Cell

A Movie of RNA Polymerase II Transcription

Alan C.M. Cheung[1] and Patrick Cramer[1,*]
[1]Gene Center and Department of Biochemistry, Center for Integrated Protein Science Munich (CIPSM), Ludwig-Maximilians-Universität München, Feodor-Lynen-Str. 25, 81377 Munich, Germany
*Correspondence: cramer@lmb.uni-muenchen.de
DOI 10.1016/j.cell.2012.06.006

We provide here a molecular movie that captures key aspects of RNA polymerase II initiation and elongation. To create the movie, we combined structural snapshots of the initiation-elongation transition and of elongation, including nucleotide addition, translocation, pausing, proofreading, backtracking, arrest, reactivation, and inhibition. The movie reveals open questions about the mechanism of transcription and provides a useful teaching tool.

RNA polymerase (Pol) II is a 12 subunit enzyme that depends on additional factors for transcription initiation, elongation, and termination (Orphanides et al., 1996; Reines et al., 1996; Roeder, 1996; Sims et al., 2004; Svejstrup, 2004; Thomas and Chiang, 2006; Vannini and Cramer, 2012). Transcription initiation begins with the formation of a closed promoter complex, which contains the 10 subunit Pol II core, the Pol II subcomplex Rpb4/7, and the transcription factors (TF) IID (which includes the TATA-box binding protein TBP and TBP-associated factors), TFIIB, TFIIE, TFIIF, and TFIIH.

Isomerisation of the closed to the open promoter complex involves separation of the DNA strands (i.e., DNA "melting") to form an unwound DNA region (the transcription "bubble") and positioning of the emerging template single strand in the active center of Pol II, which allows RNA synthesis to initiate from the transcription start site (Saunders et al., 2006; Wade and Struhl, 2008). The initially transcribing complex (ITC) is unstable and releases short RNAs during abortive initiation (Luse and Jacob, 1987). When the RNA reaches a critical length, initiation factors are released, and a stable elongation complex (EC) is formed (Hieb et al., 2006; Holstege et al., 1997), which contains a DNA-RNA hybrid of eight to nine base pairs (Kireeva et al., 2000; Gnatt et al., 2001).

During transcription elongation, the EC repeatedly performs the nucleotide addition cycle (NAC) to attach a nucleotide to the growing messenger RNA (mRNA) chain by catalyzing DNA template-directed formation of an RNA phosphodiester bond (Brueckner et al., 2009). The EC can also adopt "offline" states when it transiently pauses at certain DNA sequences (Landick, 2006), backtracks and arrests (Cheung and Cramer, 2011; Nudler et al., 1997; Wang et al., 2009), encounters a DNA lesion in the template strand (Brueckner et al., 2007; Damsma et al., 2007; Damsma and Cramer, 2009; Tornaletti, 2009), or misincorporates a noncomplementary nucleotide (Sydow and Cramer, 2009). Elongation factors, such as TFIIS and Spt4/5, are required to deal with these obstacles. TFIIS can reactivate an arrested EC by stimulating RNA cleavage (Reines et al., 1989; Rudd et al., 1994), and Spt4/5, which has a bacterial homolog, can increase transcription processivity (i.e., the property of the polymerase to stay associated with a transcribed template) (Burova et al., 1995; Martinez-Rucobo et al., 2011; Werner, 2012). To learn more about the prokaryotic RNA polymerase, see the Perspective by Nudler (2012) in this issue of *Cell*.

From Static Structures to a Movie

Crystal structures of multisubunit RNA polymerases from all three domains of life—bacteria (Vassylyev et al., 2002; Zhang et al., 1999), archaea (Hirata et al., 2008; Korkhin et al., 2009), and eukaryotes (Cramer et al., 2000, 2001) —have been determined. Based on these data, the initiation and elongation phases of transcription were delineated over the last decade (Cramer et al., 2008; Hahn, 2004; Lane and Darst, 2010). Most of the structural information was derived from X-ray analysis, but other techniques were also used, including fluorescence resonance energy transfer (FRET), electron microscopy, and protein cross-linking. Here, we integrated structural information on Pol II complexes with nucleic acids and transcription factors into a movie that visualizes Pol II initiation and elongation (Movie S1 available online). The movie was assembled from a collection of crystal structures and models (Table 1) that were combined into functional polymerase complexes (Figures 1 and 2).

All structures and models were superimposed onto the structure of the 12 subunit Pol II (PDB code 1WCM) (Armache et al., 2005), which was used as a reference. Modeling, prior to animation, was performed with Coot (Emsley et al., 2010). We used UCSF Chimera to generate all animations, labeling, and morphing interpolations between structures (Pettersen et al., 2004), and then we used FFMPEG (http://ffmpeg.org) to encode the resulting image frames into video. Initiation and elongation factors are colored green and orange, respectively, and flexible polypeptide chains are represented by dotted lines. The orientations of Pol II complexes shown in the movie are restricted to either the front or side views used in earlier publications (Cramer et al., 2001) (Figure 2). See Movie S1 online to download for teaching purposes.

Initiation Complex Formation

The movie starts with the formation of the initiation-competent Pol II-TFIIF complex (Figures 1A and 2A). Binding of the 10

Table 1. Structures Used to Generate the Transcription Movie

Structure	Organism	PDB	References
Rpb4/7	*S. cerevisiae*	1Y14	(Armache et al., 2005)
10 subunit Pol II	*S. cerevisiae*	1I50	(Cramer et al., 2001)
12 subunit Pol II	*S. cerevisiae*	1WCM	(Armache et al., 2005)
TFIIF dimerization module	*H. sapiens*	1F3U[a]	(Gaiser et al., 2000)
Pol II-TFIIF complex model	*S. cerevisiae*	[a,b]	(Chen et al., 2010)
TBP-TFIIB-DNA complex	*H. sapiens*/*A. thaliana*	1VOL[c]	(Nikolov et al., 1995)
Pol II-TFIIB complex	*S. cerevisiae*	3K1F[c]	(Kostrewa et al., 2009)
Pol II partial open complex with DNA	*S. cerevisiae*	4A3I	(Cheung et al., 2011)
Minimal initially transcribing complexes	*S. cerevisiae*	[d]	(Cheung et al., 2011)
Archaeal RNA polymerase clamp-Spt4/5 complex	*P. furiosis*	3QQC[e]	(Martinez-Rucobo et al., 2011)
Eukaryotic Spt4/5	*S. cerevisiae*	2EXU[e]	(Guo et al., 2008)
Pol II EC with exiting RNA, upstream DNA duplex, and nontemplate strand	*S. cerevisiae*	[e,f]	(Andrecka et al., 2008, 2009)
Posttranslocated complete EC	*S. cerevisiae*	1Y1W[e,g]	(Kettenberger et al., 2004)
Bacterial EC substrate complex, preinsertion state	*T. thermophilus*	2PPB[g]	(Vassylyev et al., 2007b)
EC substrate complex, insertion state	*S. cerevisiae*	2E2H[g]	(Wang et al., 2006)
Pretranslocated EC	*S. cerevisiae*	1I6H[g]	(Gnatt et al., 2001)
α-Amanitin-inhibited EC	*S. cerevisiae*	2VUM[g]	(Brueckner and Cramer, 2008)
Bacterial RNA polymerase EC	*T. thermophilus*	1IW7[g]	(Vassylyev et al., 2002)
Paused Pol II with frayed RNA	*S. cerevisiae*	3HOU	(Sydow et al., 2009)
Arrested Pol II with backtracked RNA	*S. cerevisiae*	3PO2	(Cheung and Cramer, 2011)
Pol II reactivation intermediate with TFIIS	*S. cerevisiae*	3PO3	(Cheung and Cramer, 2011)

[a]Homology modeling was used to generate a model for *S. cerevisiae*.
[b]Modeled from crosslinking data.
[c]These structures were combined to create open and closed promoter complex models (Kostrewa et al., 2009).
[d]PDB codes 4A3G, 4A3J, 4A3B, 4A3M, 4A3C, 4A3E, 4A3D, 4A3F, 4A3K, and 4A3L.
[e]These structures were used to model the Pol II-Spt4/5 EC (Martinez-Rucobo et al., 2011).
[f]Positions of the RNA, upstream DNA duplex, and nontemplate strand were determined by using single-molecule FRET.
[g]Used to model the nucleotide addition cycle.

subunit Pol II core (Cramer et al., 2001) to the Pol II subcomplex Rpb4/7 (Armache et al., 2005) generates the complete, 12 subunit enzyme (Armache et al., 2003; Bushnell and Kornberg, 2003). Rpb4/7 binding stabilizes a closed conformation of the Pol II clamp domain, which only permits passage of single-stranded DNA to the active site (Armache et al., 2003). The complete Pol II is apparently relevant for initiation and elongation because Rpb4/7 is required for initiation in vitro (Edwards et al., 1991) and because the complete Pol II is associated with the genome in vivo (Jasiak et al., 2008). Subsequent binding of TFIIF to Pol II generates the complete Pol II-TFIIF complex. We positioned the dimerization domain of TFIIF on the lobe domain of Pol II based on crosslinking data (Chen et al., 2010; Eichner et al., 2010).

In the next stage of the movie, the Pol II-TFIIF complex binds the TBP-TFIIB-DNA complex, resulting in a minimal closed promoter complex, in accordance with the classic model for initiation complex formation (Buratowski et al., 1989) (Figures 1B and 2B). The closed complex model was derived by combining crystal structures of the Pol II-TFIIB complex (Kostrewa et al., 2009; Liu et al., 2010) and the TBP-TFIIB-DNA complex (Kosa et al., 1997; Littlefield et al., 1999; Nikolov et al., 1995; Tsai and Sigler, 2000). This model reveals the central role of TFIIB as a bridge between the promoter and the polymerase. Docking of the TBP-TFIIB-DNA complex onto the Pol II-TFIIF complex involves the binding of the TFIIB N-terminal ribbon domain to the Pol II dock domain and the binding of the C-terminal TFIIB core domain to the polymerase wall. The TFIIB reader and linker regions connect the N- and C-terminal domains of TFIIB and extend through the Pol II cleft (Figures 1B, 2B, and 2C). The minimal initiation complex in the movie corresponds to the essential transcription machinery in archaea (Qureshi et al., 1997; Werner and Grohmann, 2011). It is related to the core initiation complex formed by the two other eukaryotic RNA polymerases, Pol I and Pol III, which use TBP and a TFIIB-like factor (Vannini and Cramer, 2012).

Initiation-Elongation Transition

The next step in transcription is the formation of the open promoter complex, which involves DNA melting and formation of a transcription bubble. DNA melting commences above the active center cleft, ~20 base pairs downstream of the TATA box (Giardina and Lis, 1993; Kostrewa et al., 2009). DNA melting allows the template single strand to reach the active site and the downstream DNA duplex to bind near the jaws of Pol II (Cheung

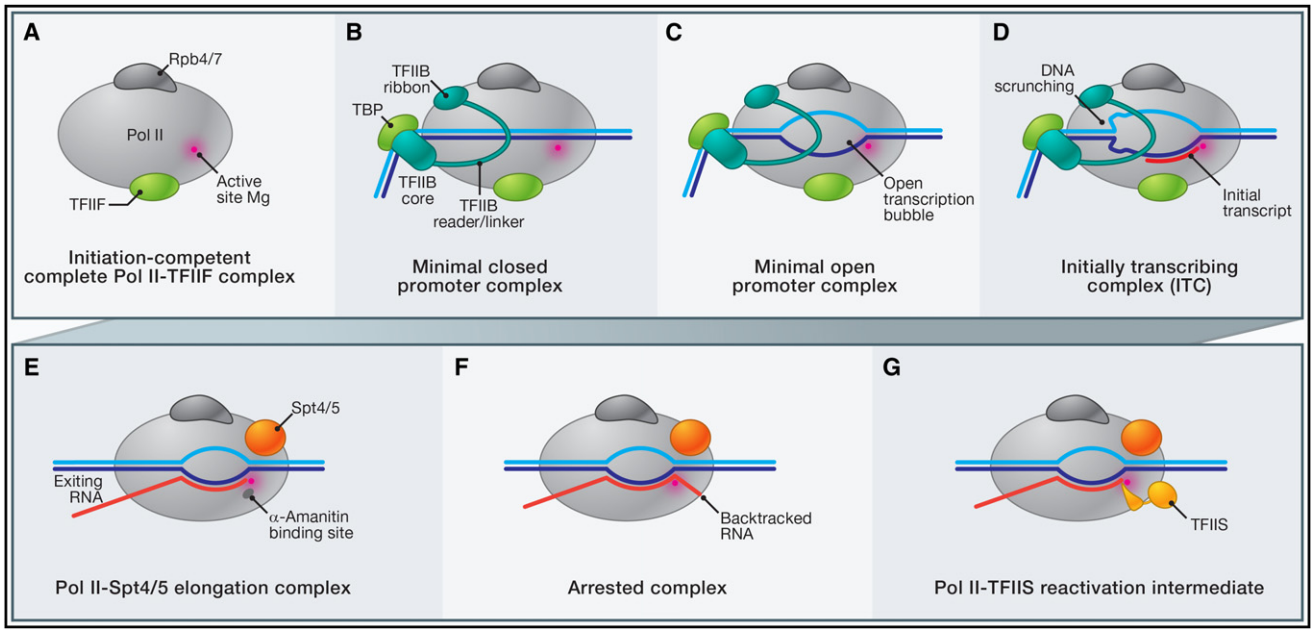

Figure 1. Functional Pol II States Described in the Movie

Schematic representation of seven Pol II functional states described in the movie (A, initiation-competent complete Pol II-TFIIF complex; B, minimal closed promoter complex; C, minimal open promoter complex; D, initially transcribing complex; E, Pol II-Spt4/5 elongation complex; F, arrested complex; G, Pol II-TFIIS reactivation intermediate). Pol II is depicted in silver, initiation factors in different shades of green, and elongation factors in different shades of orange. The DNA template strand is in dark blue, the nontemplate strand in light blue, and the RNA in red. The colors are consistent with the movie and Figure 2.

et al., 2011) (Figure 2C). The open complex then initiates RNA synthesis from DNA-templated nucleoside triphosphate (NTP) substrates, forming the ITC (Figures 1D and 2D). We animated this phase by using Pol II structures with short DNA-RNA hybrids of two to seven base pairs in length, both in the absence or presence of an NTP (Cheung et al., 2011). Similar structures were obtained by another group (Liu et al., 2011). Initial RNA synthesis may include a transient "tilting" of the short DNA-RNA hybrid in the absence of a bound NTP.

Initial RNA synthesis further involves "scrunching" of the emerging DNA strands at the upstream edge of the bubble (Figures 1D and 2D). This scrunching occurs because the upstream DNA duplex remains on the polymerase surface, whereas the downstream DNA is pulled into the active center as the DNA-RNA hybrid grows. We modeled scrunched DNA strands in accordance with single-molecule experiments of initiating bacterial RNA polymerase (Kapanidis et al., 2006; Revyakin et al., 2006). During abortive transcription, which is not shown in our movie, Pol II maintains contact with the promoter, allowing it to reinitiate RNA synthesis. Once the RNA-DNA hybrid contains eight base pairs, it is stably associated with Pol II (Kireeva et al., 2000). As the RNA extends farther, contacts to the promoter and initiation factors are broken (Van Dyke et al., 1988; Zawel et al., 1995), resulting in promoter escape and formation of the EC. We animated this transition by fading out TFIIB and TBP and adding RNA in the exit channel upstream of the hybrid (Andrecka et al., 2008; Vassylyev et al., 2007a). We also illustrate the rewinding of upstream DNA (Holstege et al., 1997; Pal et al., 2005) by fading in the upstream DNA duplex that emanates at right angles from the downstream duplex (Andrecka et al., 2009).

Transcription Elongation

In the next stage of the movie, the complete EC (Kettenberger et al., 2004) is bound by the elongation factor Spt4/5 (Klein et al., 2011; Martinez-Rucobo et al., 2011). Spt4/5 binds to the polymerase clamp and is located adjacent to the nontemplate strand of the transcription bubble (Figure 2E). Spt4/5 can associate only upon promoter escape because its binding site is occupied in the initiation complex. The N-terminal NusG homology domain of Spt5 closes the active center cleft, apparently locking in the nucleic acids and enhancing EC processivity. The view then zooms in on the active site to observe the NAC, similar to a previous animation (Brueckner et al., 2009).

During the NAC, an NTP substrate first binds to an open active center conformation, adopting a preinsertion state (Kettenberger et al., 2004; Vassylyev et al., 2007b) that was modeled based on a high-quality bacterial EC structure (Vassylyev et al., 2007b). The NTP then moves slightly to occupy the insertion site as the trigger loop folds to close the active center (Vassylyev et al., 2007b; Wang et al., 2006). Closure of the active site around the NTP generates contacts that are required for correct NTP selection and leads to catalytic nucleotide incorporation and RNA extension (Cheung et al., 2011; Vassylyev et al., 2007b; Wang et al., 2006). Release of pyrophosphate may cause trigger loop unfolding and opening of the active site (Feig and Burton, 2010). Subsequent nucleic acid translocation may follow a two-step mechanism. In the first step, the polymerase trigger loop and bridge helix cooperate to shift the nascent hybrid base pair out of the active site. This leads to translocation of the next template base into a pretemplating position above the bridge helix (Brueckner and Cramer, 2008). In the second step,

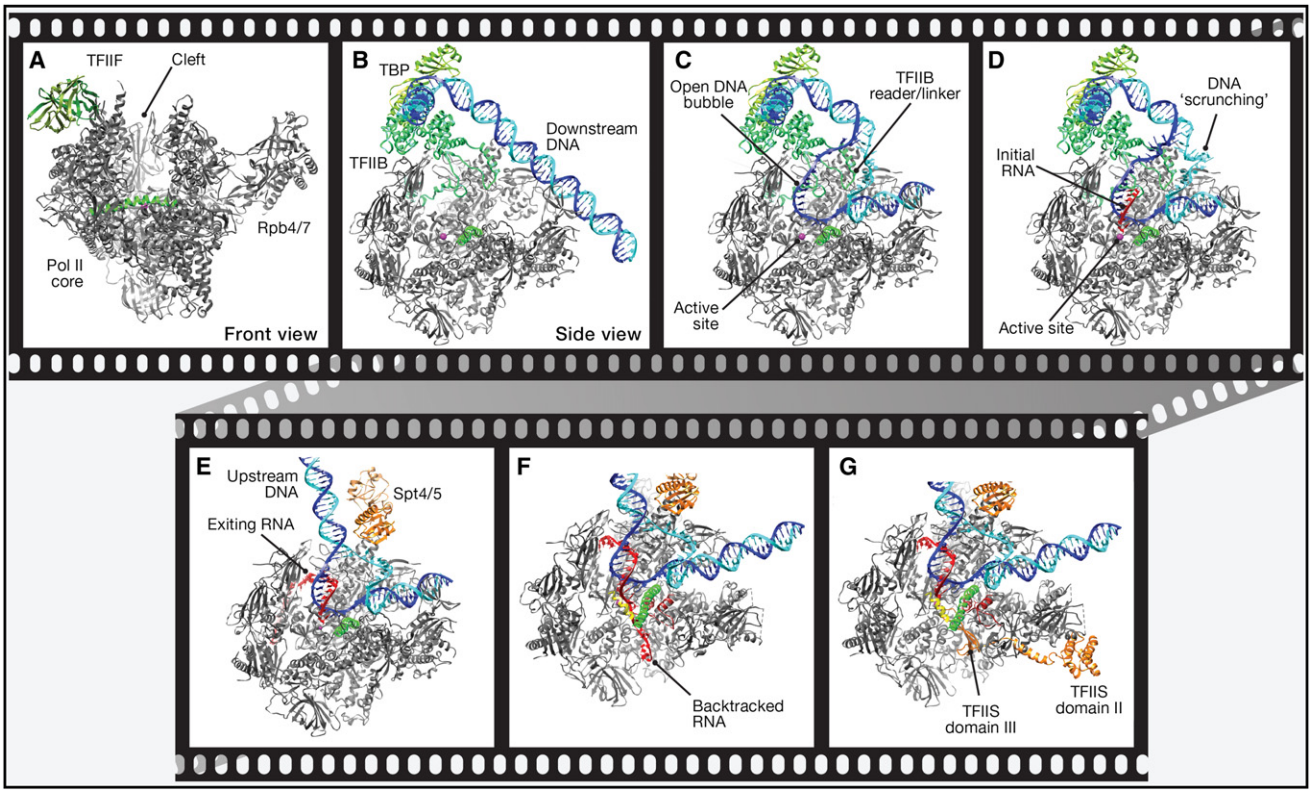

Figure 2. Snapshots from the Movie

Representative still images from the movie corresponding to the seven functional states shown in Figure 1 (A, initiation-competent complete Pol II-TFIIF complex; B, minimal closed promoter complex; C, minimal open promoter complex; D, initially transcribing complex; E, Pol II-Spt4/5 elongation complex; F, arrested complex; G, Pol II-TFIIS reactivation intermediate). Side views are depicted except for (A), which shows the front view (Cramer et al., 2001).

relaxation of the bridge helix allows the incoming template base to enter the active site, completing the NAC. A second NAC cycle in the movie shows the effect of the mushroom toxin α-amanitin, which inhibits Pol II elongation by preventing translocation (Brueckner and Cramer, 2008; Kaplan et al., 2008).

Obstacles and Reactivation

Certain DNA sequences, nucleotide misincorporation, or damaged template DNA form obstacles for NAC progression. Such obstacles induce offline states, which require reactivation before the NAC can resume. The movie illustrates how a frequent obstacle, nucleotide misincorporation, can be overcome by RNA proofreading. A purine-purine mismatch in the active site triggers Pol II pausing, which can also be induced by transcription of a weak DNA-RNA hybrid. The mismatched 3′ nucleotide frays away from the DNA template such that it stacks onto the "gating tyrosine" residue Y769 of the largest Pol II subunit (Cheung and Cramer, 2011; Sydow et al., 2009). Pol II backtracking by a single step is followed by endonucleolytic cleavage of a dinucleotide that contains the mismatched nucleotide, reactivating the EC. RNA cleavage is catalyzed by an intrinsic hydrolytic activity of the tunable Pol II active site (Kettenberger et al., 2003) and is stimulated by the RNA 3′ end (Zenkin et al., 2006). Cleavage restores a 3′-OH group in the active site, from which elongation resumes.

The movie then shows backtracking and arrest, which can occur upon attempts to elongate through specific DNA sequences (Nudler et al., 1997) or a nucleosome (Kireeva et al., 2005). During backtracking, RNA is extruded into the pore beneath the Pol II active site and is trapped in an RNA-binding site, which prevents forward translocation and NTP binding and results in arrest (Cheung and Cramer, 2011) (Figures 1F and 2F). Reactivation of arrested Pol II requires the elongation factor TFIIS, which stimulates RNA cleavage at the Pol II active site and is animated in two stages. First, domain II of TFIIS binds at the polymerase funnel and causes a conformational change in the polymerase that repositions the backtracked RNA (Cheung and Cramer, 2011). Second, domain III of TFIIS inserts into the pore next to backtracked RNA and reaches the active site with a hairpin that contains three charged residues that stimulate RNA cleavage (Figures 1G and 2G), generating a new RNA 3′ end from which transcription resumes.

Current Limitations

The movie reveals gaps in our understanding of the transcription mechanism and encourages a continuation of the current structure-function analysis of Pol II. It defines new open questions about initiation and elongation. For instance, what is the mechanism of DNA melting during the closed-to-open promoter complex transition? It is known that DNA opening requires

downstream action of TFIIE and TFIIH (Dvir et al., 2001; Kim et al., 2000), and it also involves TFIIB (Knutson and Hahn, 2011; Kostrewa et al., 2009). However, the structural mechanisms remain unclear, although they were recently elucidated for prokaryotic transcription (Feklistov and Darst, 2011). During the closed-to-open transition, some contacts between TFIIB and the polymerase are broken, resulting in movements of the upstream DNA (Treutlein et al., 2012). However, more structural restraints are required before this can be animated and included in the movie. After DNA opening, Pol II scans the template to locate the transcription start site, but the scanning mechanism is poorly understood, and it is not included in the movie. There is also no structural information for the initiating complex, which contains the first two NTP substrates prior to formation of the first phosphodiester bond. Furthermore, where exactly do other initiation factors and coactivators bind, including TFIID and the Mediator?

With respect to elongation, the animated NAC is simplified, as it does not take into account the sampling of NTPs in the active center when Pol II selects the correct substrate. Also, the animation of translocation does not take into account the real nature of the Brownian ratchet formed by the bridge helix and trigger loop. The NAC may be modified as additional conformational states of the EC are observed. For example, a recent structure of a bacterial RNA polymerase with a bound inhibitory protein revealed a new conformation of the enzyme that may be relevant for translocation (Tagami et al., 2010). Also unknown is the structure of the product complex, which contains the newly formed RNA 3′ end in the pretranslocation position and the pyrophosphate ion still bound to Pol II. This state was modeled for the movie, assuming a closed trigger loop. Only two elongation factors, Spt4/5 and TFIIS, have been located on Pol II and included in the movie, but structural details for how the many other elongation factors interact with Pol II are lacking. What are the mechanisms used by elongation factors that influence chromatin structure, such as the Paf1 complex, Spt6, or the FACT (facilitates chromatin transcription) complex? In addition, the structural mechanism of Pol II termination remains enigmatic and could not be included in the movie.

Conclusions and Future Directions

Here, we used structural information to generate a molecular movie that captures key aspects of RNA polymerase II initiation and elongation. In the future, the movie may be extended to describe transcription in its cellular context. For example, the mechanisms underlying the coordination of transcription with mRNA processing are partially understood and may be included in the movie when some remaining gaps in our knowledge are closed. This coordination relies on the C-terminal repeat domain (CTD) of Pol II, which extends from the enzyme like a long, flexible tail. The CTD is subject to posttranslational modifications and recruits many different factors at various stages of the transcription cycle. Other flexible elements, including the nascent RNA and histone tails of transcribed nucleosomes, participate in additional interactions with various factors and contribute to the coordination of transcription with other nuclear events. The intricate network of transient and cooperative interactions between these flexible elements and their target factors should eventually be understood at a level that allows for their animation. As a first step toward this goal, the movie here summarizes a wealth of structural information on the core transcription machinery and provides a useful tool for teaching the mechanism of transcription.

SUPPLEMENTAL INFORMATION

Supplemental Information includes a movie and can be found with this article online at doi:10.1016/j.cell.2012.06.006.

ACKNOWLEDGMENTS

P.C. was supported by the Deutsche Forschungsgemeinschaft, SFB646, TR5, FOR1068, SFB960, NIM, the European Molecular Biology Organization (EMBO), an Advanced Investigator Grant of the European Research Council, LMU excellent research professorship "Molecular systems biology of gene regulation," the LMUinnovativ project Bioimaging Network (BIN), and the Jung-Stiftung.

REFERENCES

Andrecka, J., Lewis, R., Brückner, F., Lehmann, E., Cramer, P., and Michaelis, J. (2008). Single-molecule tracking of mRNA exiting from RNA polymerase II. Proc. Natl. Acad. Sci. USA *105*, 135–140.

Andrecka, J., Treutlein, B., Arcusa, M.A., Muschielok, A., Lewis, R., Cheung, A.C.M., Cramer, P., and Michaelis, J. (2009). Nano positioning system reveals the course of upstream and nontemplate DNA within the RNA polymerase II elongation complex. Nucleic Acids Res. *37*, 5803–5809.

Armache, K.J., Kettenberger, H., and Cramer, P. (2003). Architecture of initiation-competent 12-subunit RNA polymerase II. Proc. Natl. Acad. Sci. USA *100*, 6964–6968.

Armache, K.J., Mitterweger, S., Meinhart, A., and Cramer, P. (2005). Structures of complete RNA polymerase II and its subcomplex, Rpb4/7. J. Biol. Chem. *280*, 7131–7134.

Brueckner, F., and Cramer, P. (2008). Structural basis of transcription inhibition by alpha-amanitin and implications for RNA polymerase II translocation. Nat. Struct. Mol. Biol. *15*, 811–818.

Brueckner, F., Hennecke, U., Carell, T., and Cramer, P. (2007). CPD damage recognition by transcribing RNA polymerase II. Science *315*, 859–862.

Brueckner, F., Ortiz, J., and Cramer, P. (2009). A movie of the RNA polymerase nucleotide addition cycle. Curr. Opin. Struct. Biol. *19*, 294–299.

Buratowski, S., Hahn, S., Guarente, L., and Sharp, P.A. (1989). Five intermediate complexes in transcription initiation by RNA polymerase II. Cell *56*, 549–561.

Burova, E., Hung, S.C., Sagitov, V., Stitt, B.L., and Gottesman, M.E. (1995). Escherichia coli NusG protein stimulates transcription elongation rates in vivo and in vitro. J. Bacteriol. *177*, 1388–1392.

Bushnell, D.A., and Kornberg, R.D. (2003). Complete, 12-subunit RNA polymerase II at 4.1-A resolution: implications for the initiation of transcription. Proc. Natl. Acad. Sci. USA *100*, 6969–6973.

Chen, Z.A., Jawhari, A., Fischer, L., Buchen, C., Tahir, S., Kamenski, T., Rasmussen, M., Lariviere, L., Bukowski-Wills, J.-C., Nilges, M., et al. (2010). Architecture of the RNA polymerase II-TFIIF complex revealed by cross-linking and mass spectrometry. EMBO J. *29*, 717–726.

Cheung, A.C., and Cramer, P. (2011). Structural basis of RNA polymerase II backtracking, arrest and reactivation. Nature *471*, 249–253.

Cheung, A.C., Sainsbury, S., and Cramer, P. (2011). Structural basis of initial RNA polymerase II transcription. EMBO J. *30*, 4755–4763.

Cramer, P., Bushnell, D.A., Fu, J., Gnatt, A.L., Maier-Davis, B., Thompson, N.E., Burgess, R.R., Edwards, A.M., David, P.R., and Kornberg, R.D. (2000). Architecture of RNA polymerase II and implications for the transcription mechanism. Science *288*, 640–649.

Cramer, P., Bushnell, D.A., and Kornberg, R.D. (2001). Structural basis of transcription: RNA polymerase II at 2.8 angstrom resolution. Science *292*, 1863–1876.

Cramer, P., Armache, K.J., Baumli, S., Benkert, S., Brueckner, F., Buchen, C., Damsma, G.E., Dengl, S., Geiger, S.R., Jasiak, A.J., et al. (2008). Structure of eukaryotic RNA polymerases. Annu. Rev. Biophys. *37*, 337–352.

Damsma, G.E., and Cramer, P. (2009). Molecular basis of transcriptional mutagenesis at 8-oxoguanine. J. Biol. Chem. *284*, 31658–31663.

Damsma, G.E., Alt, A., Brueckner, F., Carell, T., and Cramer, P. (2007). Mechanism of transcriptional stalling at cisplatin-damaged DNA. Nat. Struct. Mol. Biol. *14*, 1127–1133.

Dvir, A., Conaway, J.W., and Conaway, R.C. (2001). Mechanism of transcription initiation and promoter escape by RNA polymerase II. Curr. Opin. Genet. Dev. *11*, 209–214.

Edwards, A.M., Kane, C.M., Young, R.A., and Kornberg, R.D. (1991). Two dissociable subunits of yeast RNA polymerase II stimulate the initiation of transcription at a promoter in vitro. J. Biol. Chem. *266*, 71–75.

Eichner, J., Chen, H.-T., Warfield, L., and Hahn, S. (2010). Position of the general transcription factor TFIIF within the RNA polymerase II transcription preinitiation complex. EMBO J. *29*, 706–716.

Emsley, P., Lohkamp, B., Scott, W.G., and Cowtan, K. (2010). Features and development of Coot. Acta Crystallogr. D Biol. Crystallogr. *66*, 486–501.

Feig, M., and Burton, Z.F. (2010). RNA polymerase II with open and closed trigger loops: active site dynamics and nucleic acid translocation. Biophys. J. *99*, 2577–2586.

Feklistov, A., and Darst, S.A. (2011). Structural basis for promoter-10 element recognition by the bacterial RNA polymerase σ subunit. Cell *147*, 1257–1269.

Gaiser, F., Tan, S., and Richmond, T.J. (2000). Novel dimerization fold of RAP30/RAP74 in human TFIIF at 1.7 A resolution. J. Mol. Biol. *302*, 1119–1127.

Giardina, C., and Lis, J.T. (1993). DNA melting on yeast RNA polymerase II promoters. Science *261*, 759–762.

Gnatt, A.L., Cramer, P., Fu, J., Bushnell, D.A., and Kornberg, R.D. (2001). Structural basis of transcription: an RNA polymerase II elongation complex at 3.3 A resolution. Science *292*, 1876–1882.

Guo, M., Xu, F., Yamada, J., Egelhofer, T., Gao, Y., Hartzog, G.A., Teng, M., and Niu, L. (2008). Core structure of the yeast spt4-spt5 complex: a conserved module for regulation of transcription elongation. Structure *16*, 1649–1658.

Hahn, S. (2004). Structure and mechanism of the RNA polymerase II transcription machinery. Nat. Struct. Mol. Biol. *11*, 394–403.

Hieb, A.R., Baran, S., Goodrich, J.A., and Kugel, J.F. (2006). An 8 nt RNA triggers a rate-limiting shift of RNA polymerase II complexes into elongation. EMBO J. *25*, 3100–3109.

Hirata, A., Klein, B.J., and Murakami, K.S. (2008). The X-ray crystal structure of RNA polymerase from Archaea. Nature *451*, 851–854.

Holstege, F.C.P., Fiedler, U., and Timmers, H.T.M. (1997). Three transitions in the RNA polymerase II transcription complex during initiation. EMBO J. *16*, 7468–7480.

Jasiak, A.J., Hartmann, H., Karakasili, E., Kalocsay, M., Flatley, A., Kremmer, E., Strässer, K., Martin, D.E., Söding, J., and Cramer, P. (2008). Genome-associated RNA polymerase II includes the dissociable Rpb4/7 subcomplex. J. Biol. Chem. *283*, 26423–26427.

Kapanidis, A.N., Margeat, E., Ho, S.O., Kortkhonjia, E., Weiss, S., and Ebright, R.H. (2006). Initial transcription by RNA polymerase proceeds through a DNA-scrunching mechanism. Science *314*, 1144–1147.

Kaplan, C.D., Larsson, K.M., and Kornberg, R.D. (2008). The RNA polymerase II trigger loop functions in substrate selection and is directly targeted by alpha-amanitin. Mol. Cell *30*, 547–556.

Kettenberger, H., Armache, K.J., and Cramer, P. (2003). Architecture of the RNA polymerase II-TFIIS complex and implications for mRNA cleavage. Cell *114*, 347–357.

Kettenberger, H., Armache, K.J., and Cramer, P. (2004). Complete RNA polymerase II elongation complex structure and its interactions with NTP and TFIIS. Mol. Cell *16*, 955–965.

Kim, T.-K., Ebright, R.H., and Reinberg, D. (2000). Mechanism of ATP-dependent promoter melting by transcription factor IIH. Science *288*, 1418–1422.

Kireeva, M.L., Komissarova, N., Waugh, D.S., and Kashlev, M. (2000). The 8-nucleotide-long RNA:DNA hybrid is a primary stability determinant of the RNA polymerase II elongation complex. J. Biol. Chem. *275*, 6530–6536.

Kireeva, M.L., Hancock, B., Cremona, G.H., Walter, W., Studitsky, V.M., and Kashlev, M. (2005). Nature of the nucleosomal barrier to RNA polymerase II. Mol. Cell *18*, 97–108.

Klein, B.J., Bose, D., Baker, K.J., Yusoff, Z.M., Zhang, X., and Murakami, K.S. (2011). RNA polymerase and transcription elongation factor Spt4/5 complex structure. Proc. Natl. Acad. Sci. USA *108*, 546–550.

Knutson, B.A., and Hahn, S. (2011). Yeast Rrn7 and human TAF1B are TFIIB-related RNA polymerase I general transcription factors. Science *333*, 1637–1640.

Korkhin, Y., Unligil, U.M., Littlefield, O., Nelson, P.J., Stuart, D.I., Sigler, P.B., Bell, S.D., and Abrescia, N.G.A. (2009). Evolution of complex RNA polymerases: the complete archaeal RNA polymerase structure. PLoS Biol. *7*, e102.

Kosa, P.F., Ghosh, G., DeDecker, B.S., and Sigler, P.B. (1997). The 2.1-A crystal structure of an archaeal preinitiation complex: TATA-box-binding protein/transcription factor (II)B core/TATA-box. Proc. Natl. Acad. Sci. USA *94*, 6042–6047.

Kostrewa, D., Zeller, M.E., Armache, K.-J., Seizl, M., Leike, K., Thomm, M., and Cramer, P. (2009). RNA polymerase II-TFIIB structure and mechanism of transcription initiation. Nature *462*, 323–330.

Landick, R. (2006). The regulatory roles and mechanism of transcriptional pausing. Biochem. Soc. Trans. *34*, 1062–1066.

Lane, W.J., and Darst, S.A. (2010). Molecular evolution of multisubunit RNA polymerases: structural analysis. J. Mol. Biol. *395*, 686–704.

Littlefield, O., Korkhin, Y., and Sigler, P.B. (1999). The structural basis for the oriented assembly of a TBP/TFB/promoter complex. Proc. Natl. Acad. Sci. USA *96*, 13668–13673.

Liu, X., Bushnell, D.A., Wang, D., Calero, G., and Kornberg, R.D. (2010). Structure of an RNA polymerase II-TFIIB complex and the transcription initiation mechanism. Science *327*, 206–209.

Liu, X., Bushnell, D.A., Silva, D.-A., Huang, X., and Kornberg, R.D. (2011). Initiation complex structure and promoter proofreading. Science *333*, 633–637.

Luse, D.S., and Jacob, G.A. (1987). Abortive initiation by RNA polymerase II in vitro at the adenovirus 2 major late promoter. J. Biol. Chem. *262*, 14990–14997.

Martinez-Rucobo, F.W., Sainsbury, S., Cheung, A.C.M., and Cramer, P. (2011). Architecture of the RNA polymerase-Spt4/5 complex and basis of universal transcription processivity. EMBO J. *30*, 1302–1310.

Nikolov, D.B., Chen, H., Halay, E.D., Usheva, A.A., Hisatake, K., Lee, D.K., Roeder, R.G., and Burley, S.K. (1995). Crystal structure of a TFIIB-TBP-TATA-element ternary complex. Nature *377*, 119–128.

Nudler, E. (2012). RNA polymerase backtracking in gene regulation and genome instability. Cell *149*, this issue, 1438–1445.

Nudler, E., Mustaev, A., Lukhtanov, E., and Goldfarb, A. (1997). The RNA-DNA hybrid maintains the register of transcription by preventing backtracking of RNA polymerase. Cell *89*, 33–41.

Orphanides, G., Lagrange, T., and Reinberg, D. (1996). The general transcription factors of RNA polymerase II. Genes Dev. *10*, 2657–2683.

Pal, M., Ponticelli, A.S., and Luse, D.S. (2005). The role of the transcription bubble and TFIIB in promoter clearance by RNA polymerase II. Mol. Cell *19*, 101–110.

Pettersen, E.F., Goddard, T.D., Huang, C.C., Couch, G.S., Greenblatt, D.M., Meng, E.C., and Ferrin, T.E. (2004). UCSF Chimera—a visualization system for exploratory research and analysis. J. Comput. Chem. *25*, 1605–1612.

Qureshi, S.A., Bell, S.D., and Jackson, S.P. (1997). Factor requirements for transcription in the Archaeon Sulfolobus shibatae. EMBO J. *16*, 2927–2936.

Reines, D., Chamberlin, M.J., and Kane, C.M. (1989). Transcription elongation factor SII (TFIIS) enables RNA polymerase II to elongate through a block to transcription in a human gene in vitro. J. Biol. Chem. *264*, 10799–10809.

Reines, D., Conaway, J.W., and Conaway, R.C. (1996). The RNA polymerase II general elongation factors. Trends Biochem. Sci. *21*, 351–355.

Revyakin, A., Liu, C., Ebright, R.H., and Strick, T.R. (2006). Abortive initiation and productive initiation by RNA polymerase involve DNA scrunching. Science *314*, 1139–1143.

Roeder, R.G. (1996). The role of general initiation factors in transcription by RNA polymerase II. Trends Biochem. Sci. *21*, 327–335.

Rudd, M.D., Izban, M.G., and Luse, D.S. (1994). The active site of RNA polymerase II participates in transcript cleavage within arrested ternary complexes. Proc. Natl. Acad. Sci. USA *91*, 8057–8061.

Saunders, A., Core, L.J., and Lis, J.T. (2006). Breaking barriers to transcription elongation. Nat. Rev. Mol. Cell Biol. *7*, 557–567.

Sims, R.J., III, Belotserkovskaya, R., and Reinberg, D. (2004). Elongation by RNA polymerase II: the short and long of it. Genes Dev. *18*, 2437–2468.

Svejstrup, J.Q. (2004). The RNA polymerase II transcription cycle: cycling through chromatin. Biochim. Biophys. Acta *1677*, 64–73.

Sydow, J.F., and Cramer, P. (2009). RNA polymerase fidelity and transcriptional proofreading. Curr. Opin. Struct. Biol. *19*, 732–739.

Sydow, J.F., Brueckner, F., Cheung, A.C.M., Damsma, G.E., Dengl, S., Lehmann, E., Vassylyev, D., and Cramer, P. (2009). Structural basis of transcription: mismatch-specific fidelity mechanisms and paused RNA polymerase II with frayed RNA. Mol. Cell *34*, 710–721.

Tagami, S., Sekine, S., Kumarevel, T., Hino, N., Murayama, Y., Kamegamori, S., Yamamoto, M., Sakamoto, K., and Yokoyama, S. (2010). Crystal structure of bacterial RNA polymerase bound with a transcription inhibitor protein. Nature *468*, 978–982.

Thomas, M.C., and Chiang, C.M. (2006). The general transcription machinery and general cofactors. Crit. Rev. Biochem. Mol. Biol. *41*, 105–178.

Tornaletti, S. (2009). DNA repair in mammalian cells: transcription-coupled DNA repair: directing your effort where it's most needed. Cell. Mol. Life Sci. *66*, 1010–1020.

Treutlein, B., Muschielok, A., Andrecka, J., Jawhari, A., Buchen, C., Kostrewa, D., Hog, F., Cramer, P., and Michaelis, J. (2012). Dynamic architecture of a minimal RNA polymerase II open promoter complex. Mol. Cell *46*, 136–146.

Tsai, F.T., and Sigler, P.B. (2000). Structural basis of preinitiation complex assembly on human pol II promoters. EMBO J. *19*, 25–36.

Van Dyke, M.W., Roeder, R.G., and Sawadogo, M. (1988). Physical analysis of transcription preinitiation complex assembly on a class II gene promoter. Science *241*, 1335–1338.

Vannini, A., and Cramer, P. (2012). Conservation between the RNA polymerase I, II, and III transcription initiation machineries. Mol. Cell *45*, 439–446.

Vassylyev, D.G., Sekine, S., Laptenko, O., Lee, J., Vassylyeva, M.N., Borukhov, S., and Yokoyama, S. (2002). Crystal structure of a bacterial RNA polymerase holoenzyme at 2.6 A resolution. Nature *417*, 712–719.

Vassylyev, D.G., Vassylyeva, M.N., Perederina, A., Tahirov, T.H., and Artsimovitch, I. (2007a). Structural basis for transcription elongation by bacterial RNA polymerase. Nature *448*, 157–162.

Vassylyev, D.G., Vassylyeva, M.N., Zhang, J., Palangat, M., Artsimovitch, I., and Landick, R. (2007b). Structural basis for substrate loading in bacterial RNA polymerase. Nature *448*, 163–168.

Wade, J.T., and Struhl, K. (2008). The transition from transcriptional initiation to elongation. Curr. Opin. Genet. Dev. *18*, 130–136.

Wang, D., Bushnell, D.A., Westover, K.D., Kaplan, C.D., and Kornberg, R.D. (2006). Structural basis of transcription: role of the trigger loop in substrate specificity and catalysis. Cell *127*, 941–954.

Wang, D., Bushnell, D.A., Huang, X., Westover, K.D., Levitt, M., and Kornberg, R.D. (2009). Structural basis of transcription: backtracked RNA polymerase II at 3.4 angstrom resolution. Science *324*, 1203–1206.

Werner, F. (2012). A nexus for gene expression-molecular mechanisms of Spt5 and NusG in the three domains of life. J. Mol. Biol. *417*, 13–27.

Werner, F., and Grohmann, D. (2011). Evolution of multisubunit RNA polymerases in the three domains of life. Nat. Rev. Microbiol. *9*, 85–98.

Zawel, L., Kumar, K.P., and Reinberg, D. (1995). Recycling of the general transcription factors during RNA polymerase II transcription. Genes Dev. *9*, 1479–1490.

Zenkin, N., Yuzenkova, Y., and Severinov, K. (2006). Transcript-assisted transcriptional proofreading. Science *313*, 518–520.

Zhang, G.Y., Campbell, E.A., Minakhin, L., Richter, C., Severinov, K., and Darst, S.A. (1999). Crystal structure of Thermus aquaticus core RNA polymerase at 3.3 A resolution. Cell *98*, 811–824.