Chapter 9
Structure and Function of RNA Polymerases and the Transcription Machineries

Joachim Griesenbeck, Herbert Tschochner, and Dina Grohmann

Abstract In all living organisms, the flow of genetic information is a two-step process: first DNA is transcribed into RNA, which is subsequently used as template for protein synthesis during translation. In bacteria, archaea and eukaryotes, transcription is carried out by multi-subunit RNA polymerases (RNAPs) sharing a conserved architecture of the RNAP core. RNAPs catalyse the highly accurate polymerisation of RNA from NTP building blocks, utilising DNA as template, being assisted by transcription factors during the initiation, elongation and termination phase of transcription. The complexity of this highly dynamic process is reflected in the intricate network of protein-protein and protein-nucleic acid interactions in transcription complexes and the substantial conformational changes of the RNAP as it progresses through the transcription cycle.

In this chapter, we will first briefly describe the early work that led to the discovery of multisubunit RNAPs. We will then discuss the three-dimensional organisation of RNAPs from the bacterial, archaeal and eukaryotic domains of life, highlighting the conserved nature, but also the domain-specific features of the transcriptional apparatus. Another section will focus on transcription factors and their role in regulating the RNA polymerase throughout the different phases of the transcription cycle. This includes a discussion of the molecular mechanisms and dynamic events that govern transcription initiation, elongation and termination.

Keywords RNA polymerase • Transcription • Transcription factors • Transcription cycle

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9.1 Multisubunit RNA Polymerases – Discovery of the Enzymes and Their Role Within the Central Dogma of Life

The description of the DNA structure in 1953 (Watson and Crick 1953b), together with early studies in genetics and bacterial transformation, stimulated the question how the repeating mononucleotide units that constitute DNA are assembled in the cell (Olby 2003). This led to the hypothesis that DNA replication would require DNA to function as a template for its own synthesis (Watson and Crick 1953a; Meselson and Stahl 1958). At this point, RNA was only a poorly understood molecule and, consequently, the search for a DNA replicating enzyme rather than an RNA synthesizing enzyme started soon after the double-helical structure of DNA was presented. Arthur Kornberg and colleagues were the first to describe DNA polymerase as the DNA synthesizing enzyme (Lehman et al. 1958). RNA only came into focus when the mechanism of protein synthesis was debated (Campbell and Work 1953; Dounce 1952). In 1955, Grunberg-Manago and Ochoa proposed that polynucleotide phosphorylase (PNP) is the enzyme responsible for RNA synthesis (Grunberg-Manago et al. 1955), an idea that proved wrong. The discovery of tRNAs as acceptor for amino acids (Hoagland et al. 1957) gave the first indication that RNA and protein synthesis are linked and that protein biosynthesis requires a high specificity of the tRNA. However, PNP could not fulfil this requirement for specificity as it only adds nucleotides at the RNA termini in a non-specific fashion. This led to the hypothesis that DNA could serve as a template for RNA synthesis and initiated the search for an enzyme capable of DNA-dependent RNA-polymerisation (Hurwitz 2005). RNA polymerase (RNAP) activity was first described by Weiss and Gladstone in 1959 who demonstrated that all four NTPs were incorporated into RNA when added to rat liver nuclear extracts (Weiss and Gladstone 1959). Shortly after, four laboratories independently reported on DNA-dependent RNA synthesising activity in cellular extracts of bacterial or eukaryotic origin (i.e. from *Escherichia coli* (Hurwitz and Bresler 1961; Stevens 1960), pea embryos (Huang et al. 1960) and *Micrococcus luteus* (Weiss and Nakamoto 1961)). Geiduschek et al. (1961) and Chamberlin et al. (1963) used purified RNAPs to demonstrate that *in vitro* transcribed RNA is complementary to the DNA substrate and that the DNA template remained intact after supporting RNA synthesis. Roeder and Rutter were the first to describe that not one but three different enzymes – RNAP I, II and III – transcribe the eukaryotic genome (Roeder and Rutter 1969, 1970). These early discoveries started a new research field and it became apparent that bacterial and eukaryotic RNAPs differ not only in their subunit composition, but also in their sensitivity towards small molecules, rendering RNAP a prime target for antibiotics. Moreover, the understanding of RNAP subunit composition was of critical importance to establish the archaea as an independent domain of life. Shortly after Woese suggested the existence of the third domain of life in 1977 (Woese and Fox 1977), Stetter and Zillig attempted the isolation and biochemical characterisation of RNAPs from different archaeal organisms (Schnabel et al. 1983; Zillig et al. 1978,

1979). Their work clearly demonstrated that archaeal RNAPs are composed of more than the four subunits prototypical for bacterial RNAPs and cannot be inhibited by the antibiotic Rifampicin, providing evidence that the archaeal RNAP shares more characteristics with eukaryotic than bacterial RNAPs. At this point, the subunit composition and the catalytic activity of RNAPs from all three domains could be explored in detail. But it took another 20 years until the first structure of a multisubunit (bacterial) RNAP was solved (Zhang et al. 1999), closely followed by a crystal structure of the eukaryotic RNAP enzyme from yeast (Gnatt et al. 2001; Cramer et al. 2000) and finally, in 2008, the first crystal structure of an archaeal RNAP was reported (Hirata et al. 2008).

9.2 Multisubunit RNA Polymerases and Transcription Factors in the Three Domains of Life

9.2.1 Overall Subunit Composition and Architecture of RNA Polymerase in Bacteria, Eukaryotes and Archaea

DNA-dependent RNA polymerases either belong to the family of single subunit or to the family of multisubunit RNAPs. Single subunit RNAPs can be found in chloroplasts, mitochondria and in bacteriophages, such as T7 or SP6, and are shaped like a right hand (Cheetham and Steitz 2000). In all living cells, however, DNA-dependent RNA synthesis is carried out by complex multisubunit RNAPs (Werner and Grohmann 2011). While bacteria and archaea employ a single type of RNAP, the eukaryotic genome is transcribed by at least three specialized RNAPs, which are dedicated to different subsets of genes. RNAP I transcribes the ribosomal RNA precursor for the mature 25/28S, 18S and 5.8S rRNAs, RNAP II is responsible for the transcription of messenger RNAs (mRNAs) and many non-coding RNAs, whereas RNAP III synthesises small structured RNAs like transfer RNAs (tRNA), spliceosomal U6 small nuclear RNA (snRNA), ribosomal 5S rRNA and 7 SL RNA (Sentenac 1985; Dieci et al. 2007). In plants, two additional multisubunit RNAPs, RNAP IV and V, are encoded. Both these RNAPs are involved in non-coding RNA-mediated gene silencing processes (Haag and Pikaard 2011).

The minimalistic bacterial RNAP is comprised of four different subunits (β, β′, two copies of α and ω). Homologs of the bacterial subunits can be found in all multisubunit RNAPs (Fig. 9.1, panel a: homologous core subunits are coloured in blue). These subunits form the structurally conserved RNAP core that harbours the catalytic centre with coordinated magnesium ions at the interface of the two large bacterial subunits β/β′ and archaeal-eukaryotic subunits Rpo1/2, A190/135, RBP1/2 and C160/128, respectively. Even though there is only a low sequence identity among the core subunits across the domains of life, there is a high degree of structural conservation (Fig. 9.1c). The core subunits are arranged like a "crab claw" forming the DNA cleft. The "jaws" are part of the large subunits and interact with the incoming
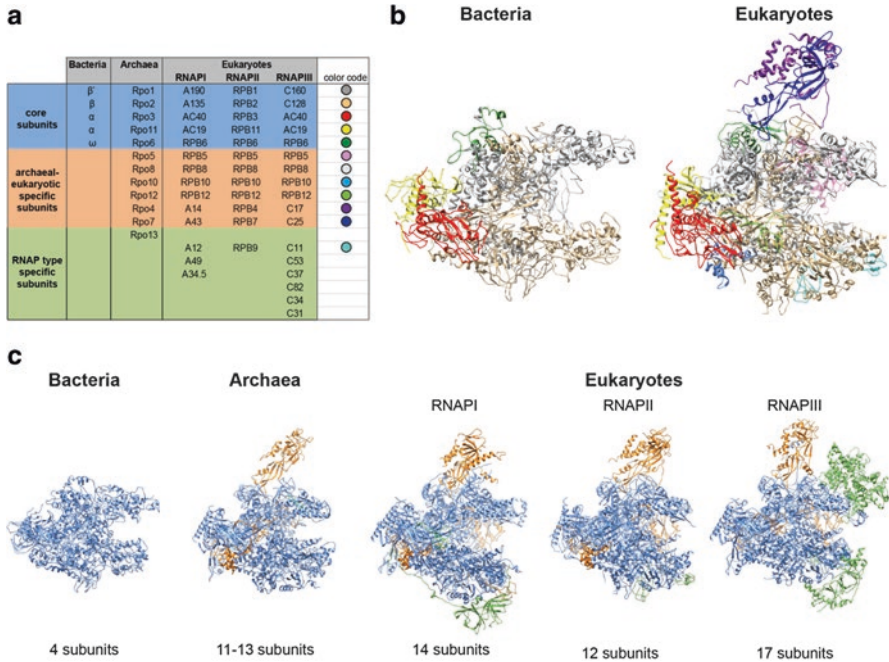
Fig. 9.1 Subunit composition and overall architecture of multisubunit RNA polymerases. (**a**) Subunit composition of multisubunit RNA polymerases in the three domains of life. Homologous core subunits are coloured in *light blue*, archaeal-eukaryotic specific subunits in *light orange* and subunits specific for the archaeal or specialized eukaryotic RNAPs I, II or II are highlighted in *light green*. (**b**) Structure of the bacterial (*left*, PDB:1I6V) and eukaryotic RNAP II (*right*, PDB: 2WAQ) with single subunits coloured according to the colour scheme given in the table. (**c**) The conserved crab-claw like architecture for multisubunit RNA polymerases from bacteria (*left*, PDB: 1I6V), archaea (PDB: 2WMZ) and eukaroytes (RNAP I: *middle*, PDB: 4C3I; RNAP II: *second right*, PDB: 2WAQ; RNAP III: *right*, PDB: 5FJ9). Universally conserved subunits are shown in *blue*, archaeal-eukaryotic specific subunits in *orange* and subunits unique of a specific RNAP type are shown in *green*

downstream DNA substrate (Fig. 9.1b). The large subunits also comprise a number of flexible elements like the bridge helix, the switch region and trigger loop, which are critically involved in the positioning of the next nucleotide and the translocation of RNA and DNA by RNAP (Zhang et al. 2010; Vassylyev et al. 2007; Brueckner et al. 2009a, b; Weinzierl 2010; Tan et al. 2008). Another mobile element of multisubunit RNAPs is the clamp domain, which can close over the DNA binding channel and adopts different conformational states in the different stages of the transcription cycle (Schulz et al. 2016; Chakraborty et al. 2012; Engel et al. 2013). The clamp represents an "interaction hotspot" as regulatory transcription factors like transcription factor E, and the elongation factor Spt4/5 bind the tip of the RNAP clamp region and adjust the position of the clamp (Grohmann et al. 2011; Grunberg et al. 2012; Klein et al. 2011; Schulz et al. 2016).

In addition to the core subunits, archaeal-eukaryotic RNAPs have an expanded set of subunits forming macromolecular assemblies up to 0.7 megadalton (MDa) in size (Fig. 9.1). Archaeal RNAPs typically contain 11–13 subunits (Grohmann et al. 2009) and the eukaryotic RNAPs 12–17 subunits (Vannini and Cramer 2012). Some of these subunits can be found in all archaeal-eukaryotic RNAPs (e.g. homologs of RNAP II subunits RPB4/5/7/8/10/12 highlighted in orange in Fig. 9.1a/c). Notably, subunits RPB5/6/8/10/12 are shared between the eukaryotic RNAPs I, II and III. Subunits 3/10/11/12 form the platform crucially important for the correct assembly of the catalytic subunits. A signature module of archaeal-eukaryotic RNAPs is the stalk domain composed of subunits Rpo4/7 (archaea), A14/A43 (RNAP I), RPB4/7 (RNAP II) and C17/C25 (RNAP III), respectively. The stalk protrudes from the core of the enzyme and is involved in a multitude of functions including the stabilisation of the initiation complex and the binding of the nascent RNA thereby increasing the elongation and termination efficiency of RNAPs. It furthermore serves as an interaction site for transcription factor E. In some archaea, subunit Rpo13 is part of the RNAP. No homologue of Rpo13 is encoded in eukaryotes or bacteria. Another example of a domain-specific subunit is RPB9, which is exclusively found in the eukaryotic domain (domain-specific subunits are color-coded in orange in Fig. 9.1c). RBP9 provides an interaction surface for transcription factor TFIIF (Ziegler et al. 2003) and is closely related to RNAP I and III subunits A12 and C11. However, A12 and C11 represent a fusion protein of the N-terminal RPB9 and the C-terminal part of transcript cleavage factor TFIIS (Ruan et al. 2011) and are considered to be "in-built" transcription factors. RNAP I and III contain additional, auxiliary subunits that are located at the surface of the RNAP thereby providing interaction platforms for factors that regulate RNAP activity. Subunits A49/A34.5 and C53/C37 are distantly related heterodimers that exhibit similarities to transcription factor F (TFIIF) (Fig. 9.2a/d) (Vannini and Cramer 2012). These TFIIF-like complexes are implicated to function in initiation complex stabilisation and occupy a location opposite of the stalk domain (Sainsbury et al. 2015). A49/34.5 furthermore enhances transcription elongation and transcript cleavage and C53/37 is important for termination. Just like A49/A34.5 and C53/C37, the protein complex C82/C34/C31 also represents an "in-built" transcription factor. C82/C34 is related to subunits alpha and beta of transcription factor E, which is associated with, but not integrated into, the archaeal RNAP and RNAP II (Fig. 9.2a/c) (Vannini and Cramer 2012; Blombach et al. 2015; Carter and Drouin 2010; Blombach et al. 2016). The archaeal TFE, TFIIE and C82/C34 all map to the RNAP clamp domain and fulfil functions during transcription initiation by stabilisation of the open DNA bubble (Blombach et al. 2016; Grohmann et al. 2011; Grunberg et al. 2012; Engel et al. 2013; Hoffmann et al. 2015). A unique feature of the largest core subunit of RNAP II is the C-terminal domain (CTD) composed of tandem heptad repeats (26 in yeast, 52 in vertebrates). The CTD is subject to extensive posttranslational modifications, most notably phosphorylation, throughout the transcription cycle, which allows a fine-tuned regulation of RNAP II activity and, among others, supports the coupling of transcription and post-transcriptional processing (for an overview see for example

a

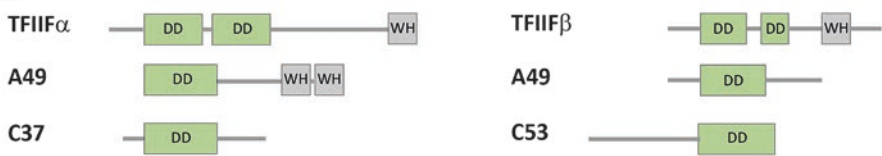| Function	Bacteria	Archaea	Eukaryotes		
			RNAPI	RNAPII	RNAPIII
promoter DNA binding	σ	TBP	TBP UAF [UBF]	TBP (TFIID) TFIIA	TBP (TFIIIB) TFIIIA; TFIIIC; [SNAPc]
& polymerase binding	σ	TFB	Rrn7 (CF), [TAF1A (SL1)]	TFIIB	Brf1 [Brf2] (TFIIIB)
polymerase binding & recruitment	σ		Rrn3 [TIF-IA]	Mediator	
initiation complex stabilisation & start site selection	σ		A49 [PAF53] (RNAP I) A34.5 [PAF49, CAST] (RNAP I)	Tfg2 [TFIIFα] (TFIIF) Tfg2 [TFIIFβ] (TFIIF)	C37 (RNAP III); Sfc1 (TFIIIC) C53 (RNAP III); Sfc7 (TFIIIC)
open complex formation	σ	TFEα (TFE) TFEβ (TFE)	A49 [PAF53] (RNAP I)	Tfa1 (TFIIEα) (TFIIE) Tfa2 (TFIIEβ) (TFIIE) TFIIH	C82 (RNAP III) C34 (RNAP III)
RNA cleavage	GreA; GreB	TFS	A12.2 (RNAP I)	TFIIS	C11 (RNAP III)
increasing processivity	NusG	Spt5 Spt4	Spt5 Spt4		
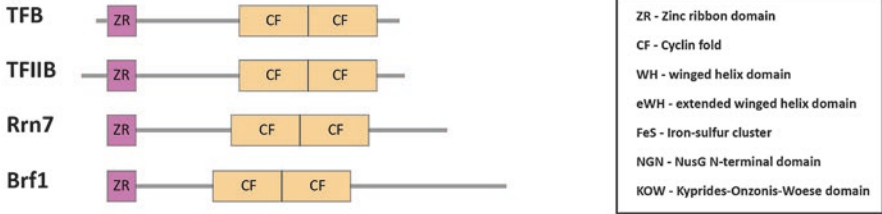
Fig. 9.2 Structure function relationship between transcription factors in bacteria, archaea and eukaryotes. (**a**) Overview of transcription factors implicated in transcription initiation and elongation in bacteria, archaea and eukaryotes. Factors with structural homologies are marked in *bold letters*. The yeast name and the mammalian name (in *squared brackets*) of the homologous protein are given. If these proteins are complex components, the name of the respective complex is depicted in *round brackets*. (**b–e**) Schematic representation of transcription factors and RNAP subunits sharing structural homologies. Structural domains are depicted as *boxes*, with similar domains having the same colour. Abbreviations are explained in the *legend on the right* and aSpt5 and eSpt5 refers to archaeal and eukaryotic Spt5, respectively

(Hsin and Manley 2012; Conaway and Conaway 2015). Taken together, multisubunit RNAPs are multiprotein complexes that are organised in conserved functional domains (e.g. core, assembly platform, stalk, clamp, jaws, DNA cleft) characterised by an increase in complexity and diversity in archaeal-eukaryotic RNAP variants.

9.2.2 Transcription Factors of RNA Polymerases in Bacteria, Eukaryotes and Archaea

Although RNAPs can synthesise RNAs on their own, they require additional factors guiding them to the promoter regions, as well as assisting transcription initiation, elongation and eventually termination. Initially, these factors were identified in different organisms owed to their ability to support different steps of transcription in highly purified *in vitro* systems. Results from these biochemical studies led to detailed models for the function of the individual factors within the transcription cycle. Many of the hypotheses derived from these biochemical studies were validated and extended by structural characterisation. Genetic analyses and the advent of technologies that allow the monitoring of the association status of the transcription machinery at virtually every gene locus *in vivo*, further helped to define a set of general factors supporting transcription in living cells.

As described before, RNAPs in the different domains of life share remarkable similarities with regard to their structure and function, without strong conservation at amino acid sequence level. This structure-function relationship is even more pronounced for transcription factors. Thus, proteins unrelated in their amino acid composition may exert similar functions, correlating with similar topology of structural elements. Consistently, in some cases, predicted structural similarities rather than sequence homologies have provided insights in a previously unknown role of a factor. This paragraph aims to put emphasis on the structure-function conservation within transcription factors in the three domains of life and the different transcription systems (Fig. 9.2). The focus will be on the so-called general transcription factors of transcription initiation, as well as on two factors implicated in transcription elongation. Details about the functional interaction of these factors with RNAPs in course of the transcription cycle are provided in paragraph 3.

9.2.2.1 Initiation Factors

RNAP needs transcription factors to: (i) Identify promoter regions, (ii) Mediate its interaction with the DNA, (iii) Determine the direction of transcription, (iv) Melt the double stranded template leading to the open complex, (v) Enter transcription elongation (Sainsbury et al. 2015; Werner and Grohmann 2011)

In bacteria, all of these tasks are accomplished by the protein family of the promoter specific sigma factors (σ factors) (Feklistov et al. 2014). σ factors were discovered as a biochemical fraction required by purified *E. coli* RNAP to transcribe selective regions of lambda DNA (Burgess et al. 1969). σ factor bound to RNAP recognises up to four out of five DNA motifs marking a prokaryotic promoter (see Sect. 9.3.2) (Bae et al. 2015). This is explained by the structure of σ proteins, organized in a variable number of folded protein domains (e.g. $\sigma_{1.1}$, σ_2, σ_3, σ_4 in the housekeeping σ^{70} in *E. coli*), which are connected by flexible linkers (Fig. 9.3a). Except $\sigma_{1.1}$, all other domains share the ability to bind to specific DNA elements. In solution, σ factors adopt a compact conformation, interfering with DNA-binding of the individual domains (Sorenson and Darst 2006; Sorenson et al. 2004). Additionally, σ^{70} has been suggested to bear an autoinhibitory N-terminal domain ($\sigma_{1.1}$)(Dombroski et al. 1992), which might stabilize the compact conformation. Upon interaction with core RNAP, σ factors undergo structural rearrangements yielding the initiation competent holoenzyme (Murakami et al. 2002a, b). After promoter binding of the holoenzyme, σ factors assist in formation and stabilisation of the nascent transcription bubble, owed to the ssDNA binding ability of the σ_2 domain (Bae et al. 2015) (see Sect. 9.3.2, Fig. 9.3a). The nascent RNA produced at the onset of transcription interferes with some of the σ-RNAP contacts resulting in stochastic dissociation of σ factors from the elongating RNAP after promoter escape (Nickels et al. 2005). The arrangement of structured domains and flexible linkers found in σ factors, which undergo functionally relevant structural changes in different phases of the transcription process, are a common feature of many transcription factors (see below).

The expression of σ variants with different promoter specificities provides prokaryotes with the possibility to express genes under specific environmental conditions (e.g. activation of genes induced under nitrogen stress conditions by σ^{54}) (Gruber and Gross 2003). Archaea and eukaryotes do not have homologs of σ factors for promoter recognition. Instead, they own the TATA-binding protein (TBP), and transcription factor B (TFB/TFIIB), which – similar to σ_4 in bacteria – bind to upstream DNA sequences in archaeal and eukaryotic promoters (Littlefield et al. 1999; Nikolov et al. 1995) (Fig. 9.3b/c). In contrast to bacterial σ factors, TBP and TFB/TFIIB may bind to promoter DNA in the absence of RNAPs, which are then recruited to the promoter-bound scaffold (Sainsbury et al. 2015). In some archaea and eukaryotes, the TBP gene underwent gene-duplication resulting in paralogs of TBP with distinct functions in gene regulation (Duttke 2015). Additionally, some archaea express multiple paralogs of TFB (Facciotti et al. 2007). Thus, in all three domains of life, gene duplication events resulting in variants of core promoter binding factors lead to an increase in the regulatory potential of gene expression.

Promoter-specific transcription by eukaryotic RNAPs I and III also strictly depends on TBP-interacting complexes containing TFB-like factors (Fig. 9.2a/b): RNAP I requires yeast core factor (CF), or its mammalian counterpart, selective factor 1 (SL1), whereas RNAP III depends on TFIIIB (Vannini and Cramer 2012). Homology between TFB/TFIIB, and the TFIIIB subunit Brf1 (and its paralog Brf2) have been described earlier (Colbert and Hahn 1992; López-De-León et al. 1992).
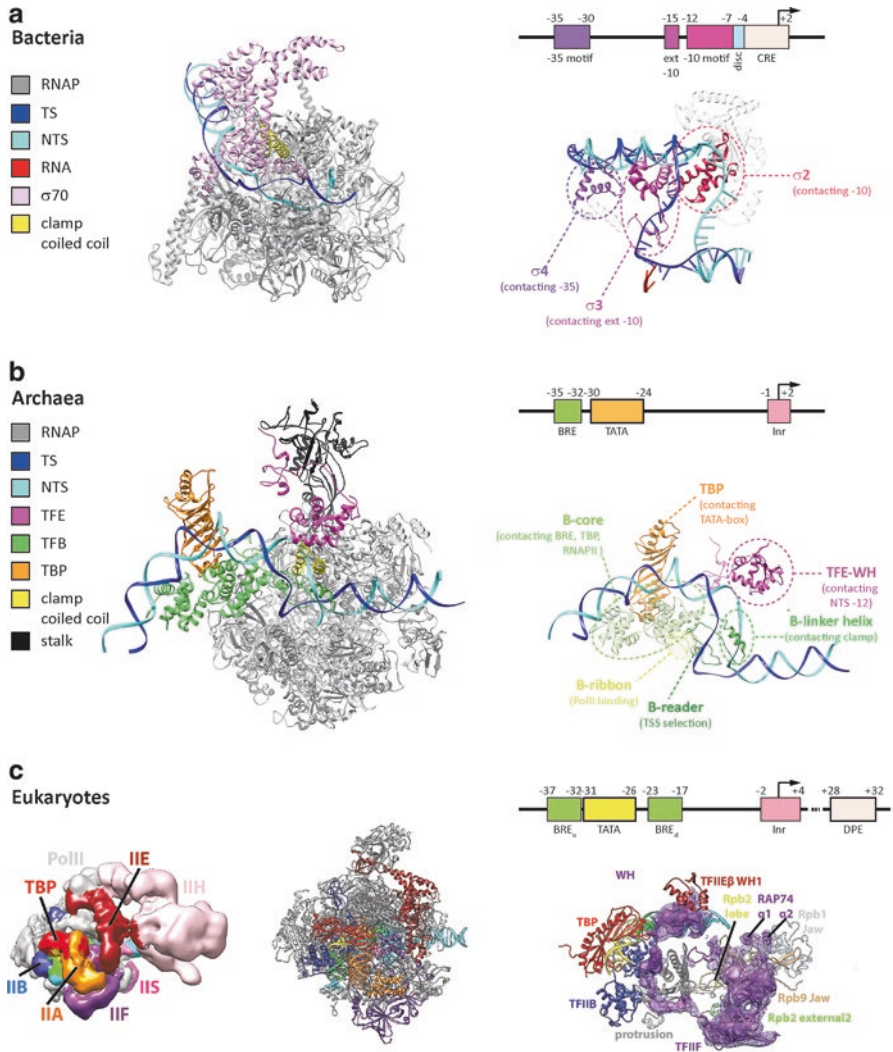
Fig. 9.3 Architecture of the open initiation complex in the three domains of life. In all cases, the template strand (TS) is inserted into the active site of the RNAP and the non-template strand (NTS) is accommodated outside the DNA binding cleft. (**a**) The bacterial initiation complex is composed of a sigma factor (here σ^{70}), the RNAP and DNA (PDB: 4YLO). σ^{70} is a multidomain protein that recognises the −35, −10 and discriminator (*disc*) motifs of the bacterial promoter and supports DNA melting. The RNAP contacts bases of the core recognition element (CRE) that surrounds the transcription start site (+1). (**b**) Model of the archaeal open pre-initiation complex (from (Nagy et al. 2015). The transcription initiation factors TBP and TFB recognise the TATA-box and B-recognition element (BRE) upstream of the transcription start site, respectively. The TFB-reader/helix domains are crucially important for promoter melting and transcription bubble stabilisation – a process furthermore supported by TFE. (**c**) Architecture of the human open pre-initiation complex (images kindly provided by Eva Nogales). EM-reconstruction of the holo-PIC (including TFIIH, *left*) and model of the core PIC (without TFIIH, *middle*). The arrangement of the transcription factors and RNAP motifs critically involved in promoter opening is shown (panel on the *right*). RAP74 is a subunit of TFIIF. Archaeal and eukaryotic promoters (shown here: human promoter) contain the initiator element (Inr) surrounding the transcription start site, and eukaryotic promoters additionally the downstream promoter element (DPE)

The homology between TFB/TFIIB and the yeast CF subunit Rrn7, or its human orthologue the SL1 subunit TAF1B, were only recently uncovered (Blattner et al. 2011; Naidu et al. 2011; Knutson and Hahn 2011). The latter was possible using a computational homology and structure prediction tool (Söding et al. 2005), in combination with functional assays. In agreement with the conserved function of σ-factors and TFB-like factors, the topology of secondary structure elements of these proteins within the different pre-initiation complexes (PICs), is remarkably similar (Burton and Burton 2014; Sekine et al. 2012) (Fig. 9.3a), despite the fact that they are unrelated in their amino acid sequence.

In addition to TBP and TFB, TFE is the third general transcription initiation factor common in the archaeal and eukaryotic transcription systems (Fig. 9.2a/c). Archaeal TFE occurs as either a monomeric TFEα (e.g. TFE from *Methanocaldococcus jannaschii*) or a dimeric TFEα/β variant (e.g. TFE from *Sulfolobus solfataricus*) (Blombach et al. 2009). TFEα is a homolog to the N-terminal part of the TFEIIEα subunit in the eukaryotic TFIIE heterodimer while the TFEβ subunit found in *Sulfolobus* is composed of one winged helix domain and one [4Fe-S] cluster containing domain that are homologous to the human RNAP III subunit RPC39 (Blombach et al. 2015, 2016) (Fig. 9.2c). TFE and TFIIE both contain winged helix (WH) domains (Meinhart et al. 2003). Interestingly, a tandem WH domain similar to a pair of WH domains in TFIIEβ has been found in the C-terminal region of the RNAP I subunit A49, and two adjacent WH domains exist in the RNAP III subunit C34 (Geiger et al. 2010). The human homolog of C82 has four WH domains, resembling the extended WH domain in TFE/TFIIEα (Lefèvre et al. 2011). As TFE/TFIIE, the WH domains of A49 and C34 may adopt a topologically similar positon over the cleft (Bischler et al. 2002; Vannini et al. 2010; Jennebach et al. 2012). As mentioned before (see Sect. 9.2.1), RNAP I and RNAP III subunits can structurally and functionally resemble RNAP II transcription factors, and may be considered as "in-built" transcription factors (Vannini and Cramer 2012).

Several transcription initiation factors are unique to the eukaryotic transcription machinery, some of which have paralogs in the different RNAP machineries. The transcription factor TFIIA is not required for RNAP II transcription *in vitro*, but it can stimulate RNA synthesis (Kang et al. 1995). This essential factor, composed of two subunits, has functions in stabilising the TBP-DNA interaction (Imbalzano et al. 1994). No TFIIA-like factor has to date been identified in the RNAP I and RNAP III transcription machineries.

Instead, RNAPs I and III contain subunits, structurally resembling TFIIF in the RNAP II system (Vannini and Cramer 2012) (see Sect. 9.2.1, Fig. 9.2a/d). Human TFIIF is a dimer of TFIIFα and TFIIFβ (Burton et al. 1988), with homologs in yeast (Chafin et al. 1991), acting together with an additional, non-essential TFIIF subunit (Henry et al. 1992). TFIIFα and TFIIFβ both contain a C-terminal WH domain (Groft et al. 1998; Kamada et al. 2001) (Chen et al. 2007; Eichner et al. 2010) (Fig. 9.2d). TFIIF binds to the lobe of RNAP II via a dimerisation module formed by the two subunits (Gaiser et al. 2000). A similar dimerization module is found in the A49/34.5 subunits of RNAP I and the C37/53 subunits of RNAP III (Geiger

et al. 2010; Kuhn et al. 2007). In both polymerases, the respective dimerization modules bind close to the lobe (Engel et al. 2013; Fernandez-Tornero et al. 2013) (Fig. 9.3c) and – as TFIIF – might be implicated in diverse steps of transcription initiation. Interestingly, two subunits of the RNAP III-specific transcription factor TFIIIC establish a structure with a TFIIF-like dimerisation module, and a WH domain (Taylor et al. 2013). It has been hypothesised that these subunits assist PIC formation, while stabilising the single stranded non-template strand (NTS) DNA. There is evidence that the DNA binding mode of the TFIIIC subunit might resemble the one observed for the bacterial σ_2 domain to the single stranded -10 region (see also Sect. 9.3.2). A similar mechanism, supporting promoter opening, was suggested for TFIIF, which shows weak homology to σ_2 (Tan et al. 1994).

The core RNAP I and III machineries lack TFIIH-related factors. This 10-subunit transcription factor is recruited by TFIIE to the RNAP II PIC, where a translocase activity is required for OC formation in an ATP-consuming step (see Sect. 9.3.2) (Grunberg et al. 2012; Kim et al. 2000). Other enzymatic activities of holo-TFIIH include the phosphorylation of the CTD of the largest subunit of RNAP II (Feaver et al. 1991; Serizawa et al. 1995). A TFIIH subcomplex has an important role in DNA repair processes, mediated by two subunits with helicase activities (Coin et al. 2007). TFIIH has been visualized by cryo EM in both, the human and the yeast PIC, and the overall topology of the complex was consistent with its suggested functions during transcription initiation (He et al. 2016; Murakami et al. 2015; Murakami et al. 2013b).

Two other megadalton-sized regulatory complexes, TFIID and Mediator, are important for the regulation and modulation of RNAP II transcription initiation (Cler et al. 2009; Poss et al. 2013; Sikorski and Buratowski 2009; Thomas and Chiang 2006). TBP was originally identified as a component of TFIID, a large complex with 13 canonical TBP associated factors (TAFs), supporting activated transcription *in vitro*. *In vivo*, TBP in the context of TFIID can be recruited to genes lacking a TATA box, due to the recognition of additional promoter elements by TAFs (Baumann et al. 2010; Kadonaga 2012). Interestingly, interaction of the transcription factor TFIIA with TBP displaces the TAF1 N-terminal domain 1 from the TBP DNA-binding surface, enabling its interaction with promoter DNA (Kokubo et al. 1994). TFIID supports activated transcription from chromatin templates *in vitro*, suggesting that this might be important for its *in vivo* function in the chromosomal context (Wu and Hampsey 1999). Several high-resolution structures of individual TAF subcomplexes are available (Sainsbury et al. 2015) helping to interpret recent cryo EM structures of the full complex in solution or bound to a promoter DNA (Bieniossek et al. 2013; Cianfrocco et al. 2013; Elmlund et al. 2009; Louder et al. 2016; Papai et al. 2010).

Mediator was identified in search of a complex that transmits regulatory signals of DNA-binding factors to the RNAP II basal transcription machinery (Casamassimi and Napoli 2007; Thomas and Chiang 2006). Mediator also supports basal transcription by assisting PIC assembly i*n vitro,* and regulates CTD phosphorylation by TFIIH (Sikorski and Buratowski 2009). As for TFIID, X-ray structures are available for individual Mediator subunits and sub-complexes and EM analyses

have been performed with both the free complex and RNAP II-bound Mediator (Larivière et al. 2012). Recently, EM structures of the human Mediator-RNAP II-TFIIF complex (Bernecky et al. 2011), and a 15 subunit core Mediator interacting with an RNAP II core initiation complex, containing TFIIB, TBP, and the Pol II–TFIIF (Plaschka et al. 2015) have been obtained. The latter analysis suggested that Mediator might enhance CTD-phosphorylation by orienting the TFIIH kinase module and part of the CTD in the PIC. It also provided insights in how Mediator might interact with the upstream DNA and RNAP II to communicate signals from regulatory DNA binding proteins (Plaschka et al. 2016b).

Interestingly, the binding of the Mediator head module to RNAP II might be topologically related to the binding of the essential transcription factor Rrn3 (TIF-IA in mammals) to RNAP I (Blattner et al. 2011). Yeast Rrn3 and CF are sufficient to support RNAP I transcription *in vitro* even in absence of TBP (Bedwell et al. 2012; Keener et al. 1998; Merkl et al. 2014). The RNAP I-Rrn3 complex is the initiation-competent form of the polymerase, which might be recruited by CF bound to the RNAP I promoter (Peyroche et al. 2000). RNAP I-Rrn3 complex formation might be important to stabilise the interaction of the polymerase with Rrn7 driving transcription initiation (Blattner et al. 2011; Knutson et al. 2014).

TFIIIA represents a transcription factor, which is conserved in all eukaryotes investigated so far and is exclusively involved in RNAP III-dependent transcription initiation (Layat et al. 2013). It was the first eukaryotic transcription factor purified to homogeneity (Engelke et al. 1980; Segall et al. 1980). TFIIIA is needed for 5S rDNA transcription by RNAP III *in vitro* and *in vivo* (Andrews and Brown 1987; Rollins et al. 1993). The protein contains multiple C_2H_2 zinc finger repeats and binds to a specific DNA-sequence upstream of the 5S rDNA. Using its zinc fingers, TFIIIA might also bind to rRNA (Brow and Geiduschek 1987). Conservation between TFIIIAs from different species is largely restricted to the zinc finger domain (Huang and Maraia 2001), and the structural basis for its interaction with nucleic acids has been provided by X-ray crystallography (Lu et al. 2003).

9.2.2.2 Elongation Factors

After initiation, RNAPs start the elongation of the nascent transcript (see Sect. 9.3.3). Specific factors have evolved that directly interact with the RNAP and assist RNAPs to efficiently promote elongation. Interestingly, two general mechanisms of elongation factor operation appear to be conserved in the three domains of life (see Sect. 9.3.3):

 I. Factors binding to the RNAP clamp domain, closing the RNAP active centre cleft and thereby stabilising the elongation complex (Fig. 9.5a)
II. Factors entering the RNAP active site via the secondary channel catalysing cleavage of backtracked transcripts and thus release arrested RNAPs (Fig. 9.5b)

Secondary channel binding proteins in bacteria belong to the GreA/B family and are unrelated in sequence and structure to the archaeal and eukaryotic secondary

channel binding TFIIS-like proteins (Nickels and Hochschild 2004; Werner and Grohmann 2011). GreA/B and TFIIS stimulate elongation *in vitro* by releasing arrested, backtracked RNAPs (see Sect. 9.3.3) (Borukhov et al. 1992; Rutherford et al. 2007; Thomas et al. 1998). As observed for σ factors and TFB-like factors, those factors share striking similarity in their overall topology in complex with RNAP in good correlation with their related function (Cheung and Cramer 2011; Tagami et al. 2010; Wang et al. 2009). TFIIS-like proteins are conserved in archaea and in RNAP I and III subunits A12.2, and C11 (see Sect. 9.2.1) (Vannini and Cramer 2012; Werner and Grohmann 2011). Apart from their function in transcription elongation, secondary channel binding proteins in bacteria and eukaryotes may play a role in transcription initiation (Sikorski and Buratowski 2009).

The NusG/Spt5 RNAP clamp binding elongation factor is the only transcription factor conserved in sequence and structure in all three domains of life (Werner and Grohmann 2011; Yakhnin and Babitzke 2014). Bacterial NusG and archaeal Spt5 bear a NusG N-terminal domain (NGN) and a C-terminal Kyprides-Onzonis-Woese (KOW) domain (Knowlton et al. 2003; Mooney et al. 2009) (Figs. 9.2e and 9.5b). In eukaryotes, Spt5 may carry multiple KOW domains and extensions at the N- and C-termini. Archaeal and eukaryotic Spt5 further form a heterodimer with Spt4 (Hirtreiter et al. 2010a; Klein et al. 2011).

Taken together, transcription factors in the three domains of life seem to fall in at least three different categories:

  I. Factors highly similar in sequence and structure (e.g. NusG/Spt5; TBP; TFB/ TFIIB, TFE/TFIIE)
 II. Factors with high structural homology (e.g. TFIIF/A34.5–49/C37–53/TFIIIC)
III. Factors, which do not share obvious sequence or structural similarities but are related in the topology of secondary structure elements in complex with RNAPs (e.g. GreA-B and TFS/TFIIS, σ-factor and TFB/TFIIB)

Thus, within the highly conserved RNAP active centre (see Sect. 9.2.1), transcription factors are required to adopt similar topologies to efficiently stimulate and regulate the catalytic process. The increasing complexity of the transcription machinery in eukaryotes, when compared to bacteria and archaea, culminates eventually in the evolution of complexes like TFIID and Mediator, comparable in size to RNAP II with associated general transcription factors. This complex network of protein-protein interactions may allow RNAP to integrate a large variety of different signals from DNA-bound regulators and from components of chromatin, the DNA-template of eukaryotic transcription.

9.3   Structural Dynamics of RNA Polymerase Throughout the Transcription Cycle

9.3.1   Overview

In the course of transcription, RNAPs repeatedly cycle through three distinct functional phases termed initiation, elongation and termination. Throughout the transcription cycle, RNAPs are associated with and regulated by transcription initiation, elongation and termination factors. During initiation, RNAP is specifically recruited to the promoter DNA located upstream of the transcription start site (TSS). Transcription initiation factors like σ factors (bacteria) or TBP and TFB-like factors (archaea/eukaryotes) specifically recognise and bind sequences in the promoter DNA, recruit the RNAP to the promoter and establish the directionality for transcription. Following RNAP recruitment, the DNA is locally melted at the TSS to allow loading of the template (TS) DNA strand into the active site of the RNAP. This process – called open complex formation – eventually yields the stable transcription bubble. RNAPs are able to start RNA synthesis *de novo* requiring no primer for RNA polymerisation. Once the RNAP escapes from the promoter, the phase of productive RNA synthesis (elongation) starts and the RNAP translocates along the TS with each new nucleotide added to the growing transcript chain. Elongation is a discontinuous process frequently interrupted by pausing events that can lead to backtracking of the RNAP. The elongation factors reduce pausing or recover backtracked arrested transcription complexes, thereby increasing the processivity of the RNAP. Eventually, the RNAP will encounter a termination signal leading to the dissociation of the elongation complex and release of the newly synthesized RNA.

9.3.2   Initiation

9.3.2.1   Bacteria

In bacteria, the RNAP core is transiently bound to a member of the σ factor family forming the bacterial RNAP holoenzyme. The number of encoded σ factors differs between bacterial species but one of the σ factor often serves a "housekeeping" factor that supports transcription initiation from the majority of promoters (Feklistov et al. 2014). *E.coli* encodes seven σ factors among which σ 70 ($σ^{70}$, also known as RpoD in *E.coli* and as $σ^A$ in many other bacteria) is the "housekeeping" factor (Paget 2015). A well-studied alternative σ factors is $σ^{54}$ (also known as RpoN or $σ^N$), which directs transcription under stress conditions and in response to environmental signals. $σ^{70}$ and $σ^{54}$ are structurally unrelated and belong to two distinct classes of σ factor that differ in their mode of action during transcription initiation (Paget 2015; Yang et al. 2015).

σ^{70} is organized in four functional domains termed $\sigma_{1.1}$, σ_2, σ_3 and σ_4 that are connected by flexible linkers (see Sect. 9.2.2). Sigma as part of the holoenzyme (but not by itself) undergoes specific interactions with conserved sequence elements of the bacterial core promoter on the one hand side and with the RNAP to perform sigma's three main functions:

I. Sequence-specific recognition of the promoter
II. Recruitment of the RNAP to the promoter
III. Promoting strand separation for initial transcription bubble formation (Paget 2015)

A helix-turn-helix motif in the highly conserved σ region 4 undergoes specific interactions with five nucleotides in the −35 region of the promoter and bends the DNA in a 30° angle (Basu et al. 2014; Murakami et al. 2002a; Zhang et al. 2012; Zuo and Steitz 2015). σ region 3 and 2 interact with the extended −10 motif and the −10 and discriminator region of the NTS, respectively (Fig. 9.3a). These interactions are critical for the site-specific recognition of the promoter DNA and only occur if the binding interfaces of sigma are exposed as a result of its interaction with the core RNAP. σ recognises the −35 and −10 motif in the double-stranded form and the nucleotides in the extended −10 and discriminator motif are "read" upon strand separation. In contrast, the bases of the NTS of the core recognition element (CRE) interact directly with the RNAP β subunit between positions −4 to +2. Here, a G at position +2 (G_{+2}) is unstacked and inserted into a deep pocket of the β subunit in a manner in which σ interacts with the bases A_{-11}, T_{-7} and G_{-6} (Zhang et al. 2012). It is thought that DNA opening starts at position A_{-11} followed by an extension of the initial melted region to yield an initial transcription bubble of 12–14 bases resulting in the open initiation complex (RP_o). The interaction of $\sigma2$ with the −10 motif results in a 90° bent in the DNA that directs the downstream DNA toward the RNAP active site cleft allowing the loading of the TS into the active site (Feklistov and Darst 2011). Upon sequence-specific recognition of the promoter DNA and loading of the DNA, $\sigma_{1.1}$ is displaced from the cleft. Relocation of $\sigma_{1.1}$ – a sigma domain that is only present in "housekeeping" sigma factors – does not occur if DNA is non-specifically bound to the RNAP rendering $\sigma_{1.1}$ a "gatekeeper" for the RNAP active site. Part of the linker region that connects σ_3 and σ_4 specifically interacts with the RNAP inserting a loop region (termed the sigma finger, $\sigma_{3.2}$) deep into the active site of the RNAP occupying the pathway of the growing RNA chain. Insertion of $\sigma_{3.2}$ stimulates binding of NTPs in the active site but when the RNA chain extends to a length of only four to five nucleotides, $\sigma_{3.2}$ contacts the nascent RNA. While this potentially stabilises short RNA-DNA hybrids (Zuo and Steitz 2015), it eventually results in a stressed intermediate state when further NTPs are added to the RNA. Single-molecule studies showed that during initial transcription, the RNAP does not translocate but remains connected to the promoter and downstream DNA is "scrunched" into the RNAP further adding to the accumulated stress (Kapanidis et al. 2006; Revyakin et al. 2006). The occurrence of the stressed intermediate state provides the mechanistic rational for an observation called "abortive

initiation". Initially, RNAP repeatedly synthesises and releases short RNA transcript 2–15 nucleotides in length (Goldman et al. 2009) without entering the productive elongation phase, as σ blocks the RNA exit pathway. RNA release instead of productive initiation beyond nucleotide 15 is one possibility to escape from the stressed intermediate state while staying connected to the promoter DNA. Alternatively, the accumulated stress provides the driving force for promoter escape leading to productive initiation. The sigma finger and σ_4 are only displaced if the RNA reaches a length of 16 nucleotides and the RNA successfully competes for the space occupied by $\sigma_{3.2}$ (Nickels et al. 2005). Open complex formation is a spontaneous energy-independent process in σ^{70} containing holoenzymes, but σ^{54} holoenzymes require an additional transcriptional activator protein that belongs to the AAA+ ATPase family before DNA melting can commence (Wigneshweraraj et al. 2008; Saecker et al. 2011; Yang et al. 2015). The coupling of ATP hydrolysis to transcriptional activation allows a tighter control of the promoter and overall transcriptional output thereby allowing a swift and precise response to environmental change. Loading and unwinding of the DNA also leads to structural changes in the RNAP. The mobile RNAP clamp is predominantly in an open conformation in the DNA-free state of the bacterial RNAP. Upon open complex formation, the clamp closes securing the DNA in the nucleic acid cleft (Chakraborty et al. 2012).

9.3.2.2   Archaea

In archaea, transcription initiation is mediated by two transcription initiation factors, TBP and TFB. While the function of TBP and TFB – e.g. the sequence-specific positioning of the RNAP at the TSS – is comparable to that of the bacterial σ factors, TBP and TFB are not homologous to sigma. TBP recognises an AT-rich region termed the TATA-box centred at position −26/−27 with respect to the TSS (Soppa 1999). Upon binding, TBP induces a severe bend of approximately 90°in the DNA (Littlefield et al. 1999; Gietl et al. 2014). The B recognition element (BRE) is another archaeal promoter element located at the upstream end of the TATA-box (Fig. 9.3b). BRE is specifically recognised by the C-terminal cyclin domain of TFB (Werner and Weinzierl 2005). The TBP-DNA interaction is of transient nature and in some archaeal species (e.g. the crenarchaeon *Sulfolobus acidocaldarius*) TFB is required to stabilise the TBP-DNA interaction (Gietl et al. 2014) and recruits the RNAP via its N-terminal domains to the promoter DNA to form the functional pre-initiation complex (PIC) (Bell and Jackson 2000; Bell et al. 1999). As TBP is composed of two symmetric repeats (Brindefalk et al. 2013), TFBs orientation at the promoter determines the directionality of the transcription initiation complex on the DNA. TFB furthermore fulfils functions at the post-recruitment stage aiding in start site selection and promoter opening (Kostrewa et al. 2009; Wiesler et al. 2013; Wiesler and Weinzierl 2011). TBP and TFB are necessary and sufficient to drive promoter-directed transcription in the archaeal transcription system and transcription bubble formation does not necessitate ATP hydrolysis (Hausner et al. 1996;

Werner et al. 2006; Werner and Weinzierl 2002; Bell et al. 1998; Qureshi et al. 1997). However, TFE stabilises the PIC and aids open complex formation (Bell et al. 2001; Grohmann et al. 2011; Grünberg et al. 2007; Werner and Weinzierl 2005). Both, TFB and TFE are comprised of multiple domains connected by flexible linkers (Fig. 9.2b/c). A high-resolution structure of the archaeal PIC is not available but structures of archaeal and homologous eukaryotic subcomplexes exist, providing insights into the structural organisation of TFE and TFB and their respective RNAP binding sites. The N-terminal part of TFB is composed of the B-ribbon, −reader and –linker domains. The B-ribbon domain interacts with the RNAP dock domain while the reader and linker protrude deep into the cleft contacting the RNAP clamp and partly occupying the RNA exit channel (Fig. 9.3b) (Kostrewa et al. 2009; Liu et al. 2010; Sainsbury et al. 2013). The B-reader follows a similar path as σ3.2 that likewise results in a clash with the emerging RNA chain. TFB contacts the TS in a way that allows the organisation of the transcription bubble and prevents a tilting of short DNA-RNA hybrids thereby stimulating transcription (Werner and Weinzierl 2005; Sainsbury et al. 2013).

TFEα/β enhance open complex formation and stimulate productive initiation (Blombach et al. 2015; Grohmann et al. 2011). Archaeal TFEα is composed of an N-terminal WH domain that interacts with the RNAP clamp and a C-terminal zinc ribbon domain that contacts the RNAP at the base of the stalk – an interaction network conserved in the eukaryotic domain (Grohmann et al. 2011; Grunberg et al. 2012; Nagy et al. 2015; Engel et al. 2013). The stalk domain is fundamentally important for the recruitment and function of TFEα. The contribution of TFEβ to TFE function is less well understood. The C-terminal domain of TFEβ from *S. solfataricus* contains a [4Fe-S] cluster that is important for dimerization with TFEα and the interaction with the RNAP clamp. TFEα executes its function by contacting the NTS at position −12 thereby stabilising the upstream edge of the transcription bubble and it is likely that TFEβ (like its eukaryotic counterpart) reaches towards the cleft to assist with the handling of DNA strands.

Due to its high flexibility, the structure of the archaeal initiation complex could not be captured at high resolution. Nevertheless, using the distance constraints derived from single-molecule FRET measurements in combination with the known partial structures allowed the modelling of the archaeal open PIC (Nagy et al. 2015). The DNA is melted between position −12 and +2 and the TS is fully loaded into the active site while the NTS is deposited outside the cleft (Fig. 9.3b). The location of the downstream DNA is comparable to the bacterial and eukaryotic initiation complex. The upstream edge of the transcription bubble is located near the RNAP clamp and WH domain of TFEα suggesting that these structural elements collaborate to achieve the stabilisation of the transcription bubble. As observed for the bacterial RNAP clamp, a conformational change of the RNAP clamp accompanies the transition from the closed (dsDNA association without loading into the RNAP) to open complex. This transition is stimulated by TFE resulting in an equilibrium shift towards the open clamp if the PIC contains TFE (Schulz et al. 2016).

9.3.2.3 Eukaryotes

The basal transcription machineries of the archaeal and eukaryotic RNAP II tran-
scription system are highly conserved. In both systems, TBP and TFIIB (homolo-
gous to the archaeal TBP and TFB) are sufficient to drive transcription from strong
promoters and negatively supercoiled templates (Parvin and Sharp 1993). Archaeal
TFE has the eukaryotic TFIIE as a functional counterpart. However, RNAP II initia-
tion complexes contain additional general transcription factors TFIIA, TFIIF and
TFIIH. Moreover, TBP is mainly part of the multiprotein complex TFIID (see Sect.
9.2.2). Like the archaeal PIC, the eukaryotic initiation complex most likely forms
from individual factors in a stepwise manner *in vivo* (Fig. 9.3c). Initially, TBP (as
part of TFIID) recognises the promoter DNA approximately 30 base pairs upstream
of the TSS (in humans). While TBP is also required for transcription from all
eukaryotic promoters, the presence of a TATA box is not mandatory for TBP bind-
ing. In fact, only 10–15% of the mammalian promoters contain a TATA-box
(Huisinga and Pugh 2004; Lee et al. 2000). Nevertheless, TBP is found to be associ-
ated with the majority of TATA-containing and TATA-less promoters in yeast (Rhee
and Pugh 2012; Juo et al. 2003) demonstrating the general role of TBP in transcrip-
tion initiation. The mechanism of DNA-bending differs from the archaeal system,
as eukaryotic TBP bends the DNA in a two-step process (Blair et al. 2012; Gietl
et al. 2014; Tolic-Norrelykke et al. 2006; Wu and Hampsey 1999). Eukaryotic TBP
forms long-lived complexes with TATA-containing promoter DNAs that are stable
for minutes to hours. TFIIB stabilises the TBP-DNA complex in its fully bend form
(Gietl et al. 2014). The auxiliary transcription factor TFIIA binds to the upstream
side of the TATA-box and stabilises the TBP-DNA complex without changing the
overall architecture of the complex (Blair et al. 2012). Like archaeal TFB, TFIIB
associates with the TBP-DNA complex via sequence-specific interactions with the
BRE element. In eukaryotes, BRE sequences line both sides of the TATA-box,
termed upstream and downstream BRE (Deng and Roberts 2006). TFIIB is also
responsible for the recruitment of RNAP in the eukaryotic system executing its role
in a highly analogous manner to the archaeal system. When RNAP II engages with
the TFIID-TFIIB-TFIIA-DNA complex it is already associated with TFIIF, a het-
erodimeric complex formed by an alpha and beta subunit (also known as Tfg1 and
Tfg2 in yeast where a third protein, Tfg3, is part of the complex) (Chen et al. 2007).
Around 50% of RNAP II are in complex with TFIIF (Rani et al. 2004), which may
help to recruit the polymerase to the promoter by interfering with its non-specific
DNA binding (Conaway et al. 1991). TFIIF fulfils various functions including the
reinforcement of the PIC via a stabilisation of TFIIB within the PIC and stabilisa-
tion of the transcription bubble. The WH domain of TFIIFβ appears to be mobile
and may adopt different positions to interact with the DNA during PIC formation
(Chen et al. 2007; Eichner et al. 2010). TFIIF is also involved in TSS selection and
stimulates early RNA synthesis (Khaperskyy et al. 2008; Ren et al. 1999; Tan et al.
1995; Yan et al. 1999; Rani et al. 2004; Fishburn and Hahn 2012). Association of
TFIIE and TFIIH completes the PIC to form the closed initiation complex (Murakami
et al. 2013a; Sainsbury et al. 2015). TFIIH and TFIIE seem to bind the initiation

complex in a cooperative mode (He et al. 2016). No X-ray structure of the complete eukaryotic PIC is available but electron microscopy and crosslinking studies revealed the overall organisation of the human and yeast PIC (Fishburn and Hahn 2012; He et al. 2016; Murakami et al. 2013b, 2015; Plaschka et al. 2015) (Fig. 9.3c). TFIIE occupies a comparable position as archaeal TFE at the clamp coiled coil and another binding site close to the stalk domain. TFIIEβ stretches over the cleft. In contrast to σ^{70}-dependent bacterial initiation and initiation in archaea, open complex formation is an energy-dependent process in the RNAP II system. The ATPase activity resides in TFIIH, a 10-subunit factor that harbours an ATPase (XPB in humans, Ssl2 in yeast). TFIIH contacts the PIC in proximity to TFIIE and interacts with the downstream DNA but not with the transcription bubble. Mechanistically, TFIIH appears to act as an ATP-dependent dsDNA translocase that rotates the downstream DNA leading to torsional stress and the unwinding of dsDNA as the upstream DNA is fixed due to the tight interaction of the TATA/BRE-TFIIB-TBP complex with the RNAP (Fishburn et al. 2015). The RNAP II initiation complex unwinds approximately 11–15 bp of DNA. Recent cryo-EM reconstruction showed that the TFIIB linker is disordered in the closed human PIC but becomes ordered in the open PIC and directly contacts the NTS (He et al. 2016). A comparable scenario is found in the structures of PIC from *S. cerevisiae*. Here, the B-linker shows weak density and the B-reader is mobile (Plaschka et al. 2016a). An intricate network of TFIIB, the clamp coiled coil, the TFIIE "E-ribbon" domain and the RNAP rudder is likely to stabilise the transcription bubble. TFIIF and TFIIE bind the promoter from the opposite sites of the cleft working in concert to encircle, retain and open the DNA (He et al. 2016; Plaschka et al. 2016a). The RAP30 subunit of TFIIF, which contacts Rpb2 (external 2 and protrusion domain), TFIIB, TBP and the downstream BRE, is another factor critically involved in bubble stabilization. Just as observed for the bacterial and eukaryotic system, the clamp domain of RNAP II adopts different conformations at the different stages of PIC assembly (He et al. 2013, 2016; Plaschka et al. 2016a). In the closed complex, the clamp is found in an open state while the clamp is closed over the DNA cleft in the open PIC. Notably, the tip of the clamp coiled coil changes its interaction partner when progressing from the open to closed transition. In the closed PIC the tip contacts WH2 of TFIIEβ but relocates to interact with the WH domain of TFIIEα during open complex formation (personal communication Eva Nogales).

The process of initiation complex assembly and open complex formation is well described for the eukaryotic RNAP II system but less well understood for RNAP I and RNAP III transcription. TBP seems to be associated with all eukaryotic initiation complexes and TFIIB-like factors are integral parts of the RNAP I and III initiation complexes (Vannini and Cramer 2012; Knutson and Hahn 2013) (see Sect. 9.2.2). In the RNAP I system, Rrn7 (TAF1 B in humans) represents the TFIIB-like factor. Rrn7, together with Rrn11 and Rrn6, is part of the CF which recruits RNAP I to the core element (CE) of the promoter. In yeast, recruitment of RNAP I additionally requires the recognition of a sequence stretch upstream of the CE, the upstream element (UE), by the upstream activation factor (UAF). UAF is comprised of histones H3 and H4, UAF30 and the factors Rrn5, Rrn9 and Rrn10. In higher

eukaryotes, the unrelated HMG-box protein upstream binding factor (UBF) might at least in part have a similar function (Sanij and Hannan 2009). RNAP I itself is associated with Rrn3 (TIF-IA in humans), which contacts subunit A43 and the CF fulfilling a bridging function.

In the RNAP III system, Brf1 represents the TFIIB-like factor when the RNAP III machinery is assembled at type I (e.g. 5S rRNA) and type II promoters (tRNAs). Here, Brf1 is found in complex with TBP and Bdp1, which together form the TFIIIB complex. Initiation at these promoter types also requires TFIIIC, a 6-subunit complex (Male et al. 2015). TFIIIC contains subunits Sfc1/Sfc7, which contains similar domains and an overall domain architecture comparable to the RNAP II-specific general transcription factor TFIIF Rap30/Rap74 (Taylor et al. 2013). At the 5S rRNA promoter, TFIIIA recruits the TFIIIC complex, whereas TFIIIC directly recognises the A and B box in tRNA promoters. RNAP III utilises a third promoter type represented for example by the human promoter for the U6 snRNA gene. Instead of Brf1, Brf2 is integral part of TFIIIB and the multisubunit SNAPc complex is crucially involved in promoter recognition upstream of the TATA-box. A structural characterisation of the Brf2/TBP/promoter DNA complex revealed the high degree of structural conservation as compared to the equivalent archaeal and RNAP II complex (Gouge et al. 2015).

Homologs of TFIIF (A49/A34.5 in RNAP I, C37/C53 in RNAP III) and TFIIE (A49 is homologous to TFIIEβ in RNAP I and C82/C34 in RNAP III) are stably integrated into RNAP I and RNAP III (see Sect. 9.2.2 and Fig. 9.2) and play a comparable role in open complex stabilisation (Hoffmann et al. 2015; Engel et al. 2013; Fernandez-Tornero et al. 2013). In the RNAP I and RNAP III system, open complex formation occurs without TFIIH-like factors rendering the melting of DNA independent of ATP hydrolysis.

9.3.3   Elongation

9.3.3.1   Promoter Clearance

Before a fully committed transcription elongation complex is established, an intermediate phase of transcription occurs in which RNAPs loose contact with initiation factors. Additionally, as noted above, the ternary RNAP-DNA-RNA complex is gradually stabilised when the first phosphodiester-bonds are synthesized. On the other hand, a few nucleotides (nt) long transcripts of bacterial RNAP are blocked by the $\sigma_{3.2}$ loop positioned at the RNA exit channel, which might lead to abortive transcription. To enter elongation, the $\sigma_{3.2}$ loop is displaced by the nascent RNA leading to its release when the DNA-RNA hybrid reaches a length of about 12 nt. The displacement of the $\sigma_{3.2}$ loop marks the initial state of promoter escape, triggering the release of RNAP from the promoter, which is required for RNAP translocation during transcription elongation (Murakami et al. 2002a).

The 5′end of eukaryotic RNAP II generated transcripts starts to enter the RNA exit channel when the DNA-RNA hybrid reaches a length of 8 nt (Westover et al. 2004b). While RNAP II scans for a transcription start site, the upstream edge of the separated strands remains fixed and the resulting transcription bubble extends downstream until it spans a length of 17–18 nt (Giardina and Lis 1993; Kuehner and Brow 2006). Immediately afterwards, the upstream region closes (Holstege et al. 1997; Pal et al. 2005) resulting in an approximately 10 nt long transcription bubble, which migrates with the translocating RNAP. Displacement of transcription factor TFIIB and initial RNA synthesis coincides with bubble collapse. It is a matter of discussion, how TFIIB destabilisation or the action of TFIIF and/or the elongation factor Spt4/Spt5 (see below) are implicated in this process (Andrecka et al. 2008; Blombach et al. 2013; Bushnell et al. 2004; Cabart et al. 2011; Fishburn and Hahn 2012; Kostrewa et al. 2009; Tran and Gralla 2008; Zawel et al. 1995). Furthermore, closing of the RNAP clamp accompanies the transition from the initiation to elongation phase (Chakraborty et al. 2012; Engel et al. 2013; Schulz et al. 2016; Gnatt et al. 2001). The stalk domain, which is present in archaea and eukaryotes but not in bacteria, is involved in this movement. It is formed by subunits homologous to the RNAP II subunits Rpb4/Rpb7 and promotes open complex formation and the processivity of the polymerase (Grohmann and Werner 2011; Hirtreiter et al. 2010b).

Transcription bubble collapse correlates with the dissociation of TFIIH and can occur after different RNA lengths have been reached, depending on the sequence of the promoter (Pal et al. 2005). Recent single-molecule analyses suggest that TFIIH-driven scanning for a TSS could result in largely extended transcription bubbles, which might either collapse back to a 10 nt containing DNA-RNA hybrid and allow promoter escape, or lead to repeated scanning, or cause dissociation of the RNAP machinery (Fazal et al. 2015).

Apparently, promoter escape can be modulated by different factors in the different RNAP systems, for example initiation factor UBF for RNAP I (Panov et al. 2006), or transcription factors TFIIF, FBP, FIR (Liu et al. 2001) and PC4 (Fukuda et al. 2004) for RNAP II. Promoter-proximal pausing represents an important regulatory step of transcription (Adelman and Lis 2012a; Jonkers and Lis 2015; Kugel and Goodrich 1998; Kwak and Lis 2013), which is relevant for a vast majority of genes in many organisms. Promoter-proximal nucleosomes or local DNA and/or RNA sequences might be implicated in this phenomenon, which might be overcome with the help of specific transcription (elongation) factors. Several detailed reviews give an overview about these important, but more specific gene-regulatory mechanisms (Liu et al. 2010, 2015, 2016; Adelman and Lis 2012b; Yamaguchi et al. 2013; Jonkers and Lis 2015).

9.3.3.2 Overview of the RNA Polymerisation Reaction

Once the promoter is cleared, RNA synthesis proceeds through repeating steps of nucleotide addition. Cooperation between RNAP subunits and precise structural refinements of the DNA-RNA hybrid in the active centre enables the RNAP to travel along the DNA, to incorporate the correct nucleotide, to remove wrongly added nucleotides and to re-enter productive transcription elongation if the enzyme is backtracked or stalled at a transcriptional barrier. Transcriptional barriers might be DNA-bound proteins like histones, or RNA-DNA hybrid structures, called R-loops. In this book chapter, we will mainly focus on the basic mechanism of RNAPs in RNA elongation and structural dynamics of RNAP domains during RNAP-movement along the naked DNA template. In addition, the function of three evolutionary conserved RNAP-associated elongation factors, (TFIIF, Spt4/5 like factors and TFIIS) will be described. Transcription elongation on a native chromatin template and the multiple interactions of RNAPs with many other factors involved in this process has been reviewed earlier in great detail (Dangkulwanich et al. 2014; Formosa 2013; Kwak and Lis 2013; Van Lijsebettens and Grasser 2014; Zhou et al. 2012).

9.3.3.3 The Nucleotide Addition Cycle

The active centre of all RNAPs is highly conserved and catalyses RNA chain elongation following a common basic mechanism, here described for RNAP II. The nucleotide addition cycle is defined as the condensation of a single nucleotide, which is incorporated as NMP in the growing RNA chain under release of pyrophosphate. Chain elongation is accompanied by the translocation of the enzyme along the DNA and by the movement of the transcription bubble. This involves both opening and closure at the 3′ and 5′ends of the 11–12 nucleotides long melted DNA strands, respectively, and annealing of 8–9 nucleotides of the nascent RNA.

In stable elongation complexes (EC), the incoming dsDNA unwinds in the downstream region, which allows the TS to contact the active centre (Gnatt et al. 2001). Positively charged residues in the RNAP II switch 2 region probably together with repellent negative charged amino acids of switch 1 and the fork loop 1 cooperate to separate the TS from the NTS (Kettenberger et al. 2004; Kireeva et al. 2011; Naji et al. 2008). Within the transcription bubble, eight to nine consecutive nucleotides hybridize with the nascent RNA, which is extended at its 3′end. Three RNAP loops-rudder, lid and fork loop – are considered to be involved in RNA-DNA strand separation (Gnatt et al. 2001; Kettenberger et al. 2004; Westover et al. 2004a). While the DNA-RNA hybrid is formed, the NTS makes a turn of about 90° near the catalytic centre and reanneals upstream with the TS to form the exiting DNA (Kornberg 2007).

Based on X-ray crystallography structures (Cheung and Cramer 2011, 2012; Kettenberger et al. 2004; Wang et al. 2006, 2009; Westover et al. 2004a; Zhang et al. 1999) six different stages can be distinguished in the nucleotide addition cycle

(NAC), which are accompanied or driven by structural rearrangements of the RNAP (Fig. 9.4) (see also Nudler 2009; Zhang et al. 2010; Cheung and Cramer 2012; Martinez-Rucobo and Cramer 2013; Dangkulwanich et al. 2014). The crucial conformational changes which are conserved through evolution (see also Bar-Nahum et al. 2005; Epshtein et al. 2002; Weinzierl 2011) concern especially two RNAP domains of the active centre, the trigger loop and the bridge helix (Fig. 9.4a). Before the nucleotide substrate (NTP) diffuses into the enzyme, RNAP II is in the post-translocation state (state I), which is characterised by an empty active site, an open trigger loop and a completely folded bridge helix (Fig. 9.4b). The NTP enters the active site between the RNA 3′end, the bridge helix and the mobile trigger loop and binds in a non-catalytic pre-insertion state (state II). A correct pairing NTP induces closure of the active centre because the trigger loop adopts an α-helical hairpin structure (state III). This conformational change brings the NTP in contact with all residues required for catalysis. Non-correct base pairing results in an equilibrium, which favours the open trigger loop formation and leads finally to dissociation of the non-pairing NTP. A two metal ion mechanism is used for catalysis (state IV). One metal ion A (Mg^{2+}) is permanently kept in the proximity of the RNA 3′end by three conserved aspartate side chains, whereas metal B (Mg^{2+}) contacts the NTP near conserved aspartate and glutamate residues of both the largest and second largest RNAP subunit. The RNA 3′hydroxyl group is deprotonated and attacks as a nucleophile the NTP-α-phosphate, which results in the formation of a new phosphodiester bond and the release of pyrophosphate (PPi) (Carvalho et al. 2011; Svetlov and Nudler 2013). Subsequently, the trigger loop opens and the elongation complex enters the pre-translocation state (state V). In this state, the nucleotide insertion site is occupied by the extended 3′end of the RNA. To incorporate the next nucleotide, the active site has to be emptied by translocation of the elongation complex by one nucleotide from the pre- to the post-translocation state (state I). Partial unfolding of the bridge helix in cooperation with trigger loop movement was suggested to push the nascent hybrid base pair out of the active centre first. In a second step, refolding of the bridge helix could guide the next DNA template base into the active centre by twisting it by 90°. However, the significance of the possible different bridge helix conformations for translocation is still discussed, especially since structural information confirming the predicted bridge helix movements is still missing (Svetlov and Nudler 2008).

To ensure transcriptional fidelity, RNAPs can move backwards (state VI). Backtracking of RNAP is a prerequisite to remove misincorporated nucleotides either by intrinsic RNAP-mediated cleavage or by cleavage supported by TFIIS-like factors.

9.3.3.4 Dynamics of Elongation

To unravel the exact molecular mechanism of transcription, crystallographic studies are not sufficient since X-ray structures are static and represent only single snapshots of the transcription cycle. Single-molecule analysis and molecular dynamics
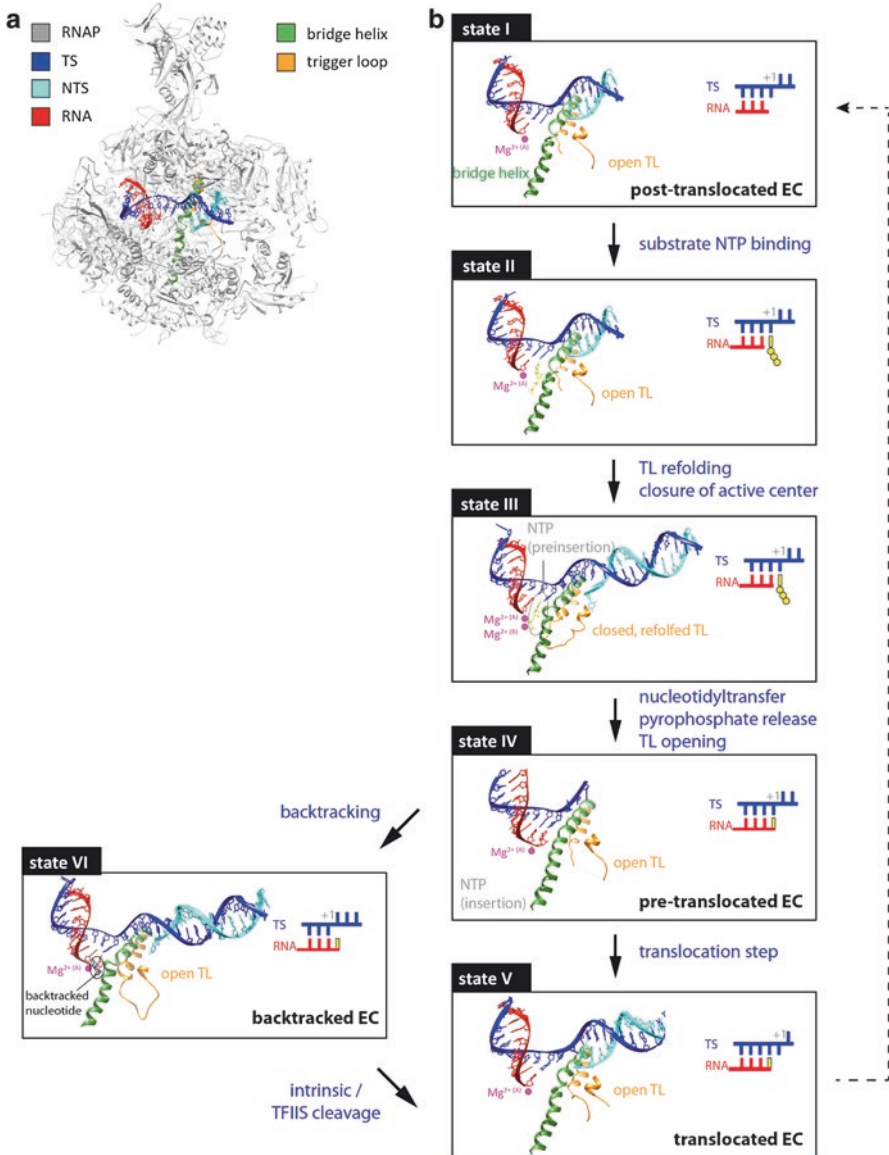
Fig. 9.4 Nucleotide addition cycle. (**a**) Overall organisation of the RNAP II transcription elongation complex (PDB: 1Y1W) including the TS (template strand), NTS (non-template strand), RNAP (RNA polymerase). Flexible elements important for the nucleotide addition cycle are highlighted. (**b**) Six distinguishable stages of the nucleotide addition cycle are shown (PDBs: 1Y1W (stage I), 1Y77 (II), 2E2H (III), 16IH (IV), 1VUM (V) and 3GTG (VI)). Dynamics of the trigger loop (TL) and the bridge helix (BH) according to published X-ray structures are indicated. Stage V represents a backtracked elongation complex. See text for details

simulation help to understand the temporal and spatial dynamics of biomolecules by considering both specific atomic interactions and the biological relevant kinetics (Zhang et al. 2016). Among other conclusions, these studies support a Brownian ratchet model to explain the dynamics of RNAP translocation. In principal, the mechano-chemical coupling that drives RNAP movement is still under debate and includes three models (Kireeva et al. 2010): a power-stroke mechanism, allosteric models and the Brownian ratchet model. Based on crystallographic analyses the power-stroke mechanism suggests that PPi release after nucleotide addition induces a structural change in the enzyme resulting in translocation and strand separation for 1 nt (Yin and Steitz 2004). Accordingly, the energy derived from the chemical reaction of NTP hydrolysis drives the motor forward. The allosteric model suggests that RNAP can exist in an activated and in a less activated state, which catalyse phosphodiester bond formation at different rates. Transition from the slow to the fast state is induced by binding of the templated NTP to the allosteric site (Foster et al. 2001; Holmes and Erie 2003; Nedialkov et al. 2003).

The Brownian ratchet model proposes that the enzyme oscillates between pre- and post-translocation states in a Brownian motion consuming thermal energy. NTP binding and formation of the phosphodiester bond favours the movement from the pre-to the post-translocation position (forward movement), but this step does not require a chemical reaction. In contrast, hydrolysis of both the pyrophosphate and the RNA transcript influences RNAP movement in the opposite direction (Abbondanzieri et al. 2005; Guajardo and Sousa 1997; Komissarova and Kashlev 1997a; Bar-Nahum et al. 2005). This model can explain the ability of RNAP to slide backwards on the DNA template without NTP utilisation when elongation is paused by specific DNA sequences or when NTPs become limiting. But it is still discussed whether such a back and forward movement occurs at all template positions during elongation. Investigating RNAP elongation kinetics via molecular dynamics simulation and single-molecule analyses using different experimental conditions clearly support the Brownian ratchet model (Kireeva et al. 2010; Zhang et al. 2016).

Another important aspect is that elongation speed is influenced by the underlying DNA sequence. RNAPs do not move at a constant speed over DNA templates with varying ATGC contents. Some sequences can cause a longer dwell time of the enzyme and two mechanisms – long and short pausing – can be distinguished. RNAP tends to backtrack during long pauses, which means that transcription is blocked until the 3′end of the nascent RNA is moved back in the active centre either by RNAP forward movement or by internal cleavage of the RNA. DNA sites provoking pauses have been identified for bacterial and human RNAPs (Imashimizu et al. 2013; Hawryluk et al. 2004; Hein et al. 2011; Herbert et al. 2006). Genome-wide analyses revealed almost 20,000 pausing sites on the *E. coli* chromosome, which are characterised by a distinct consensus sequence (Larson et al. 2014; Vvedenskaya et al. 2014).This sequence could cause pausing by interaction between the RNAP core enzyme and the 3′end of the RNA-DNA-hybrid. Accordingly, RNAPs might directly sense the shape or identity of basepairs, which could then delay enzyme translocation (Bochkareva et al. 2012).

9.3.3.5 Function of TFIIF-, Spt4/5- and TFIIS-Related Elongation Factors

One of the first factors found to be involved in RNAP II elongation was transcription initiation factor TFIIF, which associates with paused elongation complexes. During elongation, TFIIF stimulates the rate of elongation and suppresses pausing by stabilising the post-translocated elongation complex (Zhang and Burton 2004; Zhang et al. 2005). In RNAP I the heterodimer A49/34.5 and in RNAP III the subcomplex C37/53 share structural similarities, which correlates with their functional relatedness to TFIIF (Engel et al. 2013; Fernandez-Tornero et al. 2013; Geiger et al. 2010; Hoffmann et al. 2015).

Spt5/NusG-related factors are involved in elongation in all three domains of life indicating that they play an important role in supporting a basic mechanism of transcription. A common model emerged how the Spt4-Spt5-RNAP II architecture could influence transcription elongation (Fig. 9.5a). The NGN/Spt4 complex binds to the clamp, spans over the cleft and encircles the DNA in the enzyme. Binding of
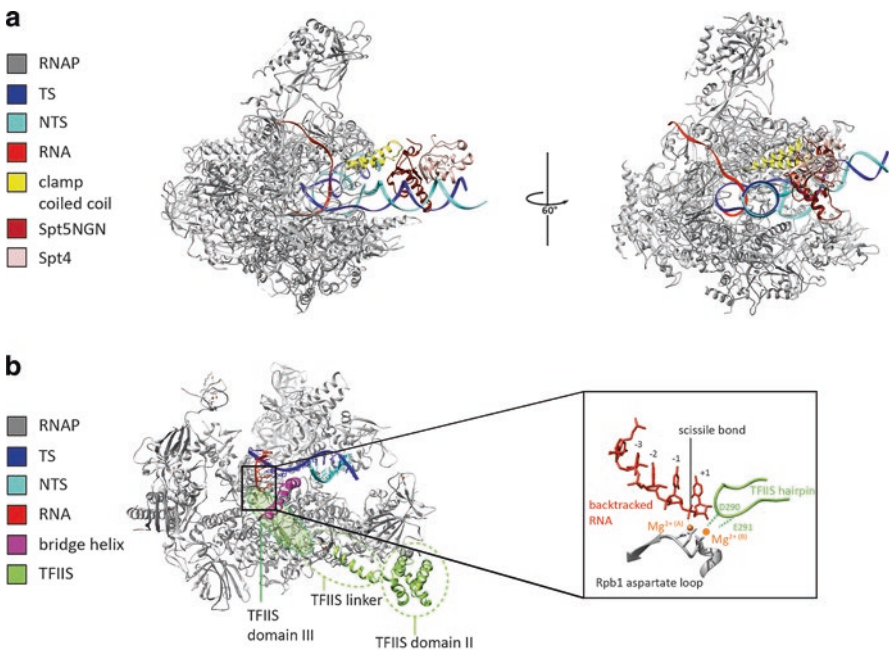
Fig. 9.5 RNAP II elongation complexes. (**a**) Structural model of a Spt5NGN/Spt4 containing RNAPII elongation complex (Model from Martinez-Rucobo et al. 2011). TS (template strand), NTS (non-template strand), RNAP (RNA polymerase), clamp coiled coil (Rpb1 domain interacting with Spt5NGN/Spt4). Spt5NGN/Spt4 bridges the cleft securing the DNA in the DNA binding channel, which contributes to RNAP processivity. (**b**) Structure of a TFIIS-containing elongation complex (PDB: 3PO3). The enlarged section shows components involved in the cleavage reaction of a backtracked RNA. See text for details

Spt4/5 induces closure of the clamp domain (Schulz et al. 2016). The downstream edge of the transcription bubble could be stabilised by interactions between the non-transcribed DNA strand and the NGN domain of Spt4/5. Biochemical and mutational analyses suggest that NusG speeds up the oscillation of RNAP between the pre- and post-translocation state (Fig. 9.4b) (Bar-Nahum et al. 2005; Borukhov and Nudler 2008). Taken together, these studies underline that the dissociation of the ternary transcription complex and release of the DNA template is prevented by Spt5/NusG elongation factors, which leads to an increase in polymerase processivity. Interestingly, cryo-EM studies indicated a shift of the Spt4/Spt5 location towards the opposite site of the cleft, interacting with the protrusion and lobe domains of RNAP II (Klein et al. 2011). This could mean that RNAP II domains (clamp and/or lobe) and the NGN domain of Spt5 interact in a dynamic fashion.

Recent structural investigations suggest that Spt4/Spt5/NusG proteins are associated with RNAP regions to which also transcription initiation factors bind. For instance, bacterial sigma factor or eukaryotic TFIIB binds to the coiled coil domain of the clamp (Arthur et al. 2000; Vassylyev et al. 2002; Kostrewa et al. 2009; Sevostyanova et al. 2008). Archaeal TFE and eukaryotic TFIIE interact with the clamp at a site that putatively overlaps with NGN domain-binding. Finally, the two large subunits of yeast TFIIF interact with RNAP domains that form one side of the cleft, but only at the periphery (Chen et al. 2007; Eichner et al. 2010). This raises the question whether competition of elongation and initiation factors for access at the clamp is a general mechanism to switch from initiation to elongation (Blombach et al. 2013). In fact, functional evidence was provided that archaeal TFE competes with Spt4/Spt5 for binding at the clamp coiled coil domain, and that this competition determines the transcription mode of the polymerase: TFE binding abolishes the inhibitory role of Spt4/Spt5 for initiation and Spt4/Spt5 removes TFE from the elongation complex (Grohmann et al. 2011). The latter could then facilitate the exchange between initiation factors and later acting RNAP-associated factors.

Pausing can lead to backtracking of the polymerase (Kireeva et al. 2005; Nudler et al. 1997; Komissarova and Kashlev 1997b). When the polymerase moves backwards along the DNA and RNA, the 3′RNA end dissociates from the DNA-RNA hybrid and is expelled from the active site through the pore underneath. Thus, RNA chain elongation is impaired. Transcription can only resume if the 3′end of the RNA is relocated into the active centre, which is mainly achieved through factor-stimulated RNA cleavage reactions. Arrested elongation complexes could be visualised by X-ray crystallography and revealed the exact position of the backtracked RNA (Cheung and Cramer 2011; Wang et al. 2009). In principal, a gating tyrosine residue Y769 can restrict the extent of backtracking, allowing the preferential cleavage at the level of dinucleotides. However, if RNAP is arrested, the DNA-RNA hybrid can become so unstable that the RNA can easily backtrack beyond the gating tyrosine into the pore keeping the trigger loop in an inactive conformation (Fig. 9.4b, stage VI). Furthermore, the bending of the bridge helix, which apparently depends on mismatches in the RNA-DNA hybrid, was reported to promote RNAP II backtracking (Da et al. 2016). Backtracked RNAPs can be rescued by intrinsic RNA cleavage activity and with the help of factors that stimulate transcript cleavage

at the active RNAP site. Upon RNA cleavage, 3–18 nucleotides long RNA stretches are released and the newly generated 3′OH of the RNA is aligned in the active site to become competent for RNA chain elongation. Bacteria, archaea and eukaryotic RNAP II have accessory factors Gre(B), TFS and TFIIS, respectively, to stimulate RNA cleavage of paused complexes (Fish and Kane 2002). Although GreB and TFIIS-related factors share no known structural homology (see Sect. 9.2.2), they function according to a similar principle (Martinez-Rucobo and Cramer 2013). In contrast to RNAP II, eukaryotic RNAP I and RNAP III employ the TFIIS-related polymerase subunits A12.2 and C11, respectively, to execute RNA cleavage (Chedin et al. 1998; Landrieux et al. 2006; Ruan et al. 2011; Jennebach et al. 2012; Kuhn et al. 2007; Lisica et al. 2016). Bacterial GreB binds to the jaw domain of the polymerase through its C-terminal globular domain while its N-terminal antiparallel α-helical coiled-coil domain approaches the active centre through the secondary channel and contacts the RNA (Opalka et al. 2003). TFIIS contains three domains. Domain II interacts with the polymerase at the RNAP jaw, and is connected via a linker domain with domain III, which is inserted into the active centre through the secondary channel (Fig. 9.5b) (Kettenberger et al. 2003, 2004). Apparently, the active site of RNAP II can be switched from polymerisation to cleavage mode and the transiently interacting TFIIS induces structural rearrangements of RNAP, which finally allow RNA processing and realign RNA in the active centre. The C-terminal parts of the RNAP I and III subunits A12.2 and C11 and the archaeal cleavage factor TFS, are homologous to the C-terminal domain of TFIIS, which is required for RNA processing. This molecular arrangement allows probably more efficient backtrack recovery and probably better proofreading (Lisica et al. 2016) for RNAP I and III. RNAP II and archaeal transcription might benefit from a dissociable cleavage factor since reversible association of TFIIS-related factors adds another level of transcriptional regulation (Ruan et al. 2011).

The mechanism of endonucleolytic cleavage is likely similar in all known TFIIS and GreB-related factors (Cheung and Cramer 2011; Kettenberger et al. 2003; Sosunov et al. 2003). Two conserved acidic residues (D290 and E291) are located at the foremost tip of the TFIIS-hairpin, which is inserted into the active centre of RNAP II. These residues position one of two Mg^{2+} ions involved in the cleavage reaction. The other Mg^{2+} ion is persistently bound to the RPB1 aspartate loop of the active site and binds the +1 RNA phosphate to align the hydrolysable phosphodiester bond (scissile bond, Fig. 9.5b). With the aid of the two acidic residues at the hairpin, the Mg^{2+} ion positions a water molecule, which then serves as a nucleophile. After RNA cleavage, the downstream RNA oligonucleotide is liberated, a new RNA 3′ end is associated to the active centre, the polymerase switches into the polymerisation mode and elongation can resume.

9.3.4   Termination

Correct transcription termination results in a length restricted transcript and in the release of RNAP and the transcript from the template. Termination of transcription requires common features for all transcription machineries, first, recognizing the end of the transcribed region, and second, overcoming related structural and energetic constraints to release the nascent transcript. Typically, terminator-complexes destabilise and release both, elongating RNA polymerases and associated transcripts. In addition, co-transcriptional RNA processing can also have a major impact on termination. No detailed structures of RNAP-containing termination complexes are yet available. Therefore, the possible mechanisms of termination are only briefly summarised.

In bacteria, two termination mechanisms are known: intrinsic termination induced by a palindromic sequence in the RNA that forms an RNA hairpin. A stem loop in the nascent RNA in which a series of U-residues follow a G-C rich element leads to pausing and destabilisation of transcription complexes (Larson et al. 2008; Peters et al. 2011; Washburn and Gottesman 2015). Mutations in the bridge helix can prevent termination, pointing at a crucial role of this RNAP domain also in the termination process (Nedialkov et al. 2013). The RNA hairpin has to be correctly folded when RNAP reaches the termination point. Furthermore, the sequence, size and length of the RNA loop influence termination efficiency.

Alternatively, termination in bacteria is mediated by the termination factor Rho (ρ). This termination factor is an RNA-dependent ATPase with helicase activity and forms a homohexameric ring. Two domains bind the nascent RNA and triggers dissociation of the RNAP from the DNA in an ATP-dependent manner. One RNA binding site binds RNA in the absence of ATP, the other site is stimulated to bind RNA transiently after the primary site is occupied. To reach the second binding site, the RNA is threaded to the central pore of Rho and the hexamer is closed when the RNA is attached. Subsequently, the transient interactions of RNA with the secondary binding site propel Rho along the RNA until it finally reaches the elongating RNAP at the release site. Dissociation of RNAP from the template can probably be achieved by unwinding of the RNA-DNA hybrid (Skordalakes and Berger 2003, 2006; Richardson 1982; Rabhi et al. 2011). The speed of elongation influences Rho dependent termination efficiency (Jin et al. 1992). In general, slowing down the elongation speed seems to be an important feature for most of the postulated termination mechanisms.

Termination in archaea and eukaryotes, however, is less well understood. Archaea and RNAP III terminate transcription after polymerizing a series of U residues (Campbell and Setzer 1992; Arimbasseri et al. 2013; Grohmann et al. 2010; Grohmann and Werner 2011; Hirtreiter et al. 2010b). This resembles the intrinsic bacteria-like termination mechanism. Whether the formation of an RNA hairpin upstream of the termination site of RNAP III is important for the mechanism of RNAP III termination is currently debated (Arimbasseri et al. 2014).

RNAP I termination involves a polymerase-specific termination factor that binds to a specific DNA sequence to pause elongation shortly upstream of its binding site, leading to transcript release (Németh et al. 2013; Jaiswal et al. 2016). In the yeast *S. cerevisiae,* this binding is sufficient to induce termination *in vitro* and *in vivo* (Merkl et al. 2014; Reiter et al. 2012).

Additionally, it was reported that factors involved in rRNA 3′end processing also participate in termination. Accordingly, co-transcriptional cleavage by rRNA processing factors generates a 5′RNA entry point for an exonuclease, which progressively degrades the nascent transcript associated with the elongating RNAP. After the exonuclease reaches the polymerase, which is slowed down at the termination site, it disrupts the ternary complex probably with the help of a helicase in a process termed the "torpedo model" (El Hage et al. 2008; Kawauchi et al. 2008; Rondon et al. 2009).

For RNAP II dependent termination either RNA 3′end processing factors or RNA-DNA helicases were suggested to disrupt the ternary transcription complexes (Kuehner et al. 2011; Mischo and Proudfoot 2013; Arndt and Reines 2015; Porrua and Libri 2015). RNAP II transcripts are often cleaved at a specific site (AAUAA) after the coding sequence and subsequently polyadenylated, which plays an important role in termination of protein-coding genes (Logan et al. 1987; Connelly and Manley 1988; Whitelaw and Proudfoot 1986). According to the "torpedo model" described above, the endoribonucleolytic cleavage allows entry of an exonuclease and rapid 5′-3′degradation, which leads to a clash between the exonuclease and the elongating RNAP II. This releases the polymerase from the template (Kuehner et al. 2011; Tollervey 2004). Alternatively, RNAP II transcription through the polyA-signal could induce structural changes of the elongation complex leading to its release from the template (Nag et al. 2006; Orozco et al. 2002). The involvement of additional factors is possible. Termination of RNAP II genes, which contain no polyA-tail, require factors containing RNA/DNA helicase activity. These factors destabilise the ternary elongation complex, thus, resembling bacterial Rho-dependent termination (Kuehner et al. 2011; Mischo and Proudfoot 2013; Porrua and Libri 2015).

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