refractory to crystallographic approaches and were finally obtained using cryo-EM analysis of in vitro reconstituted complexes (Fig. 5.6d). In order to arrive at a more complete understanding of the complete Pol II-PIC, and to map the location of all the

individual general transcription factors, the human Pol II-PIC was assembled in a step-wise fashion, following the generally accepted model for PIC assembly (Buratowski et al. 1989), and analyzed by cryo-EM (He et al. 2013). The system was simplified by substituting TFIID with just TBP (a consensus TATA box was included in the promoter DNA), and Mediator was not included in the reconstitution. Additionally, although TFIIF is thought to join the growing PIC pre-bound to Pol II (Killeen and Greenblatt 1992; Rani et al. 2004; Roeder 1996), the structure was determined for complexes with and without TFIIF to allow mapping of the density corresponding to this transcription factor.

The cryo-EM density of the TBP-TFIIA-TFIIB-Pol II complex resulting from these studies (He et al. 2013) could be fully accounted for by the crystallographic structures of isolated sub-complexes (see above) and showed the general transcription factors clustered on the upstream promoter DNA near the TATA box, with density for the DNA approaching the Pol II cleft. The otherwise poorly ordered downstream promoter DNA became stabilized upon addition of TFIIF, which binds to the cluster of general transcription factors near the TATA box (Fig. 5.6d, left), Pol II, and the upstream promoter DNA itself (He et al. 2013). The stabilization of the DNA was induced by interactions of TFIIF with DNA near the BREd element and the opening of the Pol II clamp, allowing DNA to access the Pol II cleft and form interactions with the clamp head and Rpb5. These interactions are consistent with roles of TFIIF in start site selection and transcription bubble opening (Ghazy et al. 2004; Yan et al. 1999).

Addition of TFIIE, which spans from TFIIF to the Pol II stalk (Fig. 5.6d, middle), led to further stabilization of the core PIC (He et al. 2013). Winged-helix domains in TFIIE connect the Pol II stalk region to the TFIIF subunit Rap30, topologically trapping the DNA on the Pol II-PIC. The DNA in this complex is slightly bent and contacted at either side of the initiator element by Pol II elements near the clamp head and at the Rpb5 jaw (He et al. 2013, 2016).

Finally, TFIIH was seen to bind to this core-PIC (Fig. 5.6d, right) such that its XPB subunit is in contact with the downstream promoter DNA near Rpb5 (Figs. 5.5c, 5.6d, right) (He et al. 2013, 2016), in support of its proposed function as a double-stranded DNA translocase during promoter opening (Fishburn et al. 2015; Grünberg et al. 2012; Kim et al. 2000). During the closed-to-open transition, mimicked in the studies of the human system by synthetic constructs that provide single-stranded DNA at the side of the transcription bubble, the downstream promoter DNA is bent around the Rpb5 contact for insertion of the template strand into the active site, while the Pol II clamp domain closes and assumes a conformation similar to that observed in elongation complexes (He et al. 2013, 2016).

The TFIIH-containing Pol II-PIC exhibits a bi-lobed overall appearance (Figs. 5.5c, 5.6d), with TFIIH forming one lobe and Pol II and the remaining GTFs forming the other lobe (He et al. 2013, 2016). In addition to the interaction between XPB and the downstream promoter DNA, TFIIH and the core PIC are connected by two main protein-protein interfaces: (i) Mobile regions in the C-terminal half of TFIIE α interact with the N-terminal PH domain of p62 (He et al. 2016; Okuda et al. 2008; Schilbach et al. 2017) and form additional contacts with the C-terminal region of p62, and with XPB (Schilbach et al. 2017), with the latter

interaction explaining previous biochemical data that suggest a role of TFIIE in XPB regulation (Drapkin et al. 1994; Ohkuma and Roeder 1994; Maxon et al. 1994); and (ii) the N-terminal RING domain of MAT1 interacts with the Pol II stalk and TFIIE (Schilbach et al. 2017).

In spite of initial claims to the contrary (Murakami et al. 2013), the model derived from the sequential assembly of human proteins on promoter DNA (He et al. 2013) is in good agreement with subsequent structures from both the human (He et al. 2016) and the yeast Pol II system (Fig. 5.6d, e) (Murakami et al. 2015; Plaschka et al. 2015, 2016; Schilbach et al. 2017). However, slight differences between the human and yeast systems were visualized in the higher-resolution structures. For example, the promoter DNA in the human closed complex is contacted by the Rpb1 clamp and Rbp5, while the DNA is suspended further away from Pol II in the yeast complexes, where such contacts are not present (Nogales et al. 2017b) (for further discussion see next section).

High-Resolution Analysis of the Pol II Core-PIC and Mechanism of Open

Complex Formation—High-resolution cryo-EM analysis of human and yeast Pol II core-PICs (the latter excluding TFIIH) (Figs. 5.6f, 5.7a–c) have revealed the intricate architecture of closed, open, and initially transcribing complexes in near-atomic detail (He et al. 2016; Plaschka et al. 2016). In the human open Pol II-PIC, the B-reader and B-linker elements of TFIIB contact the single stranded DNA of the open transcription bubble (Fig. 5.7d–f), and all of these elements are resolved in the map (He et al. 2016). In the yeast Pol II-PIC reconstructions (Plaschka et al. 2016), both the single-stranded DNA and the interacting TFIIB elements are not visualized or poorly resolved. The better definition of the open DNA bubble in the human system might be linked to the scanning mode of start site selection in yeast (Nogales et al. 2017b).

The reverse is true for interactions of TFIIE (Fig. 5.7g–i), where the human PIC lacks density for part of the TFIIE E-wing domain near the upstream end of the open bubble, while an interaction between this domain and the DNA is visible in the yeast structure (He et al. 2016; Plaschka et al. 2016). This might account for the propensity of the yeast Pol II-PIC system to spontaneously open the transcription bubble (Plaschka et al. 2016), while this has not been observed in the reconstituted human complexes. Nearby, the WH1 domain of TFIIE β (Tfa2 in yeast) that interacts with the TFIIF β (Rap30 in humans, Tfg2 in yeast) WH domain and in particular with the additional helix of this domain in yeast is, accordingly, also in a different position between the human and yeast systems. The WH2 domain of TFIIE β (Tfa2) also has a slightly different position in the two systems.

The structure of the human Pol II-ITC is very similar to the open complex (Fig. 5.7a, b, e, f), with the exception of an enlarged DNA bubble and the presence of RNA, which had been introduced with the substrate DNA template (He et al. 2016).

The Mediator-Bound Pol II PIC—Mediator is a co-activator complex composed of more than 20 subunits and with a molecular weight of approximately 1.2 MDa. Mediator integrates the signals from transcriptional activators and repressors for regulated transcription initiation (Borggreffe and Yue 2011; Brzovic et al. 2011; Flanagan et al. 1991;

Jeronimo et al. 2016; Lee et al. 1999; Natarajan et al. 1999; Soutourina 2018), a prerequisite for cellular development and differentiation. Additionally, Mediator stimulates the activity of CDK7 within the TFIIF CAK module towards the C-terminal, low-complexity region of the Pol II subunit Rpb1 (Kim et al. 1994). Mediator itself includes the CDK8-Cyclin C pair, which together with the Med12 and Med13 subunits form a dissociable module (Borggreffe et al. 2002; Tsai et al. 2013) that phosphorylates a range of targets, with broad implications for the mechanism of transcription regulation and for cellular development and disease (e.g. (Bancerek et al. 2013; Donner et al. 2010; Firestein et al. 2008; Knuesel et al. 2009)).

The overall structure of Mediator can be subdivided into four modules, termed head, middle, tail, and arm (Cai et al. 2009), as well as the dissociable kinase module (Borggreffe et al. 2002; Tsai et al. 2013). Early low-resolution electron microscopy analysis indicated that an open conformation of Mediator wraps around one side of Pol II to form the holoenzyme (Asturias et al. 1999; Davis et al. 2002; Dotson et al. 2000). Because of its size, complexity, conformational variability, and low abundance in cells, structure determination of Mediator has been pursued by X-ray crystal structures of subcomplexes, such as structures of its head and kinase modules (Imasaki et al. 2011; Lariviere et al. 2012a; Robinson et al. 2012; Schneider et al. 2011). These structures were then combined with chemical crosslinking-mass spectrometry distance restraints and low-resolution cryo-EM maps to arrive at structural models for the full complex (Lariviere et al. 2012b, 2013; Robinson et al. 2015, 2016; Tsai et al. 2014; Verger et al. 2019).

The most complete atomic model for any Mediator complex to date is that of the *Schizosaccharomyces pombe* Mediator head and middle modules (or core Mediator), determined by X-ray crystallography at 3.4 Å resolution (Fig. 5.8a) (Nozawa et al. 2017) and cryo-EM at 4.4 Å resolution (Tsai et al. 2017). Even though the architecture of the complex in these two structures is in overall agreement, the higher-resolution X-ray crystallographic analysis resulted in a more accurate and more complete model. The structure shows that the Med14 subunit spans the entire middle module and serves as a scaffold for the assembly of the remaining subunits of this module (Fig. 5.8a). The head and middle modules are bridged by the Med17 and Med6 subunits (Fig. 5.8a), each of which contributes folded domains to both modules, with flexible linkers connecting them (Nozawa et al. 2017). The head module had been visualized previously in isolation (Imasaki et al. 2011; Lariviere et al. 2012a; Robinson et al. 2012). Interestingly, one of these structures had been solved in complex with a CTD peptide bound in a groove formed by the Med6, Med8, and Med17 subunits (Fig. 5.8b) (Robinson et al. 2012). The Mediator tail module is critical for interactions with transcriptional activators, but in spite of the availability of partial X-ray crystal structures (e.g. for the human Med23 and Med25 subunits (Monté et al. 2018; Vojnic et al. 2011)), it has not been visualized in its entirety at high resolution. However, an approximate subunit mapping has been obtained from an integrative modeling approach (Robinson et al. 2015).

Detailed insight into the architecture of the Mediator-bound Pol II-PIC came from cryo-EM analysis of reconstituted complexes of yeast core-PIC (without TFIIF) or ITC complexes (Plaschka et al. 2015; Tsai et al. 2017) bound to a core-Mediator complex (Fig. 5.8c). These cryo-EM reconstructions revealed extensive density for the Mediator middle and head

modules, and initially allowed fitting of several sub-modules of the head module, aided by distance restraints from chemical crosslinking-mass spectrometry (Plaschka et al. 2015). Subsequently, with higher resolution cryo-EM structures and with the determination of the X-ray crystal structure of the head and middle modules (Nozawa et al. 2017), the entire core Mediator complex could be placed in the density of a TFIIF-containing and Mediator-bound Pol II-PIC (Schilbach et al. 2017) (Fig. 5.8d). Core Mediator binds to the PIC at three contact sites that are arranged near the Pol II Rpb4-Rpb7 stalk (Fig. 5.8e, f). First, the so-called movable jaw (Med18-Med20) forms contacts with the TFIIF B-ribbon, the Pol II dock, and the Pol II subunits Rpb3 and Rpb11 (Fig. 5.8e). Second, the Mediator head module subunits Med8 and Med22 directly contact the Rpb4-Rpb7 stalk (Fig. 5.8e). Finally, the plank domain of the middle module forms transient contacts to the Pol II foot (Fig. 5.8f), as deduced from different conformations observed in cryo-EM particle sub-classes (Plaschka et al. 2015; Schilbach et al. 2017). While the Mediator head and middle modules themselves assume a relatively constant conformation when associating with Pol II—which also does not undergo any major conformational rearrangements upon complex formation—the relative orientation of the two Mediator modules is changed upon core-Mediator-Pol II complex formation due to conformational rearrangements in the Med14 subunit (Schilbach et al. 2017; Tsai et al. 2017) (Fig. 5.8g, h).

This structural model just described, together with a low-resolution cryo-EM reconstruction of a complete Pol II-PIC, including TFIIF and the Mediator tail module (Robinson et al. 2016), showed the Mediator tail module pointing towards the upstream promoter DNA, where it may interact with bound transcriptional activators, consistent with their function in Mediator recruitment (Fig. 5.8i) (Borggreffe and Yue 2011; Brzovic et al. 2011; Jeronimo et al. 2016; Lee et al. 1999; Natarajan et al. 1999). The structures of TFIIF-containing PICs in complex with Mediator or core Mediator suggest that the hook motif of the Mediator middle module aids in positioning of the TFIIF CAK subcomplex, such that it is optimally placed to phosphorylate the Pol II CTD, which runs inside a cradle formed by the inner surface of the remainder of the Mediator middle module (Robinson et al. 2012, 2016; Schilbach et al. 2017) (Figs. 5.5d, e, 5.8b).

Possible Organization of a Complete Pol II-PIC Containing TFIID and Mediator
—The structure of the Pol II-PIC in the presence of full TFIID has not yet been obtained. An approximate architecture can be deduced by superimposing the components that are shared between the high-resolution Pol II-PIC reconstructions and the structure of promoter-bound TFIID, specifically TBP, TFIIA, and the upstream promoter DNA (Louder et al. 2016; Patel et al. 2018). Similarly, by superposition of Pol II in the Mediator-containing Pol II-PIC reconstructions (Plaschka et al. 2015; Robinson et al. 2016; Schilbach et al. 2017; Tsai et al. 2017) allows approximate positioning of Mediator in this architectural model (Fig. 5.9).

This analysis shows that Mediator and TFIID would occupy opposite sides of Pol II (Fig. 5.9), and thus would not clash with each other. However, structural rearrangements may be necessary concerning the position of TAF contacts with the DNA during PIC assembly. The region of TAF4 that, within lobe B, approaches TFIIA and the upstream promoter DNA partially overlaps with the Rap30 winged-helix domain within TFIIF in the model (Louder et al. 2016; Patel et al. 2018). Furthermore, the downstream promoter contacts formed by

TFIID lobe C partially overlap with regions of Pol II and TFIIH that contact the DNA in the PIC, and would, in any case, need to be released for Pol II to transcribe through the downstream promoter region. Indeed, it has been proposed that an isomerization of these contacts and release of TAF7, localized in the immediate vicinity of the downstream promoter DNA, is required for transcription initiation (Gegonne et al. 2006; Zhang et al. 2015). Because this isomerization is apparently required only for the first round of initiation, but not for subsequent re-initiation events (Zhang et al. 2015), it is possible that TFIID does not re-form these downstream promoter contacts after it has functioned as a molecular ruler to load TBP for PIC recruitment.

In spite of the release of downstream promoter contacts, TFIID may remain bound to the promoter if the upstream TFIID architecture can rearrange such that the sterical hindrance between the TFIID TAFs and the GTFs of the core Pol II-PIC can be mitigated. It has been shown that transcription re-initiation can be up to fourfold faster than the first round of initiation (Jiang and Gralla 1993) and activator-independent (Joo et al. 2017), suggesting that a set of general transcription factors remains bound at the promoter and allows incoming Pol II complexes to re-initiate rapidly. Transcription re-initiation depends on ATP hydrolysis, implicating the XPB or CDK7 subunits of TFIIH in the process (Yudkovsky et al. 2000). Even though much remains to be elucidated to gain a full understanding of the molecular details that distinguish the processes of initiation and re-initiation in Pol II transcription, the recent detailed structures of the Pol II-PIC and its components provide an architectural framework for future studies.

The Pol I and Pol III Pre-initiation Complexes

Structure of the Pol I-PIC and Mechanism of Initiation of rRNA Transcription

Pol I and Its General Transcription Factors—Pol I is responsible for synthesis of long rRNA precursors in the nucleolus, contributing up to 60% of total RNA synthesis in yeast (Warner 1999). The X-ray crystal structure of the 14-subunit Pol I was that of a transcription-incompetent dimer (Fig. 5.10a) with a widened cleft that was blocked by inserted protein segments (Engel et al. 2013; Fernández-Tornero et al. 2013). In solution, Pol I exists in an equilibrium of inactive dimers and functional monomers (Milkereit et al. 1997), a situation that may parallel the physiological response to nutrient starvation (Torreira et al. 2017). Binding of Rrn3, one of the Pol I transcription initiation factors, renders Pol I monomeric (Fig. 5.10b) and with a widened cleft that can be accessed by substrate DNA (Engel et al. 2016; Pilsel et al. 2016).

The Pol I initiation system differs substantially from that of Pol II. In yeast, the Pol I-PIC comprises the general transcription factors TBP, Rrn3, upstream activating factor (UAF), and the heterotrimeric core factor (CF) (Fig. 5.10c) (Bedwell et al. 2012; Hontz et al. 2008; Keener et al. 1998; Keys et al. 1996; Schneider 2012; Steffan et al. 1998). Rrn3 and CF support basal Pol I initiation, while binding of UAF and TBP precedes CF recruitment *in vivo* and is required for full activity (Bordi et al. 2001; Hontz et al. 2008; Keys et al. 1996; Steffan et al. 1998). Yeast CF is formed by Rrn6, Rrn7, and Rrn11 (Keys et al. 1994; Lalo et al. 1996; Lin et al. 1996). Its mammalian counterpart, selectivity factor 1 (SL1), harbors three subunits homologous to the yeast system and two additional mammalian-specific

factors (Comai et al. 1992; Denissov et al. 2007; Friedrich et al. 2005; Gorski et al. 2007; Learned et al. 1985; Naidu et al. 2011), suggesting a conserved core architecture with certain mammalian-specific features. The structure of CF (Fig. 5.10d), determined independently by both X-ray crystallography and cryo-EM, shows a bi-lobal assembly, with Rrn11 and Rrn7 each forming the core of one lobe, and with Rrn6 spanning across both lobes (Engel et al. 2017; Han et al. 2017; Sadian et al. 2017). In spite of sequence and structural homology between Rrn7 and TFIIB (Knutson and Hahn 2011), these two initiation factors are not functionally equivalent because, due to its position in the Pol I-PIC, Rrn7 cannot bind to DNA in the way TFIIB does (Fig. 5.10e) (Engel et al. 2017; Han et al. 2017; Sadian et al. 2017).

Structural Insight into RNA Polymerase I Pre-initiation Complexes—Three independent cryo-EM studies have provided detailed insights into the structures of the Pol I-PIC and the promoter-free Pol I-Rrn3-CF complex to elucidate the mechanism of transcription initiation by Pol I (Engel et al. 2017; Han et al. 2017; Sadian et al. 2017). In the Pol I-Rrn3-CF complex, lacking promoter DNA (Fig. 5.10f), CF binds to the upstream end of the Pol I cleft, which is partially widened (Engel et al. 2017). There are three contact sites between CF and the PIC core (Fig. 5.10f): (i) The N-terminal zinc-ribbon domain of Rrn7 contacts the Pol I dock domain and a loop in Rrn3; (ii) the Rrn7 insertion domain contacts the Pol I wall; and (iii) Rrn11 contacts the Pol I clamp and protrusion via its TPR domain (Engel et al. 2017). Notably, the N-terminal cyclin domain of Rrn7 is embedded in CF and therefore unable to form contacts equivalent to the TFIIB-Pol II wall interactions present in the Pol II system (Engel et al. 2017; Han et al. 2017; Sadian et al. 2017).

Upon binding of DNA and formation of an ITC, mimicked experimentally by introduction of a mismatch bubble with bound RNA, the Pol I active site cleft assumes a contracted conformation (Engel et al. 2017; Han et al. 2017; Sadian et al. 2017), similar to that observed for the elongation complex structures (Neyer et al. 2016; Tafur et al. 2016). The promoter DNA in the active site cleft of Pol I is stabilized by the clamp head, cleft, and jaw of Pol I subunit A190, the lobe domain of A135, and Rpb5 (Engel et al. 2017; Han et al. 2017; Sadian et al. 2017). Upon DNA binding, CF is relocated to a different binding site on Pol I (Fig. 5.10g) (Engel et al. 2017). Though CF uses the same binding motifs, its footprint on Pol I moves to the protrusion, Rpb12, and a Pol I-specific structural element of the wall (Engel et al. 2017) (Fig. 5.10g-i). Density for Rrn3 was absent in one of the Pol I-ITC cryo-EM maps, indicating that it may dissociate under conditions that might mimic a late initiation intermediate (Han et al. 2017), consistent with Rrn3 dissociation after Pol I initiation (Bier et al. 2004; Milkereit and Tschochner 1998).

In all cryo-EM reconstructions of Pol I-ITCs, the upstream promoter DNA appears bent (Engel et al. 2017; Han et al. 2017; Sadian et al. 2017). In one case, two kinks, by 35° and 45°, are observed near nucleotide -16 (Han et al. 2017); in the second, the DNA bends by approximately 30° between the CF binding site, where it runs along Rrn11 (DNA nucleotides -35 to -25), and the entry point into the Pol I cleft between the protrusion and the wall (DNA nucleotides -20 to -12) (Engel et al. 2017); and in the third, the bend localizes to approximately nucleotide -30 (Sadian et al. 2017). The ability of the DNA to bend and form these contacts is likely important for promoter recognition, along with the

ability of the region around the transcription start site to melt and form the transcription bubble.

Interestingly, the DNA is suspended above the cleft in the closed Pol II-PIC (He et al. 2013, 2016; Louder et al. 2016; Murakami et al. 2015; Plaschka et al. 2016), while it is already sandwiched between the wall and the protrusion in the CF-bound PIC within the Pol I system (Fig. 5.11a, b) (Engel et al. 2017; Han et al. 2017; Sadian et al. 2017). This difference indicates that Pol I-DNA interactions in the Pol I-PIC already assume an elongation complex-like state, while the Pol II-PIC requires the DNA to shift by approx. 20 Å between the PIC and the elongation complex to reach this state (Fig. 5.11c).

Model for Initiation by RNA Polymerase I—The model for Pol I initiation that emerges from the structural studies just described is that Rrn3 prepares Pol I for initiation by stabilizing it in a monomeric, open-cleft conformation, while CF binds the upstream promoter DNA, and, after docking to Pol I, loads the DNA into the Pol I cleft. Contacts between proximal promoter regions and Pol I specific structural elements likely contribute to promoter recognition at this stage. DNA unwinding and open complex formation leads to initiation of RNA synthesis and eventual displacement of Rrn7 and CF once the RNA reaches a suitable length (Engel et al. 2017; Han et al. 2017; Sadian et al. 2017).

Structure of the Pol III-PIC and Similarities to the Pol II System

Pol III and Its Redox-Sensing General Transcription Factor TFIIB—Pol III contains 17 subunits, 10 of which form the conserved core shared across multisubunit RNA polymerases, whereas 2 form a stalk reminiscent of that in Pol II (Fig. 5.1d, e). 5 Pol III-specific subunits, the C82-C34-C31 trimer and the C53-C37 dimer, appear to be homologous to general transcription factors in the Pol II system (Fig. 5.12), but have become stably incorporated into Pol III (Khatter et al. 2017; Vannini and Cramer 2012). Specifically, there is strong structural homology between TFIIF and the Pol III C53-C37 dimer, which is involved in both transcription initiation and termination (Arimbasseri and Maraia 2015; Kassavetis et al. 2010; Rijal and Maraia 2013; Wu et al. 2011) while the C82-C34-C31 trimer, located on the Pol III C160 clamp, shows homology to TFIIE (Vannini and Cramer 2012) and appears to be a functional fusion of TFIIE and TFIIF (Vorländer et al. 2018; Wu et al. 2012). C82 and C34 contain multiple winged-helix domains that participate in interactions with the Pol III general transcription factors and facilitate Pol III-PIC formation (Khoo et al. 2014, 2018).

Transcription initiation by Pol III requires the trimeric general transcription factor TFIIB (Fig. 5.13a–c), which is sufficient for Pol III transcription initiation *in vitro* (Kassavetis et al. 1990, 1999). TFIIB is formed by TBP, TFIIB-related factor 1 (Brf1), and the SANT-domain containing protein B-double prime 1 (Bdp1) (Colbert and Hahn 1992; Ishiguro et al. 2002; Kassavetis et al. 1995; Lobo et al. 1992; López-De-León et al. 1992; Schramm and Hernandez 2002; Schramm et al. 2000; Wang and Roeder 1995). In addition to Brf1, vertebrates encode a second TFIIB-related subunit, termed Brf2 (Cabart and Murphy 2001; Mital et al. 1996; Schramm et al. 2000; Teichmann et al. 2000), which acts at certain promoters, including the U6 small nuclear RNA (snRNA) promoter, characterized by a

strong TATA-box and an upstream proximal sequence element (PSE) that is recognized by the SNAPc complex (Henry et al. 1995; Sadowski et al. 1993). TFIIB not only functions in recruitment of Pol III to the promoter, but also supports initial promoter melting and extension of the open bubble via its Bdp1 and Brf1 subunits, respectively (Kassavetis et al. 2001).

The N-terminal halves of Brf1 and Brf2 are structurally homologous to TFIIB (Fig. 5.13a), contributing to the conservation of the overall architecture of the Pol II and Pol III-PICs (López-De-León et al. 1992; Vannini and Cramer 2012; Wang and Roeder 1995), while the C-terminal half includes a Brf-specific domain that forms tight and specific interactions with TBP (Juo et al. 2003; Khoo et al. 1994). The crystal structure of a Brf2-TBP-DNA complex (Gouge et al. 2015) shows that TBP engages DNA in the same way as in the TFIIB-TBP-DNA complex (Nikolov et al. 1995; Tsai and Sigler 2000) and induces the same strong bend at the TATA box (Gouge et al. 2015; Nikolov et al. 1996) (Fig. 5.13a). Even though details differ, the interactions between DNA and the regions conserved between Brf2 and TFIIB are similar overall, including contacts of the cyclin domains with the promoter DNA immediately upstream and downstream of the TATA box (Gouge et al. 2015; Nikolov et al. 1995; Tsai and Sigler 2000). The Brf2 C-terminal region, however, is unique, and contains three architectural regions, termed arch, anchor, and molecular pin (Gouge et al. 2015) (Fig. 5.13a). The molecular pin harbors a conserved LPPC-motif and binds to a ternary interface between the Brf2-cyclin domain, TBP, and the DNA (Fig. 5.13d). Interestingly, the cysteine in the molecular pin allows Brf2 to serve as a redox-sensing transcription factor that links oxidative stress to cellular responses, including apoptosis, because oxidation of the cysteine impairs complex assembly and leads to down-regulation of survival-promoting genes (Gouge et al. 2015).

The third component of TFIIB, Bdp1, is specific to the Pol III system and contributes to an extremely tight interaction of TFIIB with promoter DNA (Kassavetis et al. 1990, 2005; Shah et al. 1999). The crystal structure of a TFIIB (TBP-Brf2-Bdp1)-DNA complex (Fig. 5.13b, c) shows that the Bdp1 SANT domain plays a key role in association of the protein with the remainder of the complex and binds to a location similar to that of TFIIA in the Pol II system (Bleichenbacher et al. 2003; Gouge et al. 2017; Tan et al. 1996).

Once assembled on the promoter, TFIIB binds very stably (Kassavetis et al. 1990) and remains bound even after Pol III starts elongating, allowing for efficient Pol III recycling and high rates of transcription initiation on Pol III promoters (Dieci et al. 2013; Dieci and Sentenac 1996). Indeed, the TFIIB association is so stable that it serves as a roadblock that impedes pervasive Pol II transcription and leads to dissociation of lagging strand replicative polymerases (Roy et al. 2016; Smith and Whitehouse 2012).

As detailed above, TFIIB is the key transcription factor in the Pol III system. Two additional transcription factors, TFIIA and TFIIC are assembly and specificity factors that aid in positioning of TFIIB on the upstream promoter DNA (Kassavetis et al. 1990; Roberts et al. 1995). TFIIC a hexameric 0.5 MDa protein complex that binds to intragenic promoter elements (Conesa et al. 1993; Ducrot et al. 2006; Male et al. 2015; Stillman and Geiduschek

1984), but is dispensable after TFIIB binding and is displaced by transcribing Pol III (Bardeleben et al. 1994).

The Structure of the RNA Polymerase III Pre-initiation Complex—Three independent cryo-EM studies (Abascal-Palacios et al. 2018; Han et al. 2018; Vorländer et al. 2018) have revealed the mechanism of Pol III-PIC assembly and promoter opening at high resolution. Two of these structures were determined on a U6 snRNA promoter (Abascal-Palacios et al. 2018; Vorländer et al. 2018), one of them on the asparagine-tRNA promoter (Han et al. 2018). These studies used promoter DNA substrates mimicking closed and open Pol III-PICs as well as Pol III-ITCs (Abascal-Palacios et al. 2018; Han et al. 2018; Vorländer et al. 2018). Interestingly, it was found that the open Pol III-PIC spontaneously forms even from fully base-paired templates (Abascal-Palacios et al. 2018; Han et al. 2018; Vorländer et al. 2018) and even in presence of mutants deficient in promoter melting (Abascal-Palacios et al. 2018; Han et al. 2018). The RNA substrate was lost from the Pol III-ITC under some conditions, either due to washing or nucleolytic cleavage aided by the Pol III subunit C11 (Han et al. 2018; Vorländer et al. 2018), and the Pol III-OC and ITC complexes exhibited similar architecture (Han et al. 2018).

The cryo-EM reconstructions comprise Pol III, TFIIB, and promoter DNA, and show TFIIB tightly bound to and wrapped around the upstream DNA (Fig. 5.14a). Similarly to the Brf2-containing structure (Gouge et al. 2015, 2017), the cyclin folds of Brf1, present in the cryo-EM sample as a Brf1-TBP fusion protein (Kassavetis et al. 2005), bind to TBP and promoter DNA upstream and downstream of TBP (Figs. 5.13a, 5.14b). The SANT domain of Bdp1 binds TBP, DNA, and Brf1, leading to a highly stable assembly of TFIIB on the upstream promoter DNA, suitable for supporting multiple rounds of initiation by Pol III (Dieci et al. 2013; Dieci and Sentenac 1996).

In the open Pol III-PIC, Pol III itself exhibits a closed clamp conformation and tightly stabilizes the open DNA bubble (Abascal-Palacios et al. 2018; Han et al. 2018; Vorländer et al. 2018). Pol III subunits C37, C34 and C31, all of them part of the “built-in transcription factors” of Pol III are flexible in the structure of transcribing Pol III (Hoffmann et al. 2015), but ordered in the open PIC (Fig. 5.14a). Bound to the upstream promoter DNA, TFIIB sits above the Pol III cleft, where its Bdp1 and Brf1 subunits form several contacts with Pol III. Due to these contacts, parts of the general transcription factor subunits that are not visualized in the crystal structures (Gouge et al. 2015, 2017) are visible in the cryo-EM reconstructions (Abascal-Palacios et al. 2018; Han et al. 2018; Vorländer et al. 2018).

In Brf1, the N-terminal zinc-ribbon and the cyclin folds contact the Pol III dock, wall, and protrusion, similar to the TFIIB contacts observed in the Pol II system (Fig. 5.14c, d) (He et al. 2016; Murakami et al. 2015; Nikolov et al. 1995; Plaschka et al. 2016; Tsai and Sigler 2000). The zinc-ribbon is inserted through the active site cleft and contacts the Pol III dock domain (Fig. 5.14c), and an adjacent linker region interacts with the template DNA strand (Abascal-Palacios et al. 2018; Han et al. 2018; Vorländer et al. 2018). The zinc-ribbon interaction is also found in the Pol I system (Engel et al. 2017; Han et al. 2017; Sadian et al. 2017), while the interaction of the N-terminal cyclin fold with the wall and protrusion (Fig. 5.14c, d) is restricted to Pol II and Pol III.

Notably, Brf1 includes a structural element termed the helical pin, structurally homologous to the Brf2 molecular pin and occupying the same site at the interface between TBP, DNA, and the Brf1 cyclin folds (Gouge et al. 2017), but without the redox-sensing activity of its Brf2 counterpart (Abascal-Palacios et al. 2018; Han et al. 2018).

The extended SANT domain and a linker of Bdp1 bind to the major and minor grooves of the DNA, as visualized previously (Gouge et al. 2017). Additionally, several long helices of Bdp1 are seen in the cryo-EM maps (Fig. 5.14a), reaching across the Pol III C34 subunit (part of the TFIIE/TFIIF-like Pol III subunits), and together with C37 (part of the TFIIF-like Pol III subunits) form a platform that stabilizes C34 (Abascal-Palacios et al. 2018; Han et al. 2018; Vorländer et al. 2018). Notably, these interactions between Bdp1 and C34-C37 also lead to the stabilization of the initiation/termination loop of C37 (C37 residues 211–224) (Kassavetis et al. 2010; Rijal and Maraia 2013; Wu et al. 2011) by interactions with part of a Bdp1 region termed the “tether” (Bdp1 residues 360–398). After stabilization by the C37-Bdp1 platform, winged-helix domain 2 in C34 contacts the open transcription bubble near its upstream edge (Fig. 5.14b), implicating C34 in promoter melting or stabilization of the open bubble (Abascal-Palacios et al. 2018; Brun et al. 1997; Han et al. 2018; Vorländer et al. 2018). Bdp1 mutants that are defective in open complex formation (Kassavetis et al. 2001) map to this platform region, underscoring the importance of the molecular arrangement in this region for promoter opening (Abascal-Palacios et al. 2018).

The cryo-EM maps visualized the interactions that contribute to melting and stabilization of the transcription bubble at the active site. In the Pol III-PIC, the active site assumes a conformation that is reminiscent of elongating Pol III (Hoffmann et al. 2015), with a disordered trigger loop and rudder, and a bent bridge helix. Upon transition to the ITC, the upstream bubble edge is stabilized mostly by contacts from Pol III subunits, including the C34 and C82 winged helix domains and the C160 clamp, as well as the N-terminal Brf1 cyclin fold (Abascal-Palacios et al. 2018; Han et al. 2018; Vorländer et al. 2018). The arrangement of TFIIB elements, with the Brf1 N-terminal zinc-ribbon, the adjacent linker, and the N-terminal cyclin fold close to the transcription bubble, and with Bdp1 stabilizing C34 (Abascal-Palacios et al. 2018; Han et al. 2018; Vorländer et al. 2018), explains the dual function of TFIIB in promoter melting (Kassavetis et al. 2001).

Model for Transcription Initiation by RNA Polymerase III and Transition to Elongation—The structural results on the Pol III-PIC described in the previous section, combined with structures of apo-Pol III and transcribing Pol III (Hoffmann et al. 2015), suggest a model for Pol III transcription initiation. Initially, Pol III has a closed clamp, as visualized in the closed Pol III-PIC complex, with disordered downstream DNA projecting away from the polymerase (Vorländer et al. 2018). This is in contrast to e.g. the closed Pol II-PIC, where the DNA runs along the length of the Pol II active site cleft and interacts with the jaws at the downstream end (He et al. 2013, 2016; Murakami et al. 2015). The transcription factor-like modules of Pol III that later become stabilized, as well as TFIIB elements interacting with them, are initially partially disordered in closed Pol III-PIC (Vorländer et al. 2018). Upon insertion of promoter DNA into the cleft by TFIIB and the consequent Pol III cleft closure, TFIIB stabilizes the winged-helix domains of C34 above the cleft, leading to entrapment of the promoter DNA and initial promoter melting.

Subsequently, the Brf1 zinc-ribbon and an adjacent linker region become stabilized, with the linker region contacting the opening bubble and facilitating bubble extension, leading to an ITC and eventually an elongating state (Abascal-Palacios et al. 2018; Han et al. 2018; Vorländer et al. 2018).

As detailed above, TFIIB interacts with promoter DNA very stably and may participate in the Pol III transcription re-initiation pathway (Dieci et al. 2013; Dieci and Sentenac 1996; Kassavetis et al. 1990). An interesting and unexpected finding was the visualization of a transcribing Pol III complex that is still attached to promoter-bound TFIIB, with up to 33 bp of melted DNA accommodated by Pol III (Han et al. 2018). Based on these findings, and the fact that most Pol III-transcribed genes are short, it was proposed that Pol III may stay associated with TFIIB throughout the entire transcription cycle (Han et al. 2018).

Comparison of PIC Architectures

Conserved and Divergent Features of Eukaryotic Pre-initiation Complex Architectures

Now that detailed structures of pre-initiation complexes of Pol I, II, and III have been determined, it is possible to compare the shared and unique features of these three polymerase systems on an architectural level.

Universality of TFIIB-like Factors and Positioning of the Promoter DNA in the PIC—TFIIB-like factors play a critical role in all three eukaryotic RNA polymerase systems discussed in this chapter (see e.g. Figures 5.6, 5.7, 5.10, 5.11, 5.13, 5.14). While Pol II employs TFIIB itself, the Pol I core factor harbours the TFIIB homolog Rrn7, and the Pol III initiation factor TFIIB contains the TFIIB-related factors Brf1 or Brf2 (Fig. 5.15a–c). The structures of the Pol II and Pol III-PICs (Abascal-Palacios et al. 2018; Han et al. 2018; He et al. 2016; Plaschka et al. 2016; Vorländer et al. 2018) have revealed that in spite of the presence of additional domains in the Pol III initiation factors Brf1/Brf2, the general architecture and role of TFIIB and Brf1/Brf2 are conserved, with all of them forming similar interactions with the upstream promoter DNA and the polymerase, including interactions of the cyclin domains with the polymerase wall and the positioning of the zinc-ribbon domain at the polymerase dock and the adjacent linker in proximity of the transcription bubble (Figs. 5.14c, d, 5.15b, c). The structure of the Pol I-PIC revealed that the zinc-ribbon of Rrn7 assumes a similar position as its TFIIB and Brf1/Brf2 counterparts. However, in spite of the conservation of the two cyclin folds that lie at the core of all TFIIB-related initiation factors, the Rrn7 cyclin folds interact differently with both the upstream promoter DNA (Fig. 5.15a–c) and the RNA polymerase, as compared to TFIIB and Brf1/Brf2 (Fig. 5.15d–f).

As a consequence of this difference in PIC architecture, the path of the DNA differs between the Pol I-PIC on the one hand and the Pol II- and Pol III-PICs on the other (Engel et al. 2017, 2018; Han et al. 2017; Sadian et al. 2017). In the Pol I-ITC, the promoter DNA is accommodated in the active site cleft very similarly as in the Pol I elongation complex, likely assuming such a conformation as early as during closed complex formation. In contrast, in both the Pol II- and Pol III-PICs, the upstream promoter DNA in the closed complex is suspended high above the active site cleft, remains in this conformation during ITC formation, and rearranges to its final trajectory only in the elongation complex (Fig.

5.11c) (Abascal-Palacios et al. 2018; Han et al. 2018; He et al. 2016; Plaschka et al. 2016; Vorländer et al. 2018).

Built-in General Transcription Factor-like Subunits in Pol I and Pol III—Pol II is a 12-subunit enzyme, while Pol I has 14 subunits, and Pol III comprises 17 subunits (see Fig. 5.1). As noted above, the supernumerary subunits in Pol I and Pol III share structural and functional similarity to general transcription factors of the Pol II system. Specifically, the location of the Pol I A49-A34.5 and Pol III C53-C37 heterodimers is highly similar to the binding site of TFIIF on the Rpb2 lobe in the Pol II-PIC (Fig. 5.12). This suggests structural and functional overlap with TFIIF, even though A49 in the Pol I system also contains a tandem winged helix domain reminiscent of TFIIE in terms of its DNA interactions (Khatter et al. 2017; Tafur et al. 2016; Vannini and Cramer 2012). Notably, TFIIF is thought to arrive to the Pol II-PIC pre-bound to Pol II (Killeen and Greenblatt 1992; Rani et al. 2004; Roeder 1996), suggesting a relatively straightforward evolutionary path to how its Pol I and Pol III equivalents became incorporated into the multisubunit polymerase (Khatter et al. 2017). In spite of these similarities, there are also differences. The C53-C37 dimer in Pol III is involved not only in initiation, but also in termination, enabling Pol III to terminate autonomously upon encountering the poly-thymidine stretch that serves as the termination signal in this system (Khatter et al. 2017; Landrieux et al. 2006; Rijal and Maraia 2013).

The second polymerase subassembly that shows similarity to Pol II transcription factors is the Pol III C82-C34-C31 trimer. Similarly to TFIIE, the C82-C34-C31 trimer binds to the clamp head (Fig. 5.12b–d) and may contact and stabilize the transcription bubble (Vorländer et al. 2018). At the same time, the C34 winged helix domains 1 and 2 bind in a location that is highly similar to the TFIIF β (yeast Tfg2) winged helix domain (Fig. 5.7g, h), indicating that the C82-C34-C31 may be a functional fusion of TFIIE and TFIIF (Vorländer et al. 2018).

Pausing of elongating Pol II can lead to backtracking, at which stage the 3'-end of the nascent mRNA leaves the active site and is extruded through a tunnel in the enzyme (Cramer et al. 2000; Gnatt et al. 2001). At this point, critical elements of the Pol II active centre are trapped in inactive conformations and the nascent RNA can no longer be elongated (Cheung and Cramer 2011; Kettenberger et al. 2003), unless the polymerase either advances to place the 3'-end in the active site again, or the transcript is cleaved. In the Pol II-system, TFIIS aids in transcript cleavage and permits resumption of elongation (Izban and Luse 1992; Reines 1992; Wang and Hawley 1993). While the intrinsic Pol II nuclease activity is weak and requires enhancement by TFIIS, the Pol I and Pol III systems harbour strong intrinsic nuclease activity, residing in a TFIIS-like extension domain of their A12.2 and C11 subunits (Ruan et al. 2011; Vannini and Cramer 2012), which for the Pol III system has also been implicated in the Pol III re-initiation pathway (Iben et al. 2011; Khatter et al. 2017). Lacking or unexpectedly weak density for the product RNA in some cryo-EM reconstructions of Pol I and Pol III complexes that were formed with a synthetic DNA bubble scaffold and bound RNA may be due to cleavage of initially bound RNA by intrinsic nuclease activity (Han et al. 2018; Sadian et al. 2017; Vorländer et al. 2018).

Opening of the Transcription Bubble

The Pol II system is unique in its ability to exploit the activity of a DNA-dependent ATPase, XPB within TFIID, for opening of the transcription bubble. Bacterial RNA polymerases and eukaryotic Pol I and Pol III are able to independently melt the promoter to form open PICs, as strikingly illustrated by structural studies in which attempted structure determination of closed complexes resulted in the formation of open complexes (Abascal-Palacios et al. 2018; Engel et al. 2017; Han et al. 2018; Vorländer et al. 2018). Recent structural, biochemical, and computational analyses (Alekseev et al. 2017; Dienemann et al. 2018; Plaschka et al. 2016) suggest that at least under some circumstances, Pol II is also able to spontaneously melt the promoter, suggesting a universally conserved mechanism of promoter opening in multi-subunit RNA polymerases.

Open Complex Formation in Yeast—For the yeast Pol II transcription initiation system, a reconstitution of the yeast Pol II core-PIC using double stranded DNA for cryo-EM analysis resulted in an open complex (Plaschka et al. 2016). A subsequent study showed that in yeast, there are two distinct promoter classes concerning the requirement for TFIID; one promoter class can be melted spontaneously without contribution from TFIID, while the second class benefits more strongly from the presence of TFIID (Dienemann et al. 2018). The two promoter classes seem to be distinguished by subtle differences in their free energy of melting. Interestingly, the *HIS4* promoter used for the structural analysis that resulted in spontaneous open-complex formation (Plaschka et al. 2016) belongs to the first, spontaneously melting class. A follow-up experiment using the more highly TFIID-responsive *GAT1* promoter resulted in structure determination of both open and closed core-PICs, indicating that indeed, promoter melting is less energetically favorable compared to *HIS4* (Dienemann et al. 2018). The DNA in one of these closed complexes assumes a different, more highly distorted and more deeply inserted conformation compared to previously determined closed PICs (Dienemann et al. 2018) and may represent an initiation intermediate. This DNA conformation is observed both in the presence and absence of TFIID.

The Mammalian RNA Polymerase System—Whether these findings can be applied to the human Pol II system is not fully established. Critically, the closed Pol II-PIC could be successfully reconstituted and structurally characterized using human proteins (He et al. 2016). However, experiments in human cells where XPB was depleted using spironolactone indicate that XPB depletion predominantly affects DNA repair, while global transcription is less affected. In contrast, when XPB is present, but its enzymatic activity is inhibited by triptolide, global transcription is reduced (Alekseev et al. 2017). A hypothesis that is consistent with these observation is that XPB may, at least in some cases, act in a checkpoint-like manner. According to this model, inactive XPB binds to the downstream promoter DNA and prevents its insertion into the Pol II active site. Activation of the enzymatic DNA translocase activity of XPB—or alternatively, its depletion—would allow the downstream promoter DNA to reach the active site of Pol II and promoter melting would occur driven by binding energy alone (Alekseev et al. 2017).

Further analysis will be required to establish whether spontaneous melting of Pol II promoters is a general feature and occurs in vivo, consistent with a universally applicable mechanism for melting of promoters by multisubunit polymerases, or if higher eukaryotes have indeed evolved a unique, TFIIF-dependent solution, possibly to allow for more precise regulation of gene expression.

Conclusion

Recent breakthroughs in the structure determination of transcriptional assemblies have revealed the detailed structures of the PICs of Pol I, Pol II, and Pol III. These structures uncovered the intricate architecture of these molecular machines in atomic detail, and have provided valuable mechanistic insight into the process of PIC assembly, transcription bubble opening, and initiation of transcription. This progress has been critically enabled by technical advances in cryo-EM, which allowed the study of highly complex but low-abundance and conformationally heterogeneous molecular assemblies at ever-higher resolution.

Future progress will likely involve the reconstitution and visualization of even larger complexes, in the presence of gene-specific transcription factors, and in the context of chromatin.

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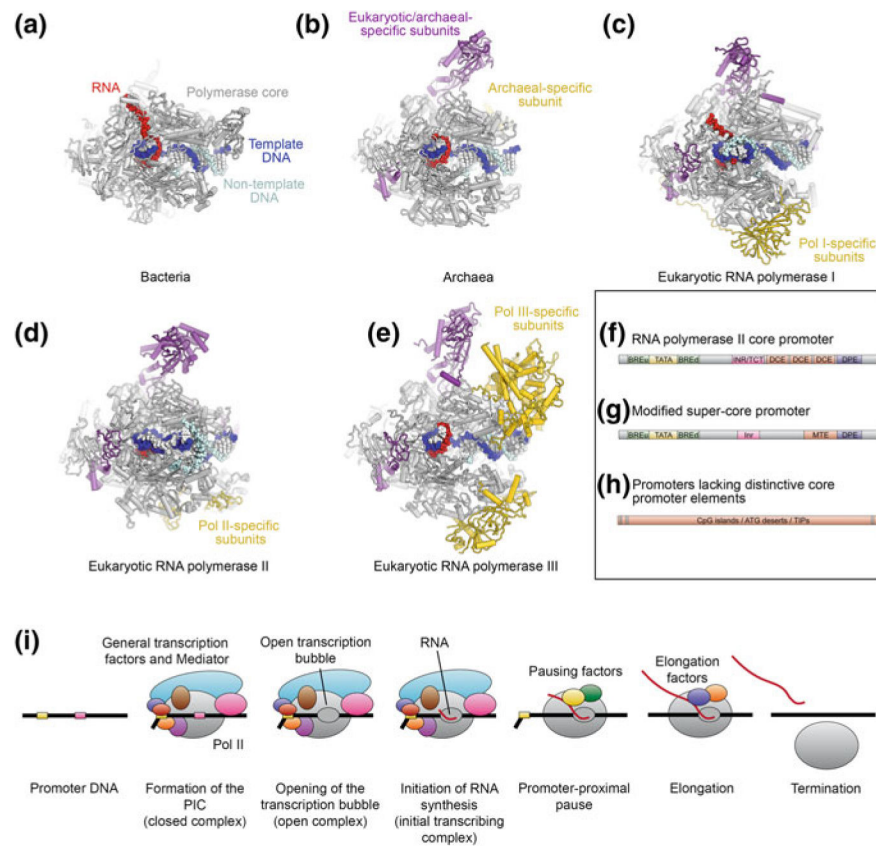


Fig. 5.1. Architecture of multisubunit RNA polymerases across kingdoms and organization of eukaryotic Pol II promoters. **a–e** Depictions of transcribing multisubunit RNA polymerases. Subunits occurring only in eukaryotes or archaea are colored purple, subunits specific to polymerase type are colored yellow. **a** Elongating bacterial RNA polymerase (Vassilyev et al. 2007). **b** Archaeal RNA polymerase (Korkhin et al. 2009), DNA superposed from (Vassilyev et al. 2007). **c** Elongating yeast Pol I (Tafur et al. 2016). **d** Elongating fungal Pol II (Ehara et al. 2017). **e** Elongating yeast Pol III (Hoffmann et al. 2015). **f–h** Organization of eukaryotic Pol II core promoters. **f** Idealized Pol II core promoter elements. Abbreviations: TATA, TATA-box; INR, initiator; TCT, initiator like element present in ribosomal protein coding genes; DCE, downstream core element; DPE, downstream promoter element (Lenhard et al. 2012; Roy and Singer 2015). **g** Modified super core promoter used for structural studies of the human Pol II-PIC and TFIID. The upstream and downstream TFIIB-response elements BREu (Lagrange et al. 1998) and BREd (Deng and Roberts 2005) were added to the super core promoter (Juven-Gershon et al. 2006), which comprises TATA-box (Gannon et al. 1979), Inr (Chen and Struhl 1985), MTE (Lim et al. 2004), and DPE (Burke and Kadonaga 1996, 1997). **h** Many eukaryotic Pol II promoters lack these distinct promoter elements and contain longer DNA segments harbouring CpG islands, ATG deserts, or transcription initiation platforms (TIPs) instead (Roy and Singer 2015). **i** Simplified schematic of the Pol II transcription cycle from PIC assembly to transcription termination

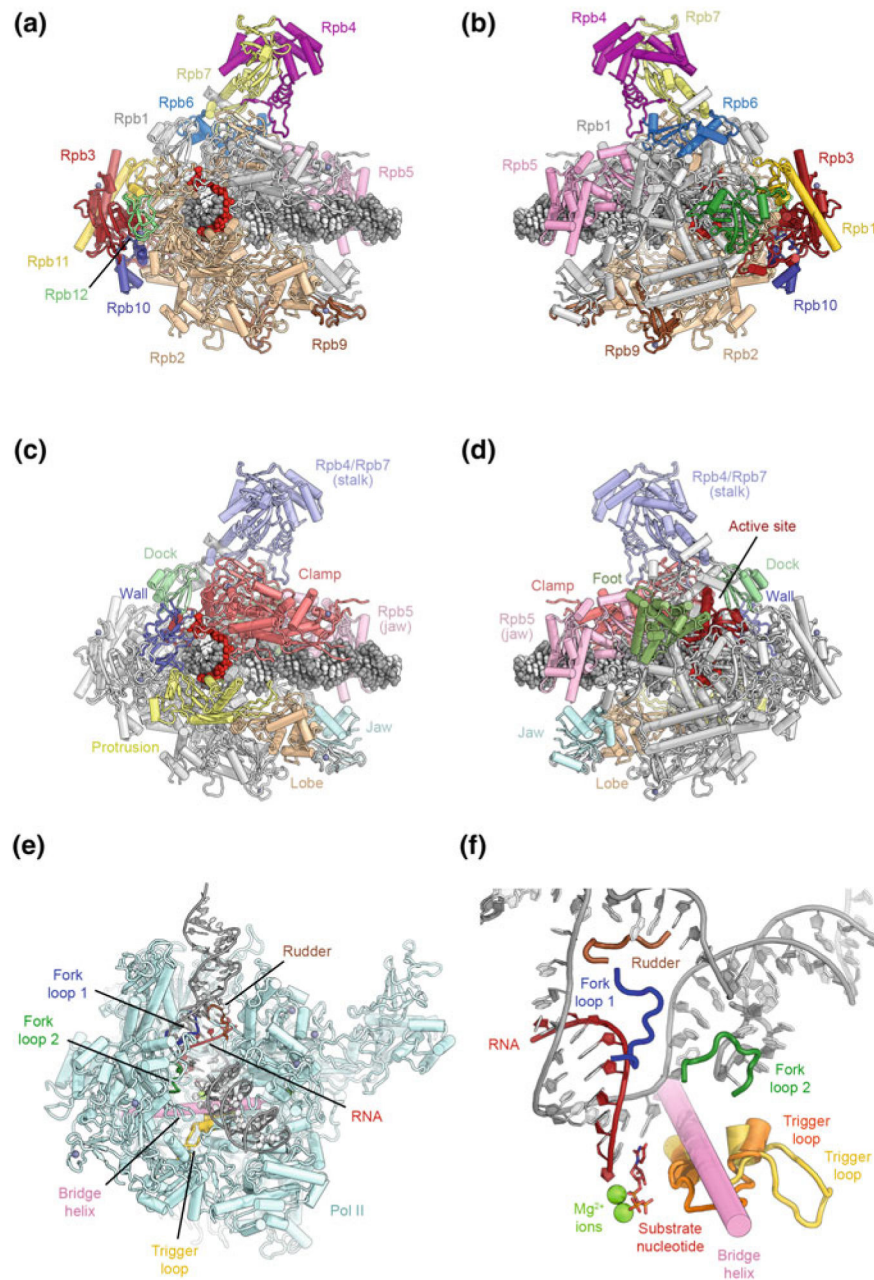
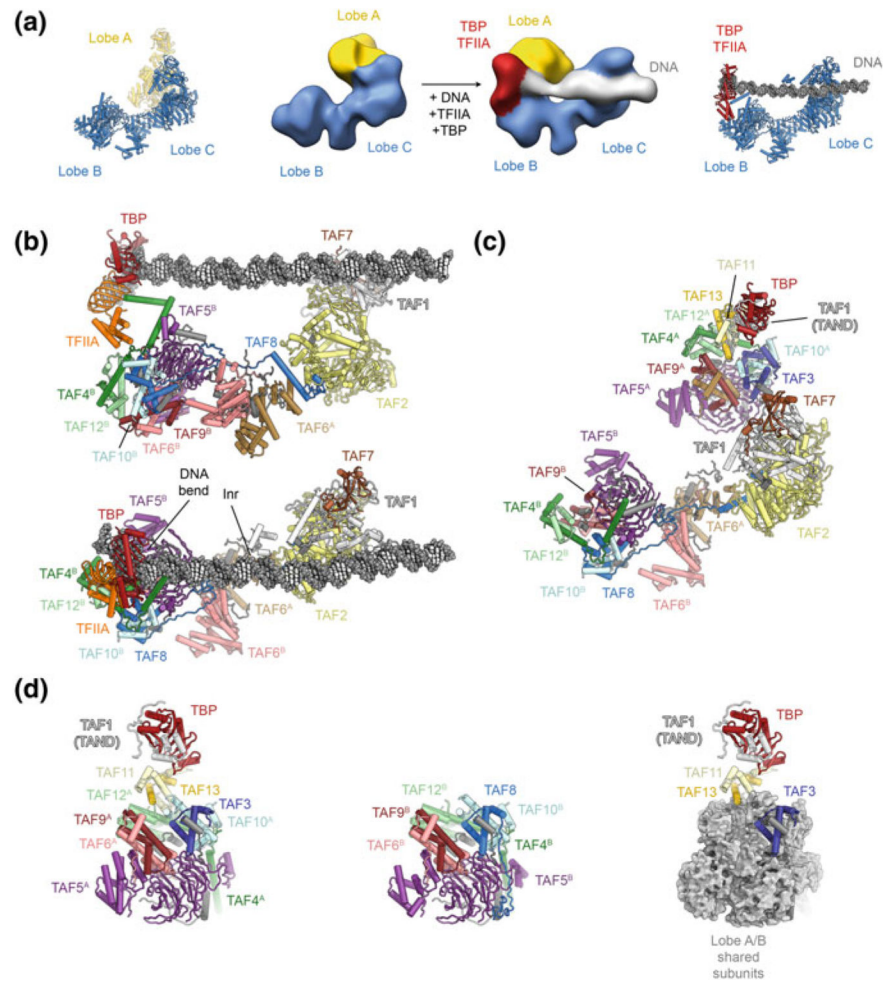
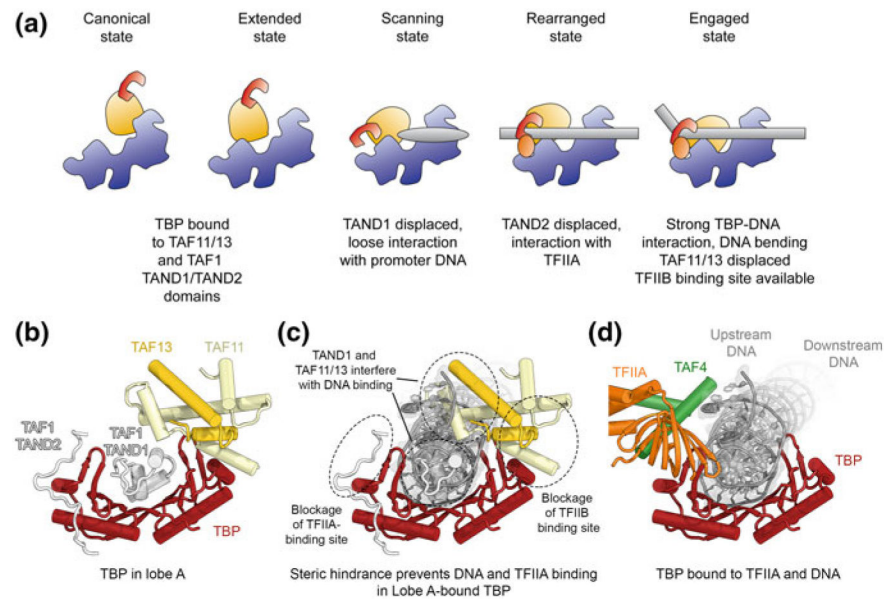


Fig. 5.2. Architecture of eukaryotic RNA polymerase II. **a, b** Depiction of transcribing Pol II extracted from the structure of an elongation complex (Ehara et al. 2017), shown from the front and back and colored by protein subunits. **c, d** Structural domains of the Pol II subunits Rpb1 and Rpb2 are highlighted in color according to (Cramer et al. 2001). Additional structural landmarks mentioned in the text are highlighted as well (protein names are indicated). **e** View of the transcription bubble at the Pol II active site (Barnes et al. 2015). Key structural and functional elements near the active site that are mentioned in the text are indicated. **f** Detailed view of the Pol II active site (Barnes et al. 2015; Wang et al. 2006). Two conformations of the trigger loop are shown in orange and yellow

**Fig. 5.3.**

The structure of human TFIID. **a** Organization of human TFIID into three lobes. Early cryo-EM maps (Cianfrocco et al. 2013) that revealed the tri-lobal overall structure of TFIID are shown in the center, with recent coordinate models (Patel et al. 2018) shown next to them for comparison. **b** Structure of TFIID bound to DNA and TFIIA (Patel et al. 2018) in side view (top) and top view (bottom). Protein subunits are shown in colour and labelled; the TBP-induced DNA bend and the location of the transcription start site (Inr) are labeled. **c** Structure of TFIID in the canonical state (Patel et al. 2018) shown in the same orientation as bottom panel in (b). **d** Composition of lobes A and B. Left: lobe A. Center: lobe B. Right: Subunits shared between lobes A and B are shown as grey surface to highlight the subunits in lobe A that distinguish between lobes A and B

**Fig. 5.4.**

Proposed conformational transitions of TFIID during DNA binding and mechanism of TBP deposition. **a** Five different conformational states (canonical, extended, scanning, rearranged, and engaged state) of TFIID as identified by cryo-EM analysis of a mixed dataset (Patel et al. 2018). For each state, a schematic depiction and the interaction environment of TBP, which is initially bound to TFIID lobe A and eventually deposited onto upstream promoter DNA in a stably bound manner, is provided. **b** TBP in lobe A is contacted by the TAF11-TAF13 dimer and the TAND1 and TAND2 domains of TAF1 (Anandapadamanaban et al. 2013; Patel et al. 2018). **c** TAF11-TAF13 and the TAF1 TAND1 and TAND2-domains block DNA- and transcription factor-binding sites on TBP. **d** TBP bound to and bending the DNA in the context of the engaged state of DNA-bound TFIID (Patel et al. 2018)

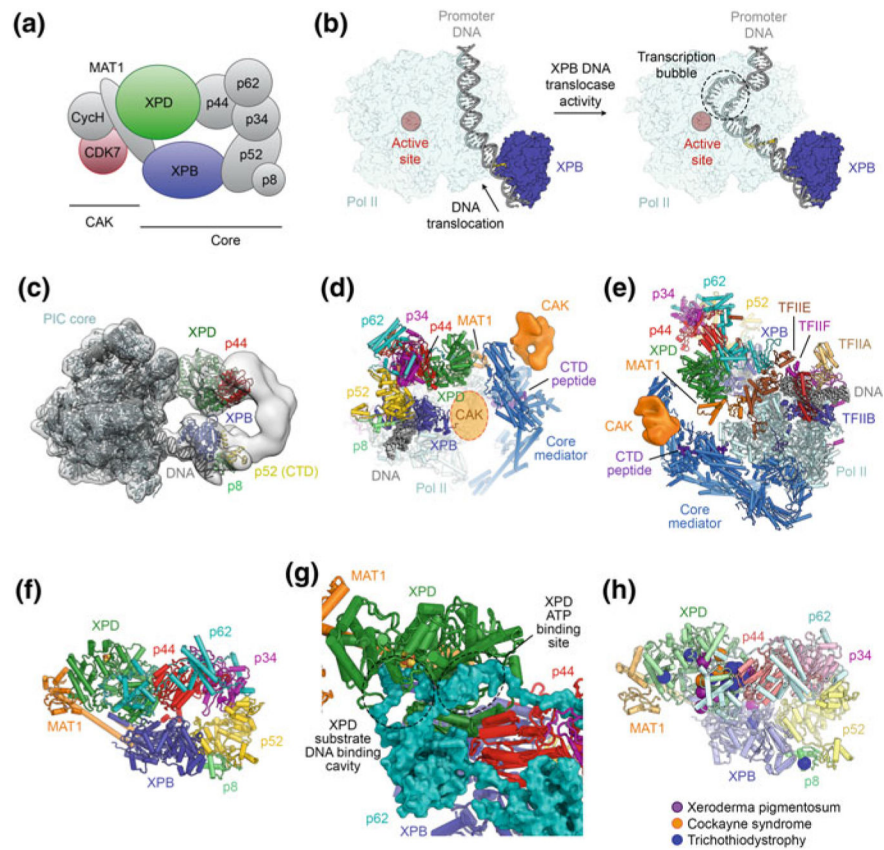
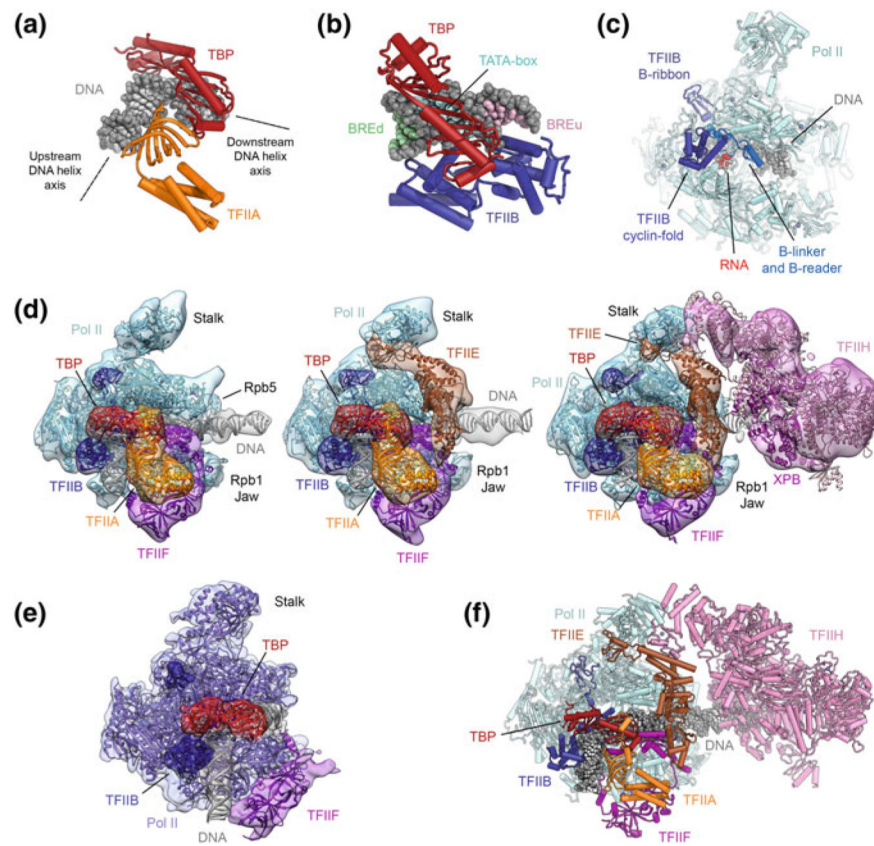


Fig. 5.5. Structure and function of TFIIH. **a** Schematic of TFIIH architecture. Main enzymatic subunits are shown in color (XPD and XPB, SF2-family DNA-dependent ATPases; CDK7, Cyclin-dependent kinase). Core and CAK subcomplexes are indicated. **b** Model for the activity of the TFIIH subunit XPB during promoter opening; depiction based on the structures in (He et al. 2016). **c** Cryo-EM reconstruction of the human Pol II-PIC (He et al. 2016) with fitted coordinate models. TFIIH subunits are highlighted in color. **d, e** Relocalization of the CAK kinase-cyclin module in the Mediator-bound Pol II-PIC (Schilbach et al. 2017): The cryo-EM volume corresponding to the CAK in the Mediator-containing PIC is shown in orange. The presumed location of the CAK in the absence of Mediator is indicated by an orange ellipse. **f** Structure of the TFIIH core complex with bound MAT1 (Greber et al. 2019). **g** Interactions of p62 with the ATP- and DNA-binding sites of XPD. **h** Mapping of human disease mutations onto the structure of TFIIH (Clever et al. 1999; Greber et al. 2019)

**Fig. 5.6.**

The structure of the Pol II-PIC and its components. **a** Structure of promoter DNA bound to TBP and TFIIA (Bleichenbacher et al. 2003). TBP introduces a pronounced bend into the TATA box (indicated by dotted black lines representing the upstream and downstream DNA helix axes). **b** Structure of promoter DNA bound to TBP and TFIIB (Tsai and Sigler 2000). The TATA box and regions corresponding to the BREd and BREu regions recognized by TFIIB are indicated by light blue, green, and pink shading of the DNA bases. **c** Structure of initially transcribing Pol II bound to TFIIB (Sainsbury et al. 2013). **d** Stepwise assembly of the human Pol II-PIC: Pol II-DNA-TBP-TFIIA-TFIIB-TFIIF (left), Pol II-DNA-TBP-TFIIA-TFIIB-TFIIF-TFIIIE (middle), and Pol II-DNA-TBP-TFIIA-TFIIB-TFIIF-TFIIIE-TFIIH (right), the latter showing a bilobal architecture. Depiction based on (He et al. 2013) with fitted coordinates from (Greber et al. 2019; He et al. 2016). **e** The structure of the yeast Pol II-ITC lacking TFIIH, TFIIA, and TFIIIE (Plaschka et al. 2015) is in good agreement with the human Pol II-PIC core (compare e.g. to leftmost complex in panel d). **f** Complete molecular structure of the human Pol II-PIC with TFIIH, assembled from coordinates for the human core Pol II-PIC and the structure of human TFIIH (Greber et al. 2019; He et al. 2016)

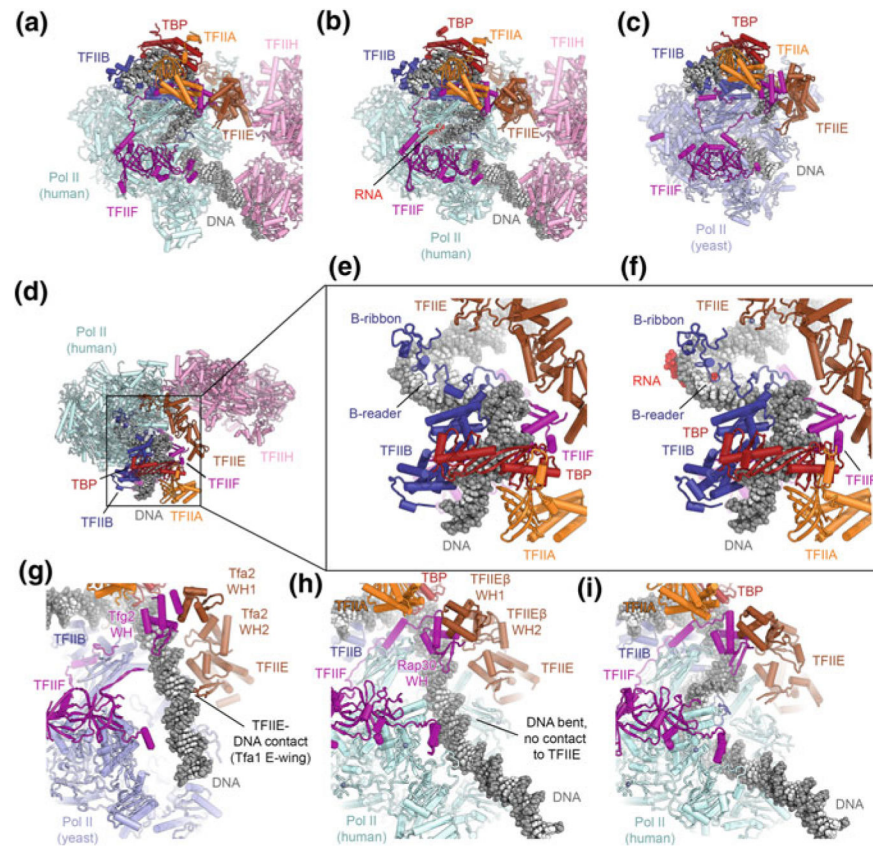
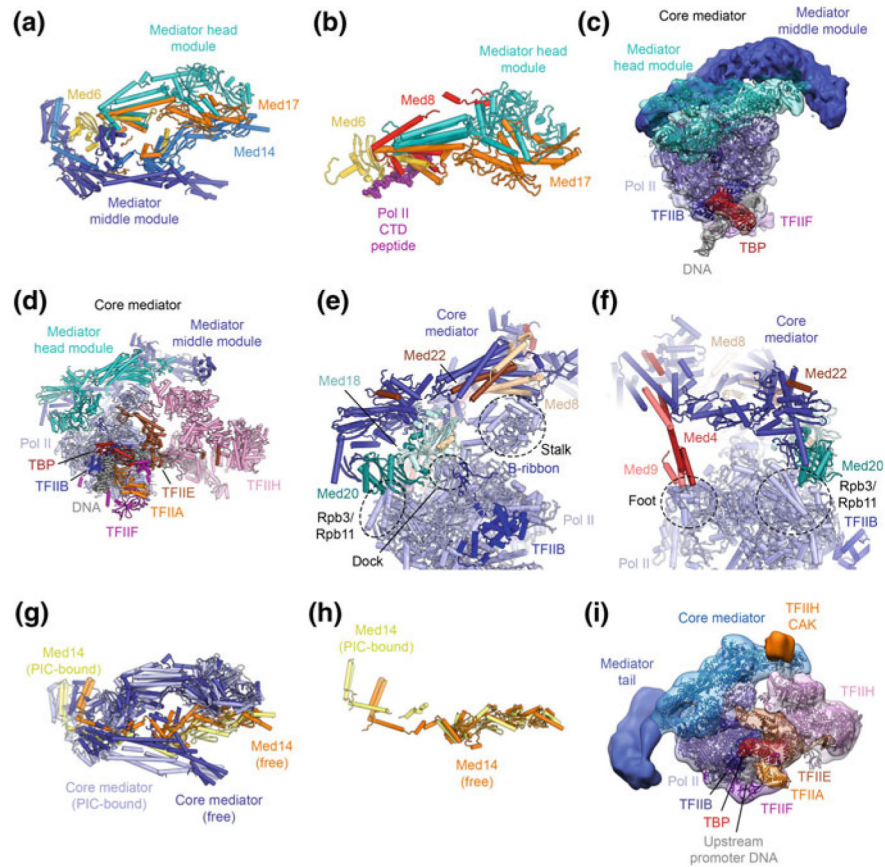


Fig. 5.7. Structure of the open and initially transcribing human Pol II-PICs and comparison with their yeast counterparts. **a** Structure of the open human Pol-II PIC (He et al. 2016). **b** Structure of the human Pol II-ITC (He et al. 2016). RNA near the Pol II active site is shown in red. **c** Comparison to the structure of the yeast open complex (Plaschka et al. 2016) reveals overall conservation of PIC architecture. **d–f** DNA interactions of TFIIB in the human Pol II open complex (**e**) and ITC (**f**) (He et al. 2016). **g** Interaction between the TFIIE E-wing domain and bound promoter DNA in the yeast closed PIC (Plaschka et al. 2016). **h** In the human Pol II-PIC (He et al. 2016), the closed promoter DNA is inserted more deeply into the Pol II cleft, and the TFIIE-DNA interaction visualized in yeast is not present. **i** Human Pol II open PIC (He et al. 2016)

**Fig. 5.8.**

The Mediator-bound Pol II-PIC. **a** Crystal structure of the Mediator head and middle modules (core Mediator) (Nozawa et al. 2017). **b** Crystal structure of the Mediator head module bound to a Pol II CTD peptide (Robinson et al. 2012). **c** Low-resolution cryo-EM structure of the core-Pol II-ITC bound to core Mediator (Plaschka et al. 2015). **d** Cryo-EM structure of the yeast Pol II-PIC, including TFIH, bound to core Mediator (Schilbach et al. 2017). **e, f** Contacts sites between core Mediator and Pol II, according to the structure shown in **(d)**. **g, h** Conformational changes in Mediator upon binding to the PIC **(g)** and associated with conformational changes in Med14 (Nozawa et al. 2017; Schilbach et al. 2017). **h** Low-resolution cryo-EM map of the yeast Pol-II PIC with Mediator (Robinson et al. 2016), including the Mediator tail module, fitted with the coordinates of the most recent yeast Pol II-core Mediator structure (Schilbach et al. 2017)

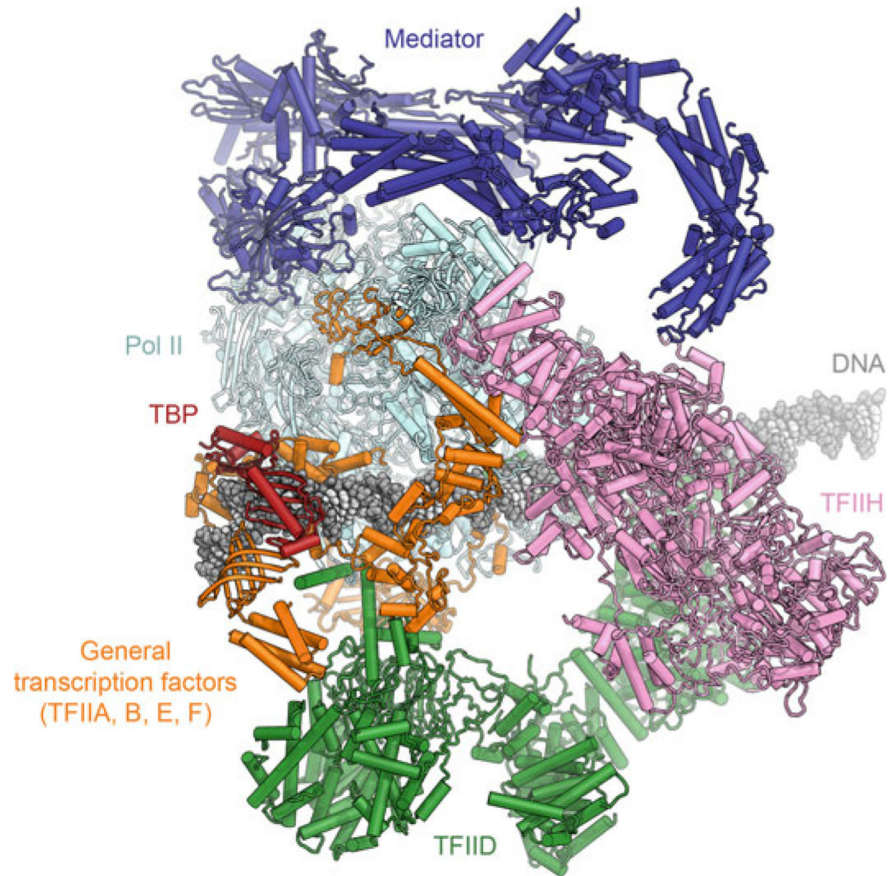
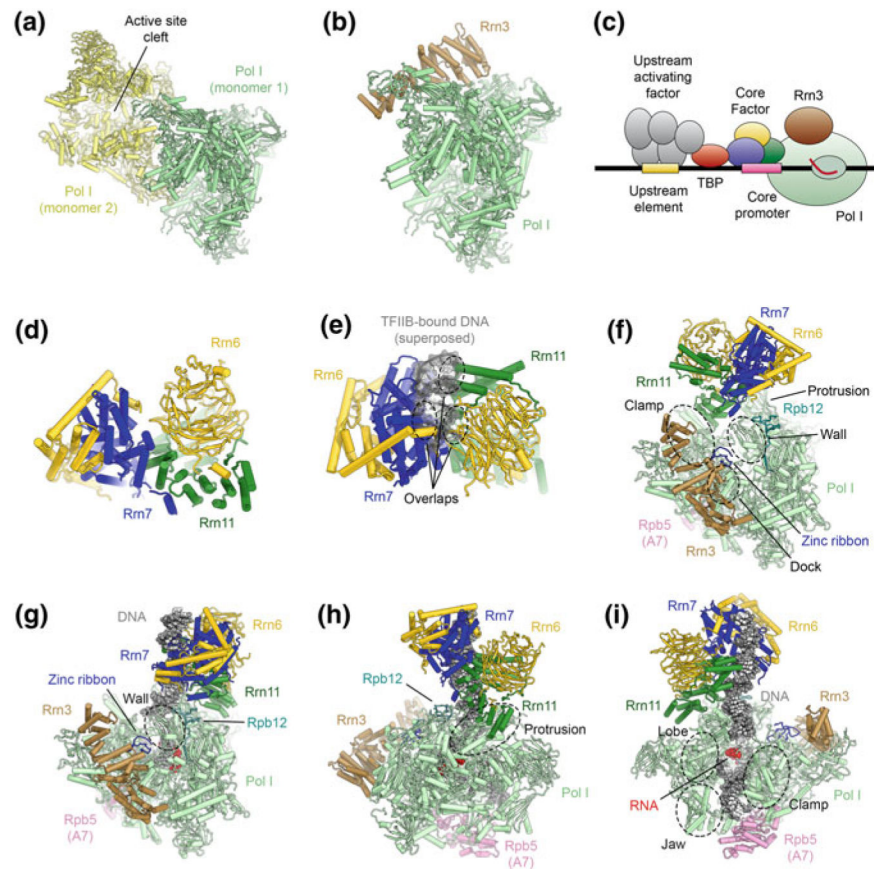


Fig. 5.9. Architectural model of the holo-Pol II-PIC with TFIID and Mediator. Proposed model of an entire Pol II-PIC, including Mediator and TFIID, generated by superimposing component structures (Greber et al. 2019; He et al. 2016; Patel et al. 2018; Schilbach et al. 2017)

**Fig. 5.10.**

The architecture of the RNA polymerase I PIC and ITC. **a** Structure of the Pol I-dimer (Engel et al. 2013), with a widened active site cleft, as observed in Pol I crystals. **b** Structure of Pol I bound with Rrn3 (Engel et al. 2016), which blocks the Pol I dimerization interface. **c** Schematic of the Pol I core promoter and general transcription factors in yeast. **d** Structure of yeast core factor (CF) (Engel et al. 2017). **e** Structure of yeast core factor shown with DNA superposed from a TFIIB-DNA structure (Tsai and Sigler 2000). The superposed DNA overlaps with CF, indicating that Rrn7 in CF cannot bind to DNA by the same mechanism as TFIIB (also see (Fig. 5.15)). **f** Structure of the Pol I-Rrn3-CF complex (Engel et al. 2017). Regions of Pol I that are in contact with CF are indicated. **g-i** Depiction of the Pol I-ITC, with bound DNA, Rrn3, and CF (Engel et al. 2017; Han et al. 2017). Contacts differ from the DNA-free complex (shown in **f**) and corresponding regions on Pol I are indicated. Downstream promoter DNA is stabilized by contacts from the Pol I clamp, lobe, and jaw domains and Rpb5

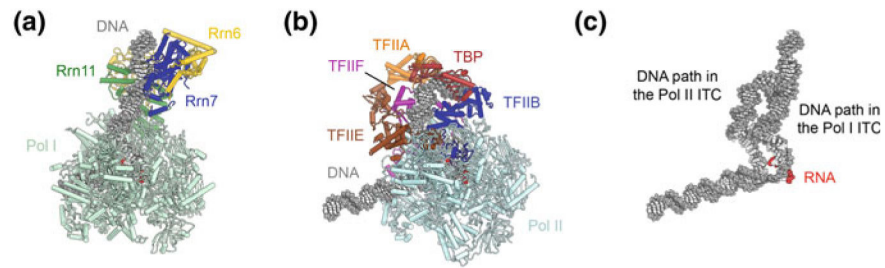


Fig. 5.11.

Comparison of DNA paths in the Pol I and Pol II ITCs. **a** Structure of the Pol I-ITC (Han et al. 2017). **b** Structure of the Pol II-ITC (He et al. 2016). **c** The path of the upstream promoter DNA is different between the Pol I and Pol II ITCs because of differences in the mode of binding of Rrn7 and TFIIIB to the DNA. See also Fig. 5.15

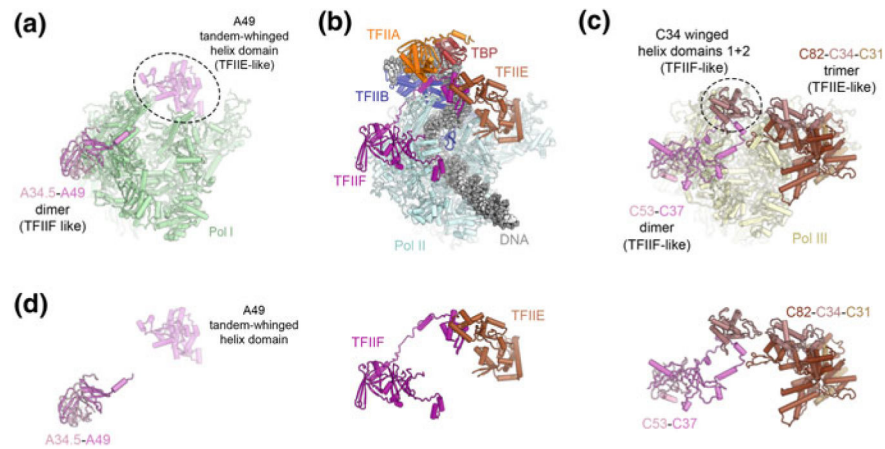
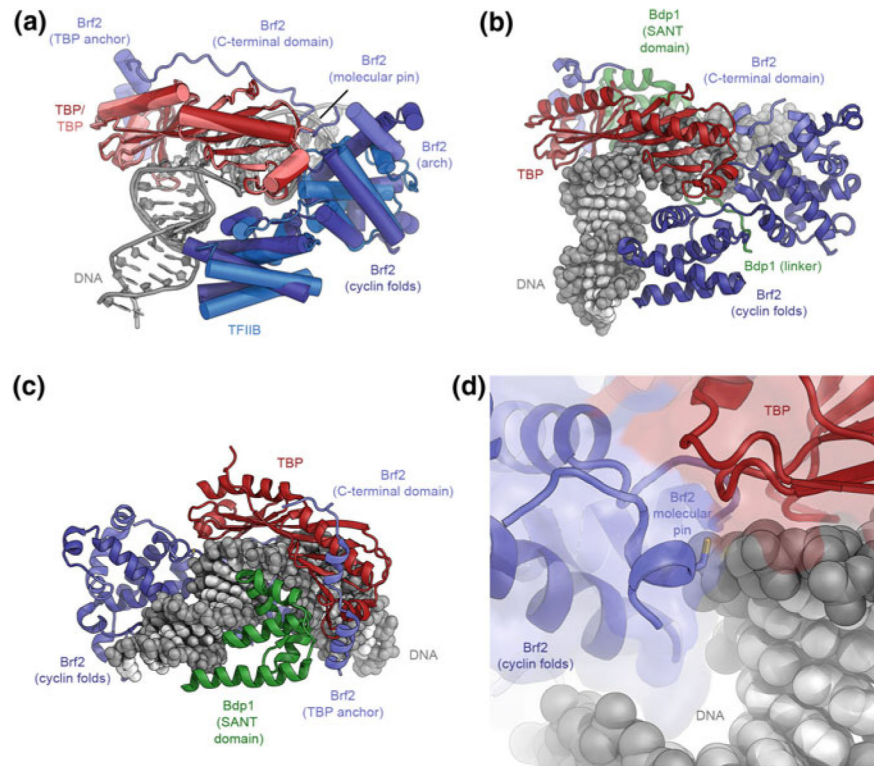


Fig. 5.12.

Transcription factor-like subunits of Pol III. **a** Structure of Pol I extracted from the structure of a Pol I elongation complex in which the tandem winged helix domain of A49 is resolved (Tafur et al. 2016). **b** Structure of the Pol II-PIC shown in the same orientation for comparison (He et al. 2016). **c** Structure of Pol III extracted from the structure of an open Pol III-PIC (Abascal-Palacios et al. 2018) showing the locations of the C82-C34-C31 trimer and the C53-C37 dimer. **d** Comparison of the overall architecture of the A34.5-A49 dimer (Pol I system, left), TFIIE and TFIIF (Pol II system, middle), and the C82-C34-C31 trimer and C53-C37 dimer (Pol III system, right). The Pol I A34.5-A49 and Pol III C53-C37 dimers are positioned similarly to TFIIF, except that the A49 tandem-winged helix domain shows similarities with TFIIE, though the binding site on the polymerase is not identical. Most of the Pol III C82-C34-C31 trimer is positioned similarly to TFIIE, with the C82 cleft loop and TFIIE E-wing domain occupying similar positions, juxtaposed to bound promoter DNA in closed PICs. The C34 winged-helix 1 domain is positioned similarly to the winged-helix domain of the TFIIF subunit Rap30

**Fig. 5.13.**

The structure of TFIIB. **a** Superposition of the structures of the DNA-bound TBP-Brf2 complex (Gouge et al. 2015) and the TBP-TFIIB-DNA complex (Tsai and Sigler 2000). TBP and the Brf2 cyclin folds in TFIIB occupy very similar positions as their Pol II-system counterparts (TBP and TFIIB, respectively). The C-terminal domain of Brf2 comprises arch, molecular pin, and TBP anchor regions (as indicated). **b, c** Structure of DNA-bound TFIIB (Gouge et al. 2017). The Bdp1 SANT domain binds to DNA at the side opposite to the Brf2 cyclin folds. **d** Structure of the redox-sensitive molecular pin in the context of bound DNA and TBP (Gouge et al. 2017)

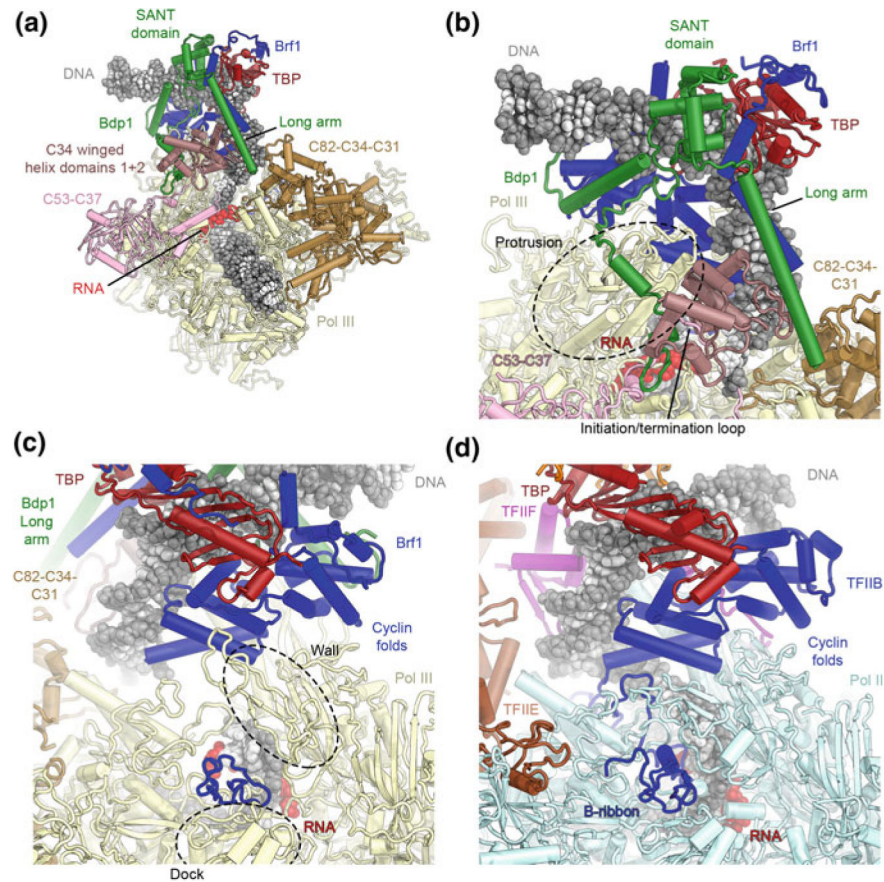


Fig. 5.14.

The structure of the Pol III-ITC. **a** Overview of the structure of the yeast Pol III-ITC (Vorländer et al. 2018). Compared to the structure of isolated TFIIB, additional elements reaching towards the polymerase are visualized in the complete PIC and ITC structures. **b** TFIIB encloses the upstream promoter. Structural elements of Bdp1 (green) contact the transcription-factor like subunits of Pol III (pink and brown), thereby contributing to a stable platform for ordering of some of these factors. **c, d** The B-ribbon domains of Brf1 (**c**) and TFIIB (**d**) occupy similar positions and interact with nucleic acids

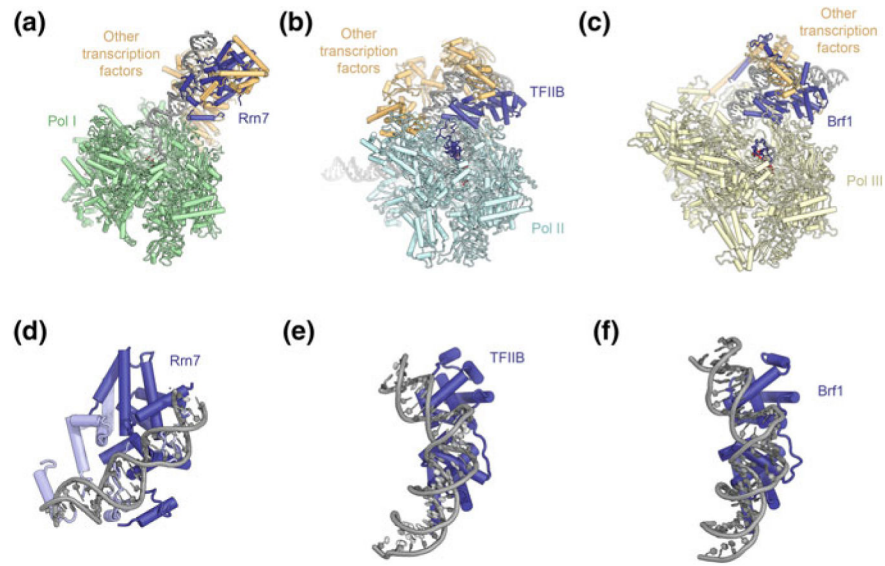


Fig. 5.15. Comparison of DNA-bound structures of TFIIB-like initiation factors. **a–c** Structure of the Pol I (**a**), Pol II (**b**), and Pol III (**c**) ITCs (Han et al. 2017; He et al. 2016; Vorländer et al. 2018). TFIIB-related factors are colored dark blue, other transcription factors orange. **d–f** Conformation of upstream promoter DNA bound to the cyclin folds of Rrn7 (**d**), TFIIB (**e**), and Brf1 (**f**)