Review

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Features of yeast RNA polymerase I with special consideration of the lobe binding subunits

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Abstract: Ribosomal RNAs (rRNAs) are structural components of ribosomes and represent the most abundant cellular RNA fraction. In the yeast *Saccharomyces cerevisiae*, they account for more than 60 % of the RNA content in a growing cell. The major amount of rRNA is synthesized by RNA polymerase I (Pol I). This enzyme transcribes exclusively the rRNA gene which is tandemly repeated in about 150 copies on chromosome XII. The high number of transcribed rRNA genes, the efficient recruitment of the transcription machinery and the dense packaging of elongating Pol I molecules on the gene ensure that enough rRNA is generated. Specific features of Pol I and of associated factors confer promoter selectivity and both elongation and termination competence. Many excellent reviews exist about the state of research about function and regulation of Pol I and how Pol I initiation complexes are assembled. In this report we focus on the Pol I specific lobe binding subunits which support efficient, error-free, and correctly terminated rRNA synthesis.

Keywords: elongation; nucleolus; proofreading; ribosomal RNA; ribosome biogenesis; transcription

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1 Introduction

Like many eukaryotes, *Saccharomyces cerevisiae* (hereafter referred to as yeast) contains three different nuclear DNA-dependent RNA polymerases (Pols): Pol I, Pol II and Pol III. They all share a common core architecture and use ribonucleotide triphosphate-molecules (NTPs) to polymerize RNA from a DNA template (Engel et al. 2018; Girbig et al. 2022; Khatter et al. 2017; Vannini and Cramer 2012). The structural features of the active centre and the molecular mechanism to synthesize RNA in the three Pols are rather similar. Nevertheless, all three Pols have adapted to fulfil specific tasks and produce different classes of cellular RNAs (Engel et al. 2018; Girbig et al. 2022; Werner and Grohmann 2011). Pol I exclusively transcribes the 35S ribosomal RNA (rRNA) gene containing the sequences for the mature 18S, 5.8S and 25S rRNAs in a membrane less substructure of the nucleus, the nucleolus. Thus, Pol I has to recognize only one specific gene promoter. Electron micrographs of chromatin spreads show an extremely high density of elongating Pol I molecules on the rRNA gene which is indicative for a high initiation rate and probably one reason for the high efficiency of rRNA production. In contrast Pol II transcribed genes are much less covered with polymerases (French et al. 2008; Laird and Chooi 1976; Osheim et al. 2004). The main products of Pol II dependent transcription are mRNAs and small non-coding RNAs. Thus, Pol II must recognize many different promoter structures, deals with distinct chromatin components, and transcribes many genes of different length. To achieve this, Pol II cooperates with many different factors involved in initiation, elongation, and termination of transcription. Pol III associates only with a few conserved promoter types and produces short untranslated RNAs such as transfer RNAs (tRNAs), the 5S rRNA, spliceosomal U6 small nuclear RNA (snRNA) and 7 SL RNA (Kessler and Maraia 2021; Moir and Willis 2013; Sentenac 1985; Vannini and Cramer 2012; Wang et al. 2022). Another significant difference between the three transcription machineries is that genes transcribed by Pol I and most Pol III are mostly devoid of nucleosomes (Conconi et al. 1989; Helbo et al. 2017; Merz et al. 2008; Parnell et al. 2008) whereas transcribed Pol II genes are usually covered with nucleosomes.

Interestingly, Pol II can substitute Pol I in genetically modified yeast strains (Nogi et al. 1991; Oakes et al. 1993). In these strains, rRNA genes remain assembled into nucleosomes (Dammann et al. 1995; Hontz et al. 2008). However, these strains grow much slower than wildtype strains, probably because of inefficient rRNA synthesis, which underlines that Pol I is optimized for rRNA production. Some molecular features which may explain the specialisation of the three Pols were recognized, whereas others are still unclear. This review aims to summarize the possible contributions of yeast Pol I specific subunits and their structural domains to the specialisation of this highly efficient transcription machinery.

2 Structural features of yeast RNA polymerase I in comparison with RNA polymerases II and III

All multisubunit Pols including bacteria and archaea share core subunits with an overall conserved structure (Table 1) which resembles a crab claw. This core structure contains the catalytic centre including a coordinated magnesium ion. In yeast Pol I the core is formed by the two largest subunits, A190 and A135, together with subunits AC40, AC19 and Rpb6 (Figure 1, Table 1). The two largest subunits contain double-ψ β-barrel motifs to build the active site and share structural homology with the two large catalytic subunits of the eubacterial RNA polymerase, the two largest subunits of the archaeal Pol, and the eukaryotic Pol II and Pol III (Cramer et al. 2001; Gnatt et al. 2001; Hirata et al. 2008; Korkhin et al. 2009; Zhang et al. 1999). Several functional important structural features can be distinguished within the Pol subunits (Figure 1). Additionally, an overview about the structural features of Pol I in correlation with similar structural features of Pol II and Pol III is given in Table 2. The two largest Pol I subunits contain the "jaws" which interact with the DNA substrate and form a cleft which opens and closes during the transcription cycle (Cramer et al. 2001; Engel et al. 2016, 2013; Fernandez-Tornero et al. 2013; Gnatt et al. 2001; Hoffmann et al. 2015; Neyer et al. 2016; Pilsl et al. 2016; Tafur et al. 2016; Torreira et al. 2017). Apart from the cleft, the active centre can be reached by two channels, the funnel and pore which represent the entry site for nucleoside triphosphates (NTPs), and the exit site for the synthesized RNA. The lobe domain and the protrusion domain are formed predominantly by subunit A135 on the core-module-side of the cleft. Subunits A190 and A135 comprise also some flexible domains. The clamp domain can partially contract over the DNA binding channel during the transcription cycle and its tip can transiently interact with Pol subunits or transcription factors (Engel et al. 2013, 2018; Fernandez-Tornero et al. 2013; Girbig et al. 2022; Gnatt et al. 2001; Grohmann et al. 2011). Mobility of two structural elements within the cleft, the trigger loop and the bridge helix, correlates with DNA translocation and NTP positioning (Brueckner et al. 2009a,b; Cramer et al. 2000; Engel et al. 2013; Fernandez-Tornero et al. 2013; Tan et al. 2008; Vassylyev et al. 2007; Wang et al. 2006; Weinzierl 2011; J. Zhang et al. 2010). Whereas the above mentioned features are similar in all eukaryotic Pols, a DNA-mimicking 'expander' loop is specific to Pol I. This element only becomes structured in inactive Pol I and was suggested to inhibit unspecific DNA binding.

In addition to the subunits shared with bacterial Pols the eukaryotic and archaeal Pols contain 6 additional common subunits (coloured subunits in Table 1). These include subunits which form the characteristic mobile stalk structure (Table 1). In Pol I the stalk consists of subunits A43 and A14 which are attached to A190. The stalk is thought to primarily play a role in transcription initiation.

In contrast to archaeal Pol and Pol II, Pol I and Pol III comprise additional subunits that are binding to the lobe structure (Figure 2). These subunits share functional and structural similarities with transcription factors associating with the lobe structure of Pol II and are therefore considered as "built-in transcription factors" (Geiger et al. 2010). The "built-in transcription factors" of Pol I are A49, A34.5 which form a heterodimer, and subunit A12.2 (Figure 1, Tables 2 and 3). The subunits A49 and A34.5 dimerize via their N-terminal dimerization domains thereby forming a stable heterodimeric complex. The N-terminal domain of A49 shares homology with the transcription factor TFIIFα of Pol II and C37 of Pol III (Tables 2 and 3). The C-terminal domain of A49 shares homology with the transcription factor TFIIEβ of Pol II and C34 of Pol III. Further, the lobe binding subunit A34.5 shares homology with the transcription factor TFIIFβ of Pol II and C53 of Pol III (Tables 2 and 3). In contrast, the transcription factor TFIIEα of Pol II and the subunits C82 and C31 of Pol III have no counterparts in Pol I (Tables 2 and 3). The N-terminal and C-terminal domains of the Pol I lobe binding subunit A12 are homologous to the Pol II subunit Rpb9, and the transcription factor TFIIS, respectively (Jennebach et al. 2012; Ruan et al. 2011). Both domains of Pol I subunit A12.2 are homologous to their counterparts in the

Table 1: Subunit composition of multi-subunit RNA polymerases in the three domains of life.

	Bacteria	Archaea	Eukaryotes (*S. cerevisiae*)		
			Pol I	Pol II	Pol III
Core subunits shared between RNA polymerases in bacteria, archaea and eukaryotes	β'	Rpo1	A190	Rpb1	C160
	β	Rpo2	A135	Rpb2	C128
	α	Rpo3	AC40	Rpb3	AC40
	α	Rpo11	AC19	Rpb11	AC19
	ω	Rpo6	Rpb6	Rpb6	Rpb6
Archaeal-eukaryotic specific subunits		Rpo5	Rpb5	Rpb5	Rpb5
		Rpo8	Rpb8	Rpb8	Rpb8
		Rpo10	Rpb10	Rpb10	Rpb10
		Rpo12	Rpb12	Rpb12	Rpb12
		Rpo4	A14	Rpb4	C17
		Rpo7	A43	Rpb7	C25
Pol-type specific subunits		Rpo13			
			A12.2	Rpb9	C11
			A49		C53
			A34.5		C37
					C82
					C34
					C31

The subunits are subdivided in core subunits, archaeal-eukaryotic-specific subunits and Pol-type specific subunits of the yeast *S. cerevisiae*. Shared subunits of all three yeast Pols are depicted in orange, common subunits of Pol I and Pol III in green. Subunits in the same lane are homologous. All coloured subunits belong to the eukaryote-specific 10 subunit-core structure (modified from Griesenbeck et al. 2017).

Pol III subunit C11. The Pol II lobe-binding subunit Rpb9 and transcription factor TFIIS as well as the Pol I lobe binding subunits are non-essential (Albert et al. 2011; Beckouet et al. 2008; Gadal et al. 1997; Hubert et al. 1983; Liljelund et al. 1992; McKune et al. 1995; Nogi et al. 1991), whereas C11, TFIIE- and TFIIF-subunits are essential proteins in yeast (Chédin et al. 1998; Feaver et al. 1994; Henry et al. 1992). The homologous subunits and transcription factors bind to similar positions in their respective Pols (see Table 3) and share also functional similarities (Vannini and Cramer 2012).

An acidic loop which is present in the C-terminal domains of A12.2, TFIIS and C11 can be positioned in close proximity to the active site of the respective Pol (Engel et al. 2013; Fernandez-Tornero et al. 2013; Neyer et al. 2016) and can support RNA cleavage activity of a mismatched RNA/DNA hybrid (Chédin et al. 1998; Kettenberger et al. 2003; Kuhn et al. 2007; Schwank et al. 2022).

3 Structural features and cellular functions of Pol I lobe binding subunits

3.1 Structural domains of A12.2

The Pol I – specific subunit A12.2 is composed of 125 amino acids and an N-terminal domain containing a zinc ribbon, a flexible linker domain including a hinge domain and a C-terminal domain comprising the evolutionary conserved motif Q.RSADE..T.F with a zinc ribbon (Nogi et al. 1993; Van Mullem et al. 2002) (Figure 3). The N-terminal part of the subunit A12.2 is positioned between the A135 lobe, the A190 jaw and the A49/A34.5 dimerization module (Figure 1). The conformation of the poorly conserved N-terminal domain of A12.2 is slightly rotated and shifted when compared to its

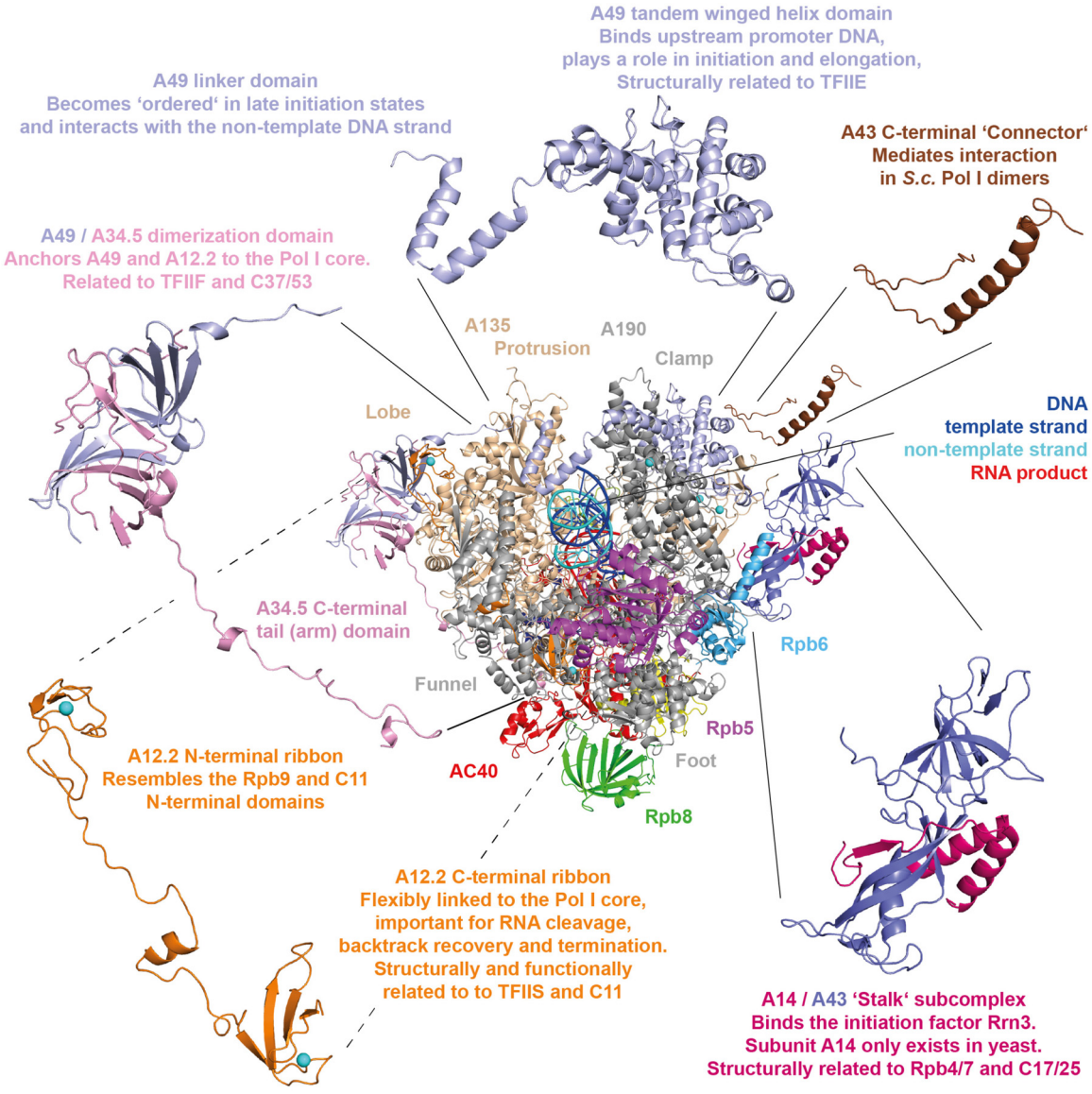


Figure 1: Structure of RNA polymerase I from *S. cerevisiae*. The overall structure of RNA polymerase I (Pol I) from *S. cerevisiae*. Ribbon model highlighting specific subunits and domains. Hybrid model constructed using COOT (Emsley and Cowtan 2004) for demonstration purposes. The structure of the Pol I elongation complex(PDB 5M3F) was extended by adding (a) a model of the C-terminal domain of subunit A12.2, (b) the "connector" domain of subunit A43 from the crystal structure (both from PDB 4C2M) and (c) the linker and tandem-winged helix domains (Geiger et al. 2010) from an ITC reconstruction (PDB 5 W66). The "front view" looks along the incoming ("downstream") DNA. Subunits not visible in the front view but present in the model are AC19, Rpb10, and Rpb12. Cyan spheres depict coordinated zinc atoms (modified figure taken with permission from (Pilsl and Engel 2022).

Pol II counterpart Rpb9. It is essential for correct Pol I assembly (Van Mullem et al. 2002) (Table 5) and required for the stable binding of A12.2 as well as for stable association of A49 and A34.5 to the lobe of Pol I (Van Mullem et al. 2002).

The A12.2 linker domain (amino acids 52–70) exhibits a helix-containing "mini-domain" that is positioned between the A190 funnel and the jaw domain. This linker contains a hinge domain that is required for the movement of the C-terminal domain of A12.2.

The C-terminal domain of A12.2 comprising the amino acids 78 to 124 is highly conserved and can occupy different positions in Pol I (Table 6). In contrast to the N-terminal domain that is exposed on the surface of Pol I, the C-terminal domain of A12.2 is either located inside the NTP entry pore close to the active site in elongation inactive complexes or is flexibly oriented when RNA synthesis is in progress (Engel et al. 2016; Neyer et al. 2016; Pilsl et al. 2016; Tafur et al. 2016). This position of the C-terminal domain inside the pore resembles almost perfectly the position of the corresponding TFIIS domain in the active site of Pol II. In the presence of the nucleotide analog GMPCPP, the heterodimer A49/34.5 was missing in the Pol I structure and the A12 C-terminal domain

Table 2: Structural domains of yeast Pol I (in alphabetical order) and their corresponding domains in Pol II and III. Positions of the domains in the Pol I structure are depicted in Figure 1.

Domain	Formed by Pol I subunit(s)	Function/localisation	Counterparts in Pol II	Counterparts in Pol III	References
Active center	A190, A135, Rpb5	Substrate binding, NTP polymerization, template movement	Rpb1, Rpb2, Rpb5	C160, C128, Rpb5	Cramer et al. (2001), Gnatt et al. (2001), Engel et al. (2013), Fernandez-Tornero et al. (2013), Hoffmann et al. (2015)
Assembly platform AC40/AC19 heterodimer	AC40, AC19	Assembly platform for the core enzyme	Rpb3, Rpb11	AC40, AC19	Vannini and Cramer (2012)
Bridge helix	A190	Unwinding-refolding dynamics required for catalysis and DNA translocation	Rpb1	C160	Cramer et al. (2001), Engel et al. (2013), Fernandez-Tornero et al. (2013)
Clamp	A190, A135	Closes on DNA and RNA	Rpb1, Rpb2	C160, C128	Gnatt et al. (2001), Werner and Grohmann (2011), Engel et al. (2013); Fernandez-Tornero et al. (2013), Enge et al. (2018), Girbig et al. (2022)
Cleft	Between A190 and A135	Opens and closes during the transcription cycle	Rpb1	C160	Cramer et al. (2001), Gnatt et al. (2001), Engel et al. (2013), Fernandez-Tornero et al. (2013), Hoffmann et al. (2015), Tafur et al. (2016)
C-terminal domain of A12.2 including linker aa 69–125 (A12.2CT)	A12.2	Required for RNA cleavage and proofreading, interferes probably with trigger loop closing	TFIIS	C11CT	Chédin et al. (1998), Cramer et al. (2001), Gnatt et al. (2001), Kuhn et al. (2007), Engel et al. (2013), Fernandez-Tornero et al. (2013), Hoffmann et al. (2015), Engel et al. (2018), Schwank et al. (2022)
C-terminal domain of A49 including the linker, A49 aa 112–415 (A49LCT)	Heterodimer A34.5/49	Mobile domain which can be anchored on top of the clamp opposite to the lobe, supports initiation and elongation	TFIIEβ/Tfa2	C34	Cramer et al. (2001), Gnatt et al. (2001), Kuhn et al. (2007), Beckouet et al. (2008), Geiger et al. (2010), Engel et al. (2013), Fernandez-Tornero et al. (2013), Hoffmann et al. (2015), Pilsl et al. (2016), Tafur et al. (2016), Engel et al. (2018)
Dimerisation domain of the heterodimer A34.5 aminoacids 1–233 (aa 1–233) A49 aa 1–186 (A34.5/A49NTL)	Heterodimer A34.5/49	Associates to the lobe and contacts the N-terminal domain of A12.2; the linker of A49 (aa 113–184) spans the cleft if A49CT is anchored at the clamp	TFIIFα/Tfg1, TFIIFβ/Tfg2	C37, C53	Cramer et al. (2001), Gnatt et al. (2001), Engel et al. (2013), Fernandez-Tornero et al. (2013), Hoffmann et al. (2015)
Expander or DNA mimicking loop	A190	Located between protrusion and clamp, only structured in inactive Pol I, probably inhibits unspecific DNA binding	No	No	Engel et al. (2013), Fernandez-Tornero et al. (2013)
Funnel and pore	A190	Entry site of nucleotides, positioning of a 3′extended RNA in a backtracked Pol	Rpb1	C160	Cramer et al. (2001), Engel et al. (2013), Fernandez-Tornero et al. (2013)
Jaws	A190, Rpb5, A12.2	Positioning of downstream DNA	Rpb1, Rpb5, Rpb9	Rpb1, Rpb5, C11	Cramer et al. (2001), Gnatt et al. (2001), Engel et al. (2013), Fernandez-Tornero et al. (2013), Hoffmann et al. (2015)
Lobe	A135	Association of A12.2, A34.5, A49	Rpb2	C128	Gnatt et al. (2001), Engel et al. (2013), Fernandez-Tornero et al. (2013), Hoffmann et al. (2015)
N-terminal domain of A12.2 including linker, aa 1–85 (A12.2NTL)	A12.2	Supports RNA cleavage and proofreading, supports elongation, required for Pol I assembly	Rpb9	C11NT	Hemming et al. (2000), Cramer et al. (2001), Van Mullem et al. (2002), Kuhn et al. (2007), Engel et al. (2013), Fernandez-Tornero et al. (2013), Knippa

Table 2: (continued)

Domain	Formed by Pol I subunit(s)	Function/localisation	Counterparts in Pol II	Counterparts in Pol III	References
					and Peterson (2013), Hoffmann et al. (2015), Engel et al. (2018), Scull et al. (2021), Schwank et al. (2022)
Protrusion	A135	Upper boundary of the cleft	Rpb2	C128	Cramer et al. (2001), Engel et al. (2013), Fernandez-Tornero et al. (2013)
Stalk domain	A43, A14	Mobile element supporting transcription initiation	Rpb4/Rpb7	C17/C25	Edwards et al. (1991), Cramer et al. (2001), Engel et al. (2013), Fernandez-Tornero et al. (2013)
Trigger loop	A190	Folding dynamics required for substrate specificity, catalysis and DNA translocation	Rpb1	C160	Wang et al. (2006), Vassylyev et al. (2007), Tan et al. (2008), Brueckner et al. (2009ab), Zhang et al. (2010), Weinzierl (2011), Engel et al. (2013), Fernandez-Tornero et al. (2013)

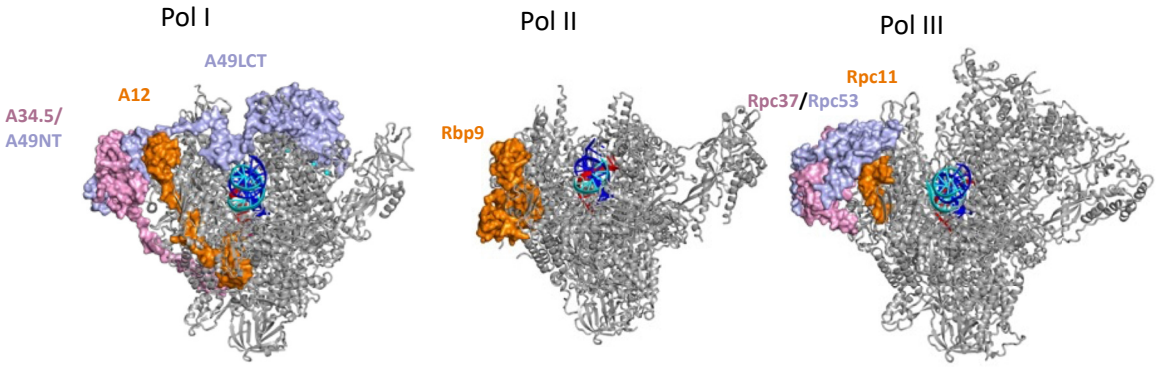


Figure 2: Pol I, II and III from *S. cerevisiae* differ in their lobe binding subunits. Structural models of Pol I, Pol II and Pol III which highlight the different lobe binding subuits. Pol I contains three lobe binding subunits A12, and the heterodimer A34.5/A49 which have homologies to the Pol III subunits Rpc11, Rpc37/Rpc53, respectively. Pol II contains only one lobe binding subunit, Rpb9 which is homologous to the N-terminus of the Pol I subunit A 12.2. Homologous subunits have the same colours. Structure models were built according to (Engel et al. 2013; Han et al. 2017; Hoffmann et al. 2015; Kettenberger et al. 2004; Neyer et al. 2016) and represent in part structure combinations of polymerases in different transcriptional states.

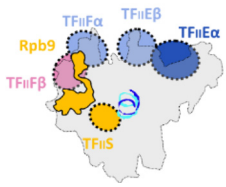
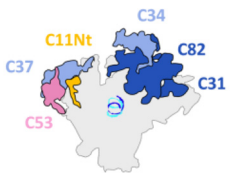
adopts a position on the surface of subunit A135. This suggests that the binding of the A12 C-terminal domain to the surface of A135 is either prerequisite or consequence of a A49/34.5 dissociation (Tafur et al. 2019). However, the physiological relevance of such a conformation remains elusive.

The C-terminal domain of A12.2 shares structural and functional similarities not only with the C-terminal domains of the Pol II transcription factor TFIIS, but also with the Pol III subunit C11 and the archaeal counterpart TFS (Chédin et al. 1998; Hausner et al. 2000; Van Mullem et al. 2002) (Figure 4). All these domains support intrinsic RNA cleavage activity of their respective Pol if the enzyme is backtracked during RNA synthesis and the RNA 3′ end became displaced from the active centre. Furthermore, they are involved in proofreading (Chédin et al. 1998; Izban and Luse 1992; Reines 1992; Reines et al. 1989; Schwank et al. 2022; Sydow et al. 2009). They all use the amino acids glutamate and aspartate within the shared Q.RSADE..T.F motif and a zinc ribbon domain to reorient bivalent metal ions in the active site to promote hydrolytic RNA cleavage (Jeon et al. 1994; Jeon and Agarwal 1996). For yeast Pol I the two crucial acidic amino acids are D105 and E106 of A12.2 which are located in an acidic loop of its C-terminal domain and correspond to the amino acids D290 and E291 of TFIIS (Schwank et al. 2022).

3.2 Structural domains of A49 and A34.5

The Pol I – specific subunit A49 is composed of 415 amino acids. The subunit can be subdivided in an N-terminal “dimerization” domain, a linker domain with an arm followed by a helix-turn-helix (HTH) motif and a C-terminal

Table 3: The lobe-binding subunits of Pol I and their counterparts in Pol II and Pol III.

Pol I	Pol II	Pol III
		
A12.2NT	Rpb9	C11NT
A12.2CT	TFIIS	C11CT
A49NT	TFIIFα/Tfg1	C37
A34.5	TFIIFβ/Tfg2	C53
	TFIIEα/Tfa1	C82
A49CT	TFIIEβ/Tfa2	C34
		C31

Schematic representation of Pol I and the lobe-binding subunits. The "built-in transcription factor" subunits of Pol I are A12.2 (orange), A34.5 (pink) and A49 (purple) according to Figure 1. The proteins TFIIEα, C82 and C31 that have no counterparts in Pol I are coloured in blue. The schematic representation of the three yeast RNA polymerase structures indicate the binding sites of the lobe-binding subunits of Pol I and their corresponding subunits and transcription factors of Pol II and Pol III.

Table 4: The Pol I specific heterodimer A49/A34.5 is conserved from human to budding yeast.

Pol I specific subunits	A49	A34.5
S. cerevisiae	A49	A34.5
M. Musculus	PAF53 (POLR1E)	PAF49 (POLR1G)
H. sapiens	hPAF53 (POLR1E)	CAST (POLR1G)
S. pombe	RPA51 (Sp-A49)	SPBC11G11.05 (Sp-A34.5)

Pol I specific subunits A49/A34.5 of *Saccharomyces cerevisiae* and their homologs in *Mus musculus*, *Homo sapiens* and *Schizosaccharomyces pombe*. Nomenclature is taken from Albert et al. (2011) and Knutson et al. (2020).

tandem winged helix (tWH) domain (Figure 3). The linker domain and the attached tWH domain are rather mobile within the Pol I enzyme.

The subunit A34.5 consists of 233 amino acids and contains an N-terminal "dimerization" domain and a C-terminal arm that is partially flexible, highly charged and required to attach the subunit-complex to the Pol I core (Geiger et al. 2010) (Figure 3). In metazoans, the C-terminal region of Pol I subunit RPA34 is extended in comparison to the yeast counterpart A34.5, attaches to the Pol I core via a rigid arm and dissociates under high salt conditions (Daiß et al. 2022). Throughout eukaryotes, both subunits, A34.5 and A49, form a stable heterodimeric complex via their N-terminal dimerization domains that binds to the lobe structure of Pol I together with subunit A12.2 (Engel et al. 2013; Fernandez-Tornero et al. 2013).

As mentioned above, homology sequence comparison and high-resolution structures show striking similarities between the Pol I subunits and general transcription factors (GTFs) of Pol II (see above and Figure 4, Tables 2 and 3). Furthermore, A49 and A34.5 from *S. cerevisiae* have orthologs in other organisms like the Pol I-associated factors PAF53/PAF49 (*Mus musculus*), hPAF53/CAST (*Homo sapiens*), Sp-A49/Sp-34.5 (*Schizosaccharomyces pombe*) (see Table 4). A49 and A34.5 share a conserved domain organization with their mouse and human counterparts, although there is only low conservation on the amino acid sequence level. Based on the stability of biochemically isolated complexes, the mouse PAF53 and PAF49 were initially described as Pol I-associated factors (Hanada et al. 1996; Penrod et al. 2012) whereas the baker's yeast and human orthologues were considered to be *bona fide* Pol I subunits. It should be mentioned, however, that also from *S. cerevisiae* and mammals Pol I can be isolated in forms lacking or containing the heterodimeric

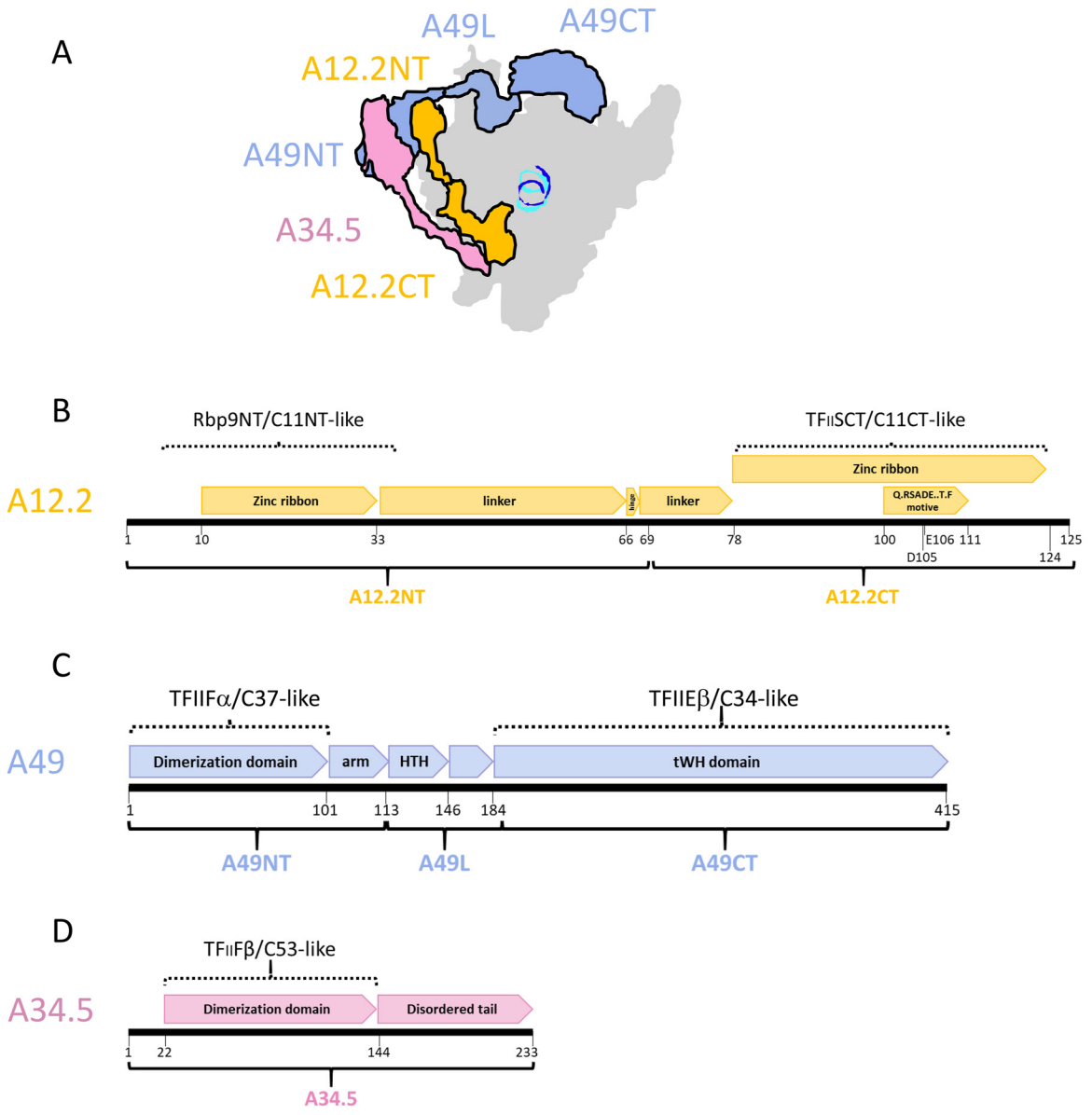


Figure 3: Schematic representation of protein domains within the lobe binding subunits of Pol I. **(**A) Schematic representation of the domains of subunits A12.2, A34.5 and A49. The N- and C-terminal domains are indicated. (B) Domains of subunit A12.2. Structural domains are depicted with respect to their position in the amino acid sequence. The N-terminal domain of A12.2 comprises a zinc ribbon and shares homology with the N-terminal domains of Rpb9 (Pol II) and C11 (Pol III). The linker domain is rather flexible and mobile. It contains a hinge domain that is required for the movement of the C-terminal domain. The C-terminal domain consisting of a zinc ribbon and an invariant Q.RSADE..T.F motif shares structural and functional similarities with the C-terminal domains of TFIIS, C11 and TFS. The amino acids D105 and E106 are suggested to correspond to the amino acids D290 and E291 of TFIIS which promote RNA cleavage of stalled Pol II. (C) Schematic representation of A49. Structural domains are depicted with respect to their position in the amino acid sequence. The N-terminal domain of A49 comprises a dimerization domain and shares homology with TFIIFα/Tfg1 (Pol II) and C37 (Pol III). The linker domain contains an arm and a helix-turn-helix (HTH) domain. The C-terminal domain is composed of a tandem winged helix (tWH) domain. It shares homology with TFIIEβ/Tfa2 (Pol II) and C34 (Pol III) (modified from Knutson et al. 2020). (D) schematic representation of A34.5. Structural domains are depicted with respect to their position in the amino acid sequence. The N-terminal terminal domain of A34.5 comprises a dimerization domain and shares homology with $TF_{II}Fβ$ (Pol II) and C53 (Pol III). The C-terminal domain is disordered (modified from Knutson et al. 2020).

complex (Hanada et al. 1996; Huet et al. 1975). Furthermore, there is evidence that the Sp-A49/Sp-34.5 sub-complex in the fission yeast *S. pombe* might be compromised in its association or rather flexibly attached to the remainder of Pol I in some conditions (Heiss et al. 2021). Thus, it remains an open question if the heterodimeric A49/A34.5 complex dynamically associates with Pol I in course of the transcription process. Nevertheless, ChIP analyses suggested that

Table 5: Overview about phenotypes observed in yeast strains expressing truncated lobe binding subunits or carrying complete deletions of the respective genes.

Mutant strain	Growth phenotype	Growth phenotype in the presence of inhibitors	Affinity-purified Pol I	Functional analysis	References
ΔRPA12	Temperature sensitive > 34 °C	Sensitive to the nucleotide reducing drugs 6-azauracil and mycophenolate	Pol I ΔA12 lacks A12.2, A34.5 and A49	Amount of A190 is decreased, synthetic lethal with A14	McCusker et al. (1991), Nogi et al. (1993), Gadal et al. (1997), Van Mullem et al. (2002)
RPA12ΔNT (expresses RPA12 aa 69–125)	Temperature sensitive > 34 °C	Sensitive to the nucleotide reducing drugs 6-azauracil and mycophenolate	Pol I A12ΔNT lacks A12.2, A34.5 and A49	Two-hybrid interaction with A135 is impaired; nucleolar integrity impaired, synthetic lethal with A14	Van Mullem et al. (2002), Girke and Seufert (2019)
RPA12ΔCT (expresses RPA12 aa 1–69)	No growth phenotype found	No sensitivity found	Pol I A12ΔCT contains all subunits, but lacks A12CT	Sufficient for interaction with Pol I	Van Mullem et al. (2002), Merkl et al. (2020)
RPA12DE/AA (ΔRPA12 pGAL-RPA12D105A/E106A)	Lethal upon growth on galactose		Not determined		Own unpublished results
ΔRPA49	Cold sensitive <24 °C, synthetic lethal with *HMO1* and *TOP3* deletion	Sensitive to the nucleotide reducing drugs 6-azauracil and mycophenolate	Pol I ΔA49 lacks subunits A49 and A34.5, but contains A12.2	Weaker interaction with A12.2 *in vivo*, changed nucleolar shape, Reduction of Pol I loading rate	Liljelund et al. (1992), Gadal et al. (1997, 2002), Beckouet et al. (2008), Albert et al. (2011)
RPA49ΔCT (expresses Rpa49 aa 1–366)	Cold sensitive <24 °C	Sensitive to the nucleotide reducing drugs 6-azauracil and mycophenolate (	Not determined	Weaker interaction with A12.2 *in vivo*, changed nucleolar shape, Reduction of Pol I loading rate	Liljelund et al. (1992), Desmoucelles et al. (2002), Beckouet et al. (2008), Albert et al. (2011)
RPA49ΔNT (expresses Rpa49 aa 119–416)	No growth phenotype found	No sensitivity found	Not determined		Beckouet et al. (2008)
ΔRPA34.5	No growth phenotype found; synthetic lethal with *TOP1* deletion	Hypersensitive to caffeine, not sensitive to mycophenolate	Not determined, A49 is still recruited to the rRNA gene in ChIP analysis	Decondensed nucleolus	Gadal et al. (1997), Beckouet et al. (2008), Albert et al. (2011)

A49 and A34.5 remain attached to the Pol I core throughout the transcription of an entire pre-rRNA gene in *S. cerevisiae* cells (Beckouet et al. 2008; Rossi et al. 2021) which is clearly different from ChIP experiments with subunits of TFIIF or TFIIE (Mayer et al. 2010; Rossi et al. 2021).

3.3 Growth phenotypes and subcellular distribution of Pol I in strains expressing mutants of lobe-binding subunits or carrying deletions of the respective genes

The three yeast lobe binding subunits are all encoded by non-essential genes at 30 °C, but *RPA12* and *RPA49* deletion strains are temperature sensitive and do not survive at restrictive temperatures. Some subdomains of the lobe binding subunits were shown to be important for the subcellular localization of Pol I and mutation/deletion of the genes coding for the proteins were reported to have an impact on the morphology of the nucleolus. Growth phenotypes and cell biological features are summarized in Table 5.

By immunofluorescence microscopy, the localization of Pol I was investigated in different *rpa12* mutant strains. In *RPA12* deletion strains, Pol I was still restricted to the nucleus, but its distribution was different from the distribution in a wild type strain and it was speculated that the absence of Rpa12.2 may have an effect on nucleolar organization (Van Mullem et al. 2002). However, Δ*rpa12* strains were not affected in nuclear division or chromosome segregation (Girke and Seufert 2019). Furthermore, the above phenomena might be

Table 6: Overview about currently available cryo-EM structures of yeast Pol I with focus on domains of lobe binding subunits and the contraction state of the cleft.

Activity cycle	Author nomenclature	Reference	Functional state assigned	EMDB	PDB	A12-CTD	A34.5/A49 attached	A49 tWH/ linker	Cleft state (Å)
Initiation	CC1	Sadian et al. (2019)	Closed complex (partial)	4982	6RQH	In funnel	Yes	Flexible	35
	CC2	Sadian et al. (2019)	Closed complex (partial)	4984	6RQL	In funnel	Yes	Flexible	33
	OC1	Sadian et al. (2019)	Open complex with CF and Rrn3	10,007	6RUO	In funnel	Yes	Flexible	32
	OC2	Sadian et al. (2019)	Open complex with CF and Rrn3	10,038	6RWE	In funnel	Yes	Positioned	25
	IM1	Sadian et al. (2019)	Intermediate initiation complex with CF and Rrn3	4987	6RRD	In funnel	Yes	Flexible	32
	IM2	Sadian et al. (2019)	Intermediate initiation complex with CF and Rrn3	10,006	6RUI	In funnel	Yes	Flexible	34
	eiPIC	Pilsl and Engel (2020)	Early intermediate Pol I – Rrn3 – CF complex	10,544	6TPS	Flexible	Yes	Flexible	28
	Rrn3-free PIC1	Han et al. (2017)	Early Rrn3 free PIC	8774	5W64	Flexible	Yes	Flexible	25
	Rrn3-free PIC2	Han et al. (2017)	Rrn3 free PIC	8775	5W65	Flexible	Yes	Flexible	23
	Rrn3-free PIC3	Han et al. (2017)	Late Rrn3 free PIC	8776	5W66	Flexible	Yes	Positioned	24
	OC	Tafur et al. (2016)	CF/Rrn3 free open complex	3446	5M5W	In funnel	Yes	Flexible	31
	PIC	Sadian et al. (2017)		3727	5OA1	In funnel	Yes	Flexible	30
	ITC	Engel et al. (2017)	Initially transcripting Rrn3-CF-Pol I complex	3593	5N61	Flexible	Yes	Partially positioned	26
Elongation	EC	Neyer et al. (2016)	Elongation complex	4147	5M3F	Flexible	Yes	Flexible	25
	EC_tWH	Tafur et al. (2016)	EC with defined tWH position on initiation scaffold	3449	5M64	Flexible	Yes	Positioned	25
	EC1	Tafur et al. (2016)	Elongation complex	3447	5M5X	Flexible	Yes	Flexible	25
	EC2	Tafur et al. (2016)	EC on initiation scaffold	3448	5M5Y	Flexible	Yes	Flexible	25
	Core EC-nh	Tafur et al. (2019)	GMPCPP containing core EC	240	6HLR	Flexible	No	Flexible	24
	EC-nh	Tafur et al. (2019)	GMPCPP containing complete EC	238	6HKO	Flexible	Yes	Flexible	24
	EC*-nh	Tafur et al. (2019)	GMPCPP containing 12SU EC	239	6HLQ	On A135 external	No	Flexible	25
Initiation competent	Pol-I-Rrn3-tWH	Sadian et al. (2019)	DNA-bound Pol I-Rrn3 complex with tWH	4985	6RQT	Flexible	Yes	Positioned	24
	Pol I/Rrn3	Engel et al. (2016)	Rrn3-bound Pol I	3439	5G5L	Mostly in funnel	Yes	Flexible	33
	Pol I/Rrn3	Pilsl et al. (2016)		3443	–	Flexible	Yes	Flexible	
	Pol I/Rrn3	Torreira et al. (2017)		4086	–	Flexible	Yes	Flexible	
14-Subunit apo Pol I	Pol I apo	Neyer et al. (2016)	Free 14 subunit Pol I	4148	5M3M	In funnel	Yes	Flexible	36
	Pol I apo	Torreira et al. (2017)		4086	5LMX	Flexible	Yes	Flexible	34
	Pol I* apo	Tafur et al. (2019)		241	6HLS	On A135 external	No	Flexible	31

related to decreased A190 levels observed in *RPA12* deletion strains (Nogi et al. 1993) pointing to a role for Rpa12 in Pol I stability. Interestingly, none of the phenotypes is observed in a strain expressing a mutant Rpa12.2 lacking the C-terminal domain, whereas the N-terminal deletion mutant behaves like a *RPA12* deletion strain. This indicated that the N-terminal domain of Rpa12.2 bears its growth supporting functions (Van Mullem et al. 2002).

Two nucleolar targeting mechanisms have been identified for subunit A34.5. First, the protein may reach the nucleolus due to its association with A49 and second, a species-specific nucleolar targeting signal is located in the lysine-rich C-terminal domain of A34.5 (Albert et al. 2011). When *RPA34* is deleted, the nucleolus appeared decondensed and less electron dense although the mutant strains did not have a strong growth phenotype. In Δ*rpa49* mutants, A34.5 is

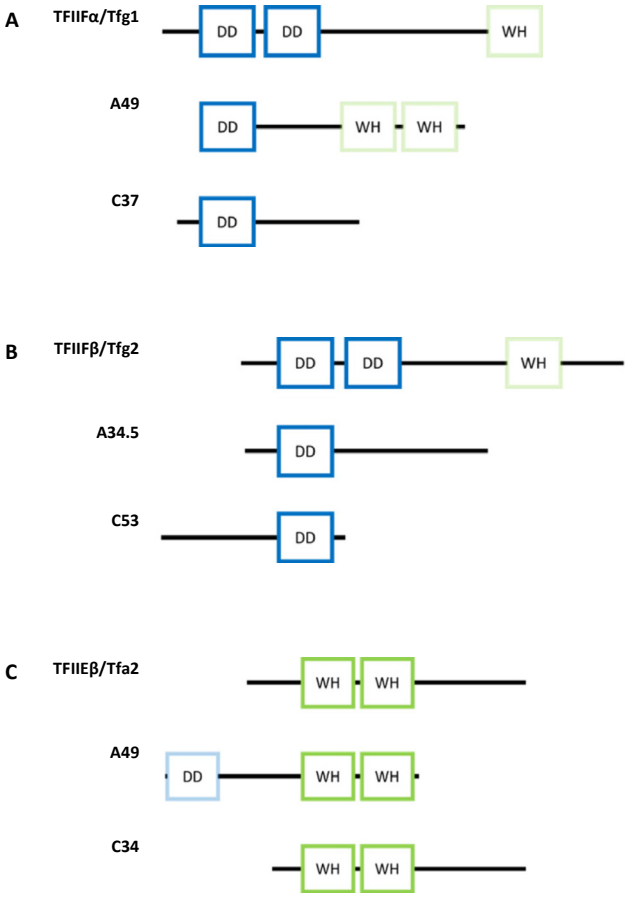


Figure 4: Schematic comparison of protein domains of transcription factors of Pol II and lobe binding subunits of Pol I and Pol III. "Built-in transcription factor" subunits of Pol I and III share structural homology with transcription factors of Pol II (A) TFIIFα (Tfg1), A49 and C37 share N-terminal dimerization domains (DD). The dimerization domains are colored in blue. (B) TFIIFβ (Tfg2), A34.5 and C53 share dimerization domains (DD). (C) TFIIEβ (Tfa2), A49 and C34 share winged-helix domains (WH). The winged-helix domains are colored in green.

still located in the nucleolus but the nucleolar morphology is drastically altered (Albert et al. 2011). The typical nucleolar crescent-like shape observed in yeast nuclei is absent in Δ*rpa49* strains and the nucleolus is enlarged. Furthermore, ultrastructural analysis suggested that the electron dense areas of the nucleolus are distributed to other areas of the nucleus. In Δ*rpa49* strains carrying a reduced number of rRNA gene copies, the size and shape of the nucleolus are as in the wild-type situation. All *in vivo* data suggest that both subunits of the heterodimer are important to maintain wild type nucleolar morphology. As observed for the N-terminal domain of Rpa12.2, yeast strains expressing the C-terminal domain of Rpa49 lacking the dimerization domain have no significant phenotype, whereas a C-terminal truncation mutant behaves like a Δ*rpa49* strain (Beckouet et al. 2008).

4 Lobe binding subunits and transcription initiation

Several auxiliary factors support yeast Pol I in transcription initiation. Upstream activating factor (UAF) consisting of Rrn5, Rrn9, Rrn10, Uaf30 and the histones H3 and H4 is bound to the upstream element (UE) at the ribosomal gene promoter (Keener et al. 1997; Keys et al. 1996; Siddiqi et al. 2001). To initiate transcription, UAF in complex with the TATA-binding protein TBP (Baudin et al. 2022) recruits the core factor (CF) which consists of Rrn6, Rrn7 and Rrn11 (Keys et al. 1994; Lalo et al. 1996; Lin et al. 1996) and binds the core element (CE) located between 15 and 38 base pairs upstream of the transcription start site (TSS). The promoter-bound factors provide a platform for the recruitment of the initiation competent Pol I in association with the Pol I specific transcription factor Rrn3 (Aprikian et al. 2001; Keener et al. 1998; Milkereit and Tschochner 1998; Steffan et al. 1996; Yamamoto et al. 1996) (see as recent review Hori et al. 2023). These factors form the Pol I preinitiation complex (PIC). Initiation efficiency is further supported by the C-terminus of Net1 (Nucleolar silencing establishing factor and telophase regulator) (Hannig et al. 2019; Shou et al. 2001) which might be functionally related to the C-terminal part of the metazoan Pol I transcription factor UBF (Hannig et al. 2019). UAF, TBP and Net1 significantly enhance promoter-dependent transcription *in vitro*, but they are not required for basal transcription, whereas CF, Rrn3 and Pol I are sufficient and required for a basal *in vitro* initiation activity (Engel et al. 2017; Keener et al. 1998; Pilsl et al. 2016). Transcription factor Rrn3 is released from the transcribing Pol I immediately after rRNA synthesis has started (Beckouet et al. 2008; Bier et al. 2004; Milkereit and Tschochner 1998). Whether phosphorylation of Rrn3 and/or Pol I (Blattner et al. 2011; Fath et al. 2001) or a different mechanism triggers the dissociation of Rrn3 from the elongating Pol I needs more experimental evidence.

The initiation process is accompanied by several structural rearrangements of Pol I, in which also the lobe binding subunits are involved. Transcriptional inactive Pol I dimers were observed *in vitro* (Milkereit et al. 1997) and *in vivo* (Torreira et al. 2017) and structurally characterized by X-ray analysis (Engel et al. 2013; Fernandez-Tornero et al. 2013; Kostrewa et al. 2015). Dimeric Pol I contains a structured expander or DNA mimicking loop, an expanded cleft, a partially unwound bridge helix and a positioned A12.2 C-terminal domain near the active centre (Engel et al. 2013; Fernandez-Tornero et al. 2013; Kostrewa et al. 2015). The inactive dimers have been discussed to represent "hibernating" Pol I as a possible regulation of its activity in

non-favourable growth conditions in *S. cerevisiae* (Torreira et al. 2017). Pol I dimers that contain structural features of an inactive enzyme were also observed in *S. pombe* indicating an evolutionary conserved mechanism, at least in yeast species (Heiss et al. 2021). Several structures of monomeric Pol I were determined using single particle cryo-EM which may represent steps from an inactive Pol I molecule to an initiation competent and actively initiating form, and finally to an elongating enzyme (Table 6). In general, the following transcriptional Pol I-states can be distinguished: Apo Pol I is monomeric and inactive and has similar structural features as the dimeric enzyme, although subtle cleft contraction is observed, the A12.2 C-ribbon is partially detached and the expander and connector domains are flexible (Neyer et al. 2016; Torreira et al. 2017). After recruitment to the promoter in the presence of Rrn3 and CF, Pol I forms the closed complex (CC), promoter melting yields the open complex (OC), which is converted to an initially transcribing complex (ITC) and finally to an elongating complex (EC) (Engel et al. 2018; Girbig et al. 2022; Hori et al. 2023; Neyer et al. 2016; Tafur et al. 2016). Cryo-EM analyses revealed also several intermediates which allow comprehensive insights into the structure of the Rrn3-Pol I complex (Engel et al. 2016; Pilsl et al. 2016; Torreira et al. 2017) as well as in Pol I promoter binding and opening (Pilsl and Engel 2020; Sadian et al. 2019). The step-by-step transition from an initiation inactive to an elongation competent enzyme is accompanied by gradual cleft contraction, and the removal of the A12.2 C-terminal domain from the active site (Table 6). The transition also involves disordering of the expander, clamp closure, and folding of the bridge helix. Furthermore, the A49 C-terminal tWH domain and the linker which appear to be rather flexible and are absent in many structures adapt defined positions in structural snapshots of OC formation in good correlation with clamp contraction (Sadian et al. 2019). Since the linker spans the cleft and binds the non-template strand in an open complex it might assist stable PIC formation. The A49 tWH domain was localized on top of the clamp and was shown to contact the upstream DNA at defined residues within the CE in the OC structure (Sadian et al. 2019) further pointing to a role in transcription initiation. Accordingly, promoter-dependent *in vitro* experiments confirmed that the tWH domain and the linker region of A49 is required to achieve efficient initiation (Pilsl et al. 2016). Clear evidence that A49 and in particular the tWH domain of A49 support Pol I association with the rDNA promoter *in vivo* were provided by Miller spreading (Albert et al. 2011) and ChIP experiments (Beckouet et al. 2008). Upon initial transcription the A49 tWH alters its location within the complex which was discussed to assist promoter escape and Rrn3 release (Sadian et al. 2019). Positioning of the A49 tWH towards the A135 wall domain presumably triggers the dissociation of the transcription initiation factor Rrn3 from the pre-initiation complex. Along these lines, Chromatin immunoprecipitation analysis (ChIP) revealed that Rrn3 is exclusively bound at the rRNA gene promoter in wildtype cells whereas Rrn3 remains associated with the elongating Pol I in mutant strains lacking either A49 or its C-terminal domain (Beckouet et al. 2008). Additionally, *in vitro* experiments confirmed in yeast (Bier et al. 2004; Milkereit and Tschochner 1998) and *in vivo* experiments in mammals (Herdman et al. 2017) that Rrn3 travels a short distance with Pol I in the initial phase of transcription and gradually dissociates. The latter might be mediated by the altered association of the A49 tWH with Pol I upon initial transcription perhaps in combination with the loss of CF-Rrn3 contacts (Sadian et al. 2019).

The A49 tWH was also localized on top of the clamp in an elongation complex again correlating with cleft contraction which indicated that A49 tWH may stabilize a contracted cleft also in this transcription phase (Tafur et al. 2016). In support, the tWH of RPA49 was detected at a very similar position in an elongation complex of human Pol I (Misiaszek et al. 2021). It is tempting to speculate that the tWH (and perhaps the linker spanning the cleft) are important to stabilize elongating Pol I by cleft contraction leading to a processive enzyme. Since the A49 tWH and linker cannot be seen in other EC structures (Table 6) (Neyer et al. 2016; Tafur et al. 2016) it could be that the position of these domains is subject of dynamic alterations. Interestingly, first evidence that A49 might localize at the position on top of the clamp has already been proposed more than 20 years ago based on EM analyses of apo Pol I with A49 specific antibodies (Bischler et al. 2002).

Whereas several reports propose an active role of TFIIS in Pol II-dependent transcription initiation, not much is known whether its Pol I homolog A12.2 functions in a similar way (Guglielmi et al. 2007; Kim et al. 2007; Malagon et al. 2004; Prather et al. 2005). RNA-Seq data of *RPA12* deletion strains showed increased Pol I occupancy at the 5′end of the rRNA gene (Clarke et al. 2021). This indicates that Pol I is more often paused in vicinity of the promoter. Since lack of A12.2 reduces the tight binding of the heterodimer A34.5/49 to the Pol I core (Merkl et al. 2020; Schwank et al. 2022; Van Mullem et al. 2002) (Table 5), a pleiotropic effect due to improper A49-association cannot be ruled out. Since the C-terminal domain of A12.2 is removed from the active centre during initiation (Engel et al. 2017; Tafur et al. 2016), it remains to be determined whether this movement is a prerequisite rather than a consequence of the initiation process. Experiments in which Pol I lacking all three lobe binding subunits (Pol I ΔRPA12) (Table 5) is reconstituted with recombinantly expressed domains of A12.2 and A34.5/49

(Schwank et al. 2022) should help to answer the question if any of the A12.2 domains support transcription initiation in the presence or absence of the heterodimer.

5 Lobe binding subunits and transcription elongation

5.1 Contributions of the lobe binding subunits to efficient RNA synthesis

The first indication that the heterodimer might be involved in rRNA synthesis was published in 1975 (Huet et al. 1975) when a Pol I was purified lacking two polypeptides with molecular masses similar to A34.5/49. This so called Pol A* enzyme was compromised in RNA synthesis from many different DNA templates when compared with the Pol I wildtype enzyme called Pol A with the exception of the synthetic polymer $d(A\text{-}T)_n$ templates This suggested that the missing polypeptides might participate in an important step of transcription though not interfering with nucleotide polymerisation (Huet et al. 1976, 1975). Since then, experimental data accumulated underlining the involvement of the heterodimer in several steps of transcription elongation. ChIP experiments of yeast strains lacking either A49 or A49CT showed that the C-terminal domain of A49 alone supports Pol I recruitment to the promoter and stably associates with elongating Pol I within the entire transcribed region (Beckouet et al. 2008). Pol I lacking A49 and A34.5 can transcribe DNA templates *in vitro*, though with reduced efficiency (Pilsl et al. 2016). Addition of recombinant heterodimer restores transcriptional activity (Geiger et al. 2010; Kuhn et al. 2007). Reconstitution experiments confirmed that the A49 C-terminal linker and the tWH domain are crucial to support both *in vitro* transcription initiation and elongation (Pilsl et al. 2016).

Deletion of *RPA49* leads to a decrease in Pol I loading rate per ribosomal gene (Albert et al. 2011) which can be suppressed by mutations in the jaw and lobe domains (Darriere et al. 2019). This led to the hypothesis that the mutations in lobe and jaw domains may lead to structural changes mimicking structural rearrangements induced by the presencene of the A49 tWH, maybe facilitating loading of the DNA and closing of the cleft (Darriere et al. 2019). Accordingly, these Pol I suppressor mutants express a highly processive enzyme.

Thus, the high processivity of Pol I may correlate with closing of the cleft which results in tight binding to DNA and probably involves the A49 tWH domain which dynamically associates with Pol I on top of the clamp. Interestingly, it was suggested that the *bona fide* Pol II transcription elongation factor Spt4/5, also supports Pol I transcription (Anderson et al. 2011; Huffines et al. 2021; Schneider et al. 2006; Viktorovskaya et al. 2011). Based on the Spt4/5-Pol II structure (Martinez-Rucobo et al. 2011) Spt4/5 should bind to the elongating enzyme at a similar position as the A49 tWH domain. However, Spt4/5 cannot occupy the exact same position, because the Pol I clamp core helices are extended in length when compared to Pol II. Nevertheless, it is possible that Spt4/5 and A49 tWH are exchanging at this position during specific steps of initiation and/or elongation. Yet, the low Spt4/5 enrichment at rRNA genes compared to enrichment of A34.5 and other Pol I specific factors and compared to the rather high Spt4/5 enrichment at canonical Pol II genes suggests that only very few transcribing Pol I complexes interact with Spt4/5 (Rossi et al. 2021).

Subunit A12.2 is also involved in the nucleotide addition cycle (Appling et al. 2018, 2017; Scull et al. 2021). Accordingly, it activates transcription elongation by modulating kinetics and energetics of nucleotide incorporation (Appling et al. 2017). Comparison of Pol I complexes containing no A12.2 with complexes containing A12.2 without its C-terminal domain led to the conclusion that the A12.2 N-terminal domain stimulates transcription elongation. Using native elongating transcript sequencing (NET-seq), Pol I occupancy along the 35S rRNA gene and the intragenic spacer (IGS) was investigated *in vivo* (Clarke et al. 2018; Huffines et al. 2021; Scull et al. 2020). The deletion of A12.2 significantly alters the Pol I occupancy throughout the 35S rRNA gene and at the IGS. Pol I occupancy was increased at the 5′ and 3′end of the 35S rRNA gene when compared to the WT strain. The elevated Pol I occupancy at the 5′ end was explained as a defect in transcription elongation (Clarke et al. 2021). How these findings match with the proposed stabilisation of elongation complexes when A12.2 is lacking (see paragraph termination) remains to be studied in more detail. Furthermore, subunit A12.2 influences also Pol I pausing at AT-rich sequences (Scull et al. 2021).

It is possible that dynamic interactions between A12.2 and the heterodimer A34.5/49 are required to promote efficient elongation, explaining above mentioned results. First evidence for such a dynamic interplay came from structural studies using the non-hydrolysable NTP analogue GMPCPP to arrest the elongating Pol I (Tafur et al. 2019) (Figure 5). In the resulting Pol I EC* complex the N-terminal domain of A12.2 is still associated with the lobe domain of Pol I at the known position but its C-terminal domain binds Pol I at a position where the dimerization domain of A34.5/49 is associated with the wild-type enzyme. Since the heterodimer was absent in this structure it was suggested that the A12.2 C-terminal domain might help to displace the heterodimer

from the A135 lobe (Figure 5). Initial *in vitro* experiments indicated that the heterodimer cannot be removed by subunit A12.2 (Tafur et al. 2019). Thus, it remains unclear whether and how the A12.2 C-terminal domain and the heterodimer compete for the same lobe binding site and/or cooperate to ensure efficient elongation. Since ChIP data clearly show that the heterodimer is associated with the core Pol I over the entire ribosomal gene from the initiation to the termination site (Beckouet et al. 2008; Rossi et al. 2021), such an interaction must be either short-lived or only a minor Pol I fraction use such a lobe binding exchange mechanism during elongation. In such a scenario it is also possible that the heterodimer is removed by elongation factors like Spt4/5 which would otherwise clash with the A49 tWH domain (see before) and thereby allows binding of A12.2 CT to the lobe. On the other hand, it cannot be ruled out that the EC* complex does not resemble a physiological Pol I complex due to the presence of the non-hydrolysable nucleotide GMPCPP.

5.2 Lobe binding subunits and RNA cleavage

During RNA-synthesis elongating RNA polymerases move forward along the DNA template, they may pause or stall, and they can also backtrack on the DNA. During deeper backtracks, the 3′end of the RNA is displaced from the active site leading to a transcriptionally inactive state (Cheung and Cramer 2011; Kettenberger et al. 2003; Komissarova and Kashlev 1997; Nudler et al. 1997; Wang et al. 2009). As a trigger, physical barriers like nucleosomes or incorporation of wrong nucleotides may slow down elongating polymerases and promote pausing (Cheung and Cramer 2011; Erie et al. 1993; Jeon and Agarwal 1996; Lisica et al. 2016; Luse and Studitsky 2011; Sydow et al. 2009; Thomas et al. 1998).

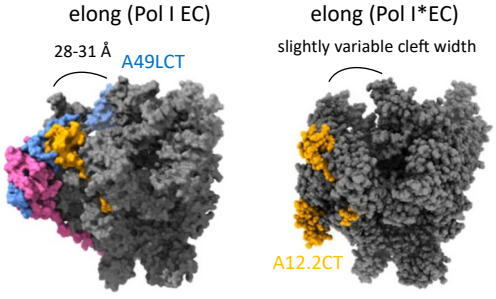


Figure 5: Structures of Pol I EC and of Pol I* EC (formed in the presence of GMPCPP). The 14-subunit Pol I bound to a DNA-RNA scaffold is shown. In this conformation, only the first 67 amino acids of A12.2 can be observed (A12.2 hinge) while the C-terminal domain (A12.2CT) is disordered. In the Pol I* EC, which was obtained in the presence of GMPCPP, A49 and A34.5 are absent and the A12.2 C-terminal domain adopts a position on the A135 surface (Tafur et al. 2019).

According to Pol II dependent transcription, mismatched 3′-RNA termini could cause a distortion in the RNA/DNA duplex and either adopt a frayed state or promote backtracking of the enzyme (Cheung and Cramer 2011; Sydow et al. 2009; Wang et al. 2009). To overcome the backtracked state to restart transcription elongation, Pols have to realign the 3′end of the RNA with the active site either by one dimensional diffusion or by RNA cleavage (Depken et al. 2009; Galburt et al. 2007; Izban and Luse 1993; Lisica et al. 2016; Reines 1992). The efficiency of backtrack recovery is influenced by the depth of the backtracked state. The presence of TFIIS enables Pol II to recover from backtracks by its RNA cleavage activity and accelerates this process. When compared with Pol II, Pol I can recover from backtracking more efficiently: Pol I recovers from deeper backtracks and is faster in backtrack recovery (Lisica et al. 2016). Nevertheless, the mean pause durations were similar for both enzymes. Pol I recovers from short backtracks (up to ~3 nt for Pol I) mainly by diffusion and from intermediate backtracks mostly by cleavage with the help of subunit A12.2. Pol II overcomes short backtracks (up to ~7 nt for Pol II) mainly by diffusion and from intermediate backtracks equally by diffusion and cleavage. The Pol I structure of the open complex with the C-terminal domain of the subunit A12.2 in the active centre (Table 6) might represent Pol I in a backtracked stage which is prerequisite for RNA cleavage. Alternatively, the structure could represent Pol I before RNA synthesis has started (Sadian et al. 2019; Tafur et al. 2016).

The C-terminal domain of A12.2 is required but not sufficient for efficient Pol I cleavage. This could be shown in reconstitution experiments adding purified recombinant proteins/domains of the lobe binding subunits to purified Pol I lacking both A12.2, and the heterodimer A34.5/49 and monitoring transcription and RNA cleavage (Schwank et al. 2022). These experiments revealed that both the conserved amino acids D105 and E106 within the Q.RSADE..T.F motif and a completely folded C-terminal domain are necessary for the cleavage reaction. Furthermore, the N-terminal region of A12.2 supported the cleavage reaction. This domain probably helps to position the C-terminal domain correctly in the active site of Pol I to execute the cleavage reaction. Accordingly, addition of the separated two domains to Pol I lacking lobe binding subunits cannot fully restore cleavage activity (Schwank et al. 2022). Like TFIIS mediated Pol II cleavage, cleavage by Pol I occurs mainly in steps of dinucleotides until a correct 3′ RNA/DNA hybrid pair resides within the active centre of the enzyme. The high conservation between TFIIS and A12.2 in structure and function is underlined by swap experiments which showed that A12.2 supports Pol II RNA cleavage to some extent. Whereas A12.2 removes two nucleotides from misaligned RNA/DNA hybrids

together with Pol II, TFIIS could not substitute for A12.2--dependent Pol I cleavage. It is likely that the partial complementation is due to the structural differences between TFIIS and A12.2 and that the same reason prevents a functional interaction of TFIIS with Pol I (Schwank et al. 2022).

Only the fused N-and C-terminal domains of A12.2 can promote re-extension of cleaved RNA. Without the N-terminal domain misaligned DNA/RNA hybrids were often shortened only by 2 nucleotides and still contained an incorrect base pair. Nevertheless, they were extended (Schwank et al. 2022). Usage of the reconstituted Pol I transcription system helped also to reinvestigate the role of the heterodimer in the cleavage reaction more in detail. The heterodimer stimulated neither cleavage nor backtracking if the N-terminal or C-terminal domain of A12.2 was absent or inactive. But in the presence of the complete subunit A12.2 both the heterodimer and the dimerization module including the linker increased cleavage efficiency (Geiger et al. 2010; Kuhn et al. 2007; Schwank et al. 2022). Interestingly, in the presence of either heterodimer or the dimerization module including and the linker, a longer backtrack of the reconstituted Pol I enzyme on misaligned DNA/RNA hybrids was observed. Accordingly, a correct assembled dimerization domain together with the N-terminal domain of Rpa12.2 might represent a prerequisite to stimulate deep backtracking and efficient RNA cleavage mediated by the Rpa12.2 C-terminus.

5.3 Transcription fidelity and proofreading

Transcription fidelity is required to ensure the production of functional active RNAs. Transcription fidelity can be achieved by two different mechanisms: (1) Proofreading and (2) selectivity for the correct nucleotide. Proofreading is required after the incorporation of a mismatching nucleotide. After recognition of the mis-incorporated nucleotide, RNA polymerase II backtracks, cleaves off the mis-incorporated nucleotide with the support of TFIIS and re-enters transcription elongation (Jeon and Agarwal 1996; Thomas et al. 1998; Wang et al. 2009). The Pol II subunit Rpb9 stimulates the cleavage reaction by enhancing the response of Pol II to TFIIS (Awrey et al. 1997; Knippa and Peterson 2013). Furthermore, Rpb9 is important for transcription fidelity *in vivo* (Nesser et al. 2006) and *in vitro* (Knippa and Peterson 2013).

The second mechanism relies on the selectivity for the correct nucleotide. Before nucleotide addition, the enzyme must discriminate between nucleotides and select the correct nucleotide that forms a Watson–Crick base pairing with the DNA template strand. The substrate selection requires two steps. First, the nucleotide binds to an open active centre and establishes Watson–Crick interactions. Then, the nucleotide is transferred to the insertion site and a phosphodiester bond is formed between the nascent RNA and the nucleotide (Brueckner et al. 2009b; Sydow et al. 2009; Vassylyev et al. 2007; Westover et al. 2004).

Like TFIIS, the Pol I subunit A12.2 is involved in transcription fidelity *in vitro* and *in vivo*. Yeast strains carrying a deletion of *RPA12* have an 11-fold increase in the error-rate upon RNA synthesis *in vivo* (Gout et al. 2017). Pol I lacking the C-terminal domain of A12.2 or the entire full length A12.2 mis-incorporates NTPs *in vitro* and loses its cleavage activity (Kuhn et al. 2007; Schwank et al. 2022). Only the presence of the complete subunit A12.2 but not the presence of the isolated A12.2 domains can prevent significant nucleotide mis-incorporation of Pol I *in vitro* and lead to resumption of transcription after the cleavage reaction (Schwank et al. 2022). In the presence of full-length A12.2 backtracking in combination with cleavage and re-extension is probably the predominant mechanism to reactivate a paused ternary Pol I complex rather than reactivation through one dimensional diffusion kinetics. Furthermore, the release of a backtracked Pol I by RNA cleavage minimalizes erroneous transcripts. In cleavage deficient Pol I mutants the backtracked state apparently cannot be resolved by removal of nucleotides. Therefore, one dimensional diffusion kinetics might eventually allow that a small fraction of the enzyme escapes the backtracked state and extends an error-containing RNA.

In addition to its role as RNA cleaving factor, subunit A12.2 can also influence transcription fidelity by affecting nucleotide selectivity and/or incorporation kinetics (Appling et al. 2017). Nucleotide addition rate constants of wild-type Pol I and Pol I lacking subunit A12.2 are always in the range of milliseconds (Appling et al. 2017, 2015), although the polymerization mechanism is affected in Rpa12.2 depleted Pol I (Appling et al. 2017). RNA cleavage reactions are in the same time range, though slightly delayed (Appling et al. 2015). In contrast, the elongation assays which suggested that synthesis of errorfree RNA require RNA cleavage activity, used a much longer time scale (Schwank et al. 2022). In this experimental set-up Pol I which contains a functional A12.2 subunit steadily incorporates and removes wrong nucleotides, whereas Pol I containing A12.2 mutants cannot remove mis-incorporated nucleotides which then result in the synthesis of erroneous RNA. In summary, A12.2 domains might contribute differently to elongation properties. The N-terminus might speed up elongation by appropriate nucleotide incorporation (Scull et al. 2021) or support backtracking, whereas the C-terminus is responsible for RNA cleavage of a backtracked enzyme.

Surprisingly, A12.2 mutants expressing a truncated A12.2 C-terminus show no growth phenotype even under stress-conditions like elevated or reduced temperature or depletion of the nucleotide pool (Darriere et al. 2019; Van Mullem et al. 2002). This implicates that Pol I proofreading under the tested conditions is not required to generate functional ribosomal RNAs. Mis-incorporated nucleotides in the rRNA – for example in the decoding centre of a newly synthesized ribosome – can, however, be disastrous for the translation process (Chernoff et al. 1994; Green et al. 1997; LaRiviere et al. 2006; Liebman et al. 1995; Powers and Noller 1990; Yoshizawa et al. 1999; Youngman et al. 2004). Furthermore, alterations in the rRNA sequence might also impair proper assembly of ribosome biogenesis factors or ribosomal proteins which would lead to dead-end rRNA precursors, impairing ribosome production (Raué and Planta 1995). Post-transcriptional quality control and RNA degradation mechanisms (see as review Houseley and Tollervey 2009) like the nonfunctional rRNA decay (NRD) (Cole et al. 2009; LaRiviere et al. 2006) or pre-rRNA degradation by the exosome in No-bodies (Dez et al. 2006) might explain the lack of a prominent phenotype in strains lacking Pol I proofreading activity. It remains to be investigated in which physiological situations the impaired proofreading activity of the rRNA synthesis machinery becomes crucial for cellular survival.

5.4 Transcription through nucleosomes

Transcription through nucleosomes is a special challenge for all eukaryotic RNA polymerases. If Pols encounter nucleosomal barriers, they often stall, backtrack and arrest (Cheung and Cramer 2011; Lisica et al. 2016; Luse and Studitsky 2011). Rapid relief of enzyme arrest requires additional factors including RNA cleavage stimulating proteins and cleavage of the 3′ end of the RNA which is displaced from the active centre when the enzyme is backtracked. The mechanism to overcome nucleosomal barriers seems to be similar for many RNA polymerases (Studitsky et al. 1997, 1995). Nevertheless, Pol I and Pol II probably face different challenges in their interplay with native chromatin template. The dense packing of elongating Pol I molecules is likely not compatible with a regular nucleosomal structure. Indeed, rRNA genes are nucleosome depleted, resulting in a so called “open” chromatin state at transcribed rRNA genes (Conconi et al. 1989; Merz et al. 2008). Furthermore, there is evidence that nucleosome depletion occurs upon Pol I transcription and that DNA replication is the dominant process re-assembling nucleosomes on rRNA genes in dividing cells (Wittner et al. 2011). This indicates that Pol I faces the nucleosomal barrier only once during the cell cycle and that nucleosomes might be depleted during a pioneering round of transcription.

Affinity purified Pol I and Pol III transcribe nucleosomal DNA templates in *in vitro* assays more efficiently when compared with Pol II (Merkl et al. 2020). In the absence of transcription factors like TFIIS, or TFIIF or other elongation factors which facilitate chromatin transcription (such as FACT) Pol II has difficulties to transcribe through nucleosomes (Belotserkovskaya et al. 2003; Gaykalova et al. 2015; Luse and Studitsky 2011). In Pol I and Pol III the subunits A34.5/49, A12.2 and C37/53, C11 resembling TFIIE, TFIIF, and TFIIS in Pol II are integral components of the enzymes. For Pol I, the C-terminal part of A12.2 contributes to the ability to transcribe through nucleosomes, suggesting that the RNA cleavage activity of Pol I supports passage through nucleosomes (Merkl et al. 2020). The heterodimer A34.5/49 also supported Pol I passage through a nucleosome. Interestingly, in the absence of the dimerization module formed by A34.5 and the A49 N-terminal domain, addition of the A49 linker and tWH alone improved Pol I movement through nucleosomes. Based on genetic and structural data it was discussed that the A49 linker together with the A49 tWH domain might stabilize the closed conformation of DNA-bound Pol I, thereby maintaining a contracted cleft (Darriere et al. 2019; Tafur et al. 2019). It is possible that an enzyme containing the A49 linker and tWH, but lacking A34.5 and the A49 N-terminal domain may not go into deep backtracks with the 3′ end of the RNA displaced from the active centre, and, thus, might be highly processive (Merkl et al. 2020). In summary, this suggests that cooperation between the different domains of the Pol I lobe binding subunits contributes to the competence to transcribe nucleosomal templates.

The finding that mutant strains expressing Pol I enzymes lacking either A34.5/A49 or A12.2 are viable suggests that Pol I movement through nucleosomes is supported by other factors which weaken the interaction between nucleosomes and DNA during transcription (Gadal et al. 1997; Liljelund et al. 1992; Nogi et al. 1993). Such candidates could be the above mentioned Spt4/5 (Anderson et al. 2011; Schneider et al. 2006) which might replace the A49 linker and tWH domain at the edge of clamp, or FACT (Birch et al. 2009) or Paf1c (Zhang et al. 2009; Y. Zhang et al. 2010) which both interact with chromatin. All three factors contribute to Pol II passage through nucleosomes (Belotserkovskaya et al. 2003; Crickard et al. 2017; Hsieh et al. 2013; Kim et al. 2010). The true impact of these factors on rRNA gene chromatin transcription of Pol I in comparison to their role in Pol II transcription of nucleosomal templates remains to be elucidated. None of these factors shows a strong accumulation at rRNA genes when compared to the respective association with Pol II genes (Rossi et al. 2021). Accordingly, the

next challenges are to determine *(i)* the crucial factors to allow Pol I transcription of nucleosomal rRNA genes *in vivo (ii)* whether nucleosomes are evicted, or temporally disassembled upon Pol I passage through nucleosomes and *(iii)* the possible cooperation between the participating factors and the lobe binding subunits to elucidate the minimal requirements for Pol I passage through nucleosomes.

5.5 Termination of transcription

Transcription termination is the last step of each transcription cycle. It results in a length restricted transcript and the dissociation of Pols and the transcript from the DNA. Common features for all transcription machineries in the termination process are *(i)* to recognize the site which represents the end of the transcript and *(ii)* to overcome energetic barriers to release the transcript and to dissociate from the template. Accordingly, in termination complexes both elongating Pols and associated RNAs must be destabilised. In general, the termination process includes the slowing and pausing of the elongating enzyme which can be achieved by a DNA bound protein and/or by a specific RNA (secondary) structure. Co-transcriptional RNA processing may also have a major impact on termination (see as review Nemeth et al. 2013).

In yeast, one main and several so called "fail-safe" termination sites were determined for Pol I using either *in vitro* termination assays (Lang and Reeder 1993; Lang et al. 1994) or *in vivo* analysis (Clarke et al. 2021; El Hage et al. 2008; Prescott et al. 2004; Reeder et al. 1999) (Figure 6). Most transcripts are terminated at site T1 which is located 90 bp downstream of the mature 25S rRNA sequence and includes an A/T rich sequence (Figure 6). The binding site for the DNA binding proteins Nsi1 or Reb1 (Reb1/Nsi1 binding site) is located 18 bp downstream of the external transcribed spacer element ETS2 (Lang and Reeder 1995; Reeder et al. 1999). The second termination site T2 is in the intergenic spacer IGS1 downstream of the Reb1/Nsi1 binding site. T2, which is followed by replication fork barrier (RFB) sequences (El Hage et al. 2008; Prescott et al. 2004; Reeder et al. 1999), and a recently suggested third termination sequence, T3, just upstream of a promoter-proximal Reb1 binding site of the following 35S rRNA gene (Clarke et al. 2021) are considered as fail-safe termination sites.

Proper Pol I termination *in vitro* and *in vivo* requires the Pol I specific termination factor Nsi1 which binds to a specific DNA sequence to pause elongation shortly upstream of its binding site. DNA-bound Nsi1 or the Nsi1-homolog Reb1 cooperate with a T-rich sequence upstream of the pausing site leading to destabilization and dissociation of the ternary Pol I-RNA-DNA complex *in vitro* (Lang et al. 1994; Merkl et al. 2014; Reiter et al. 2012). Whereas both Myb-related proteins Nsi1 and Reb1 recognize the same binding site and induce termination *in vitro*, predominantly Nsi1 binding to its cognate termination site was detectable *in vivo* (Goetze et al. 2010; Kawauchi et al. 2008; Reiter et al. 2012) arguing that Nsi1 is the *bona fide* Pol I termination factor. In fact, DNA-bound Nsi1 is sufficient to induce Pol I termination *in vitro* and *in vivo* (Merkl et al. 2014; Reiter et al. 2012). Additionally, factors involved in rRNA 3′end processing were reported to also contribute to transcription termination (Braglia et al. 2010a, 2011; El Hage et al. 2008; Elela et al. 1996; Kawauchi et al. 2008; Reeder et al. 1999). Accordingly, co-transcriptional cleavage by the endonuclease Rnt1 generates an entry point for the exonuclease Rat1 which progressively degrades the Pol I-associated transcript with the help of the helicase Sen1. After Rat1 reaches the polymerase, which is slowed down by Nsi1 at the termination site, it collides with the enzyme and disrupts the ternary transcription complex. This process resembles the torpedo-like model of Pol II termination (Kim et al. 2004; Luo et al. 2006; West et al. 2004). The RNA cleavage reaction, which provides the entry point for the exonuclease, is crucial for the torpedo-like termination process. However, *in vivo* and *in vitro* experiments confirmed that transcription can be terminated in the absence of the known Rnt1 cleavage site (Merkl et al. 2014; Reiter et al. 2012). According to the torpedo model, this result could be interpreted by endonucleolytic (failsafe) cleavage within the T-rich DNA stretch to support termination (Braglia et al. 2010b). However, failsafe cleavage is hard to explain since a protein factor must get access to an RNA region which is protected by Pol I bound at the terminator region. This argues that efficient termination either does not absolutely require a functional torpedo mechanism or RNA hydrolysis might be executed by an intrinsic RNA cleavage activity which might be supported by subunit A12.2.

In fact, A12.2 was previously reported to be involved in transcription termination (Clarke et al. 2021; Prescott et al. 2004) and was suggested to destabilize the Pol I elongation complex (Appling et al. 2018). Complete depletion of A12.2 resulted in stabilized elongating Pol I complexes by probably altering the electrostatic interactions within the Pol I EC (Appling et al. 2018). In contrast, Pol I containing only the N-terminal domain of A12.2 was similar to wild-type Pol I in elongation complex stability, indicating that it is an intrinsic destabilizer of the Pol I EC which might help to dissociate the ternary transcription complex at the termination site (Scull et al. 2021). Native elongating transcript sequencing (NET-seq) experiments revealed an increased Pol I occupancy downstream the rRNA gene 3′ end in mutants lacking A12.2 which was explained as terminator read-through.

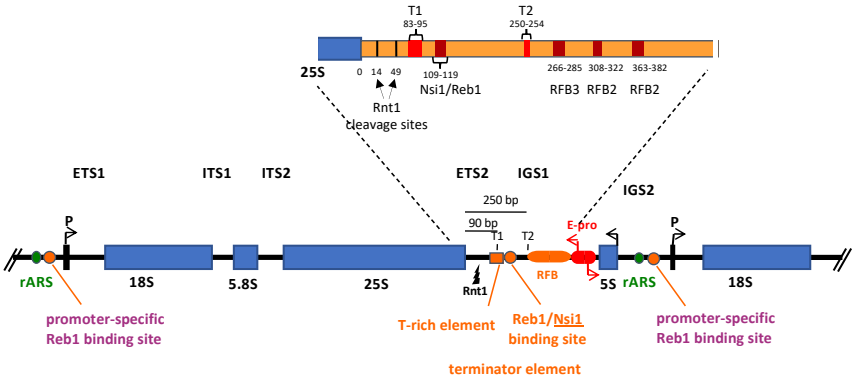


Figure 6: Structure of the *S. cerevisiae* rDNA locus with termination sites. One rDNA repeat of the 150 to 200 tandemly repeated transcription units on chromosome XII is depicted. Pol I synthesizes a 35S precursor RNA (about 9.1 kb) which extends from the promoter (P/black arrow) to the terminator element. The precursor contains the sequences of the mature 18S, 5.8S and 25S rRNA and the external and internal transcribed spacer elements ETS1, ETS2, ITS1 and ITS2, respectively. Pol I-transcribed genes are separated by an intergenic spacer (IGS) which contains the Pol III-transcribed 5S rRNA gene (opposite transcription direction), and the IGS1 and IGS2 sequences which were previously thought to be not transcribed and named NTS1 and NTS2 for non-transcribed spacer. Most of Pol I terminates 90 bp downstream of the 3′end of 25S RNA (T1). Fail-safe termination sites are reported at T2, 250 bp downstream of the 3′end and at the replication fork barrier (RFB), and at site T3 upstream of a promoter proximal Reb1 binding site. Cis elements which were described to be involved in termination are the Reb1/Nsi1 binding site, an upstream T-rich sequence and the RFB. E-pro is a bidirectional Pol II promoter, rARS marks a replication origin within IGS2, Rnt1 the position coding for the Rnt1 cleavage site (modified from Nemeth et al. 2013).

Furthermore, Pol I occupancy downstream of the termination sites T1 and T2 and throughout the IGS was observed for the *rpa12*Δ strain. These analyses suggested that Pol I lacking subunit A12.2 is not terminating transcription at the termination sites T1 and T2 but reaches a third termination site (Clarke et al. 2021). In *S. pombe* the physical interaction between the Nsi1-homolog Reb1 and subunit *Sp*A12 is crucial to efficiently terminate transcription (Jaiswal et al. 2016) which argues that yeast A12.2 and Nsi1 might function similarly in termination.

In summary, subunit A12.2 might play a dual role to support Pol I termination. RNA cleavage by the C-terminal part might either destabilize the ternary transcription complex or help to generate the entry site for exonucleolytic degradation independently of Rnt1, whereas both full length A12.2 and its N-terminal part participate to destabilize the elongating Pol I (Appling et al. 2018; Scull et al. 2021). It will be interesting to see, whether the absence of A12.2-mediated cleavage activity impairs termination at the main termination site T1 or at any of the fail-safe termination sites if the endonuclease Rnt1 is inactivated. Furthermore, future experiments using *in vitro* termination assays in the absence and presence of the different lobe binding domains must clarify whether and how the cleavage activity of A12.2 and the backtracking activity of the heterodimer A34.5/A49 influence Pol I termination.

5.6 Pol I regulation and outlook

Not much is known whether the lobe binding subunits are involved in yeast Pol I regulation. In some mammalian cell lines the expression levels of PAF53 and PAF49, which are homolog to A49 and A34.5, can be reduced by 70 % upon serum starvation, whereas in other cell lines the expression level was not so drastically reduced (Penrod et al. 2015). In other studies treatment of serum deprived cells with insulin resulted in an increase of PAF53 production and enhanced levels of the Pol I initiation and elongation factor UBF (Hannan et al. 1998). Furthermore, posttranslational modifications (PTMs) might impact the dynamics of the PAF53/PAF49 interaction with the Pol I core enzyme (Chen et al. 2013; Penrod et al. 2015). Although all yeast lobe binding subunits have phosphorylated sites (Gerber et al. 2008; Sadowski et al. 2013), up to now no alteration in the phosphorylation pattern depending on growth phase and/or nutrient availability was observed. Although clear evidence exists that the Pol I machinery responds to nutrient availability implicating the target of rapamycin (TOR) signalling pathway (Claypool et al. 2004; Laferte et al. 2006; Li et al. 2006; Milkereit and Tschochner 1998; Philippi et al. 2010; Torreira et al. 2017) (see as review Hori et al. 2023) it is unclear how stringent and fast the reported mechanisms are. Along this line, it was shown that levels of newly

synthesized ribosomal proteins dropped drastically before the activity and integrity of the Pol I machinery was altered upon nutrient deprivation (Philippi et al. 2010; Reiter et al. 2011). It was shown that the immediate shutdown of rRNA production following TOR inhibition was rather the consequence of rapid degradation of aberrantly assembled pre-ribosomal RNAs than due to a direct effect on rRNA synthesis by the Pol I transcription apparatus. But even if rRNA gene transcription is not the target of TOR inactivation, it is still possible that modulation or modifications of transcription factors or Pol I subunits help to adjust the efficiency of rRNA synthesis according to nutrient conditions upon longer times of nutrient deprivation.

In any case, the multiple activities and the dynamics of the lobe binding subunits make them to good candidates to modulate Pol I activity at different stages of the transcription cycle. Future analyses will give further molecular insights how they cooperate to adapt Pol I for maximal efficient rRNA synthesis, how their dynamic movements are co-ordinated and regulated, and whether and how these features are conserved in different eukaryotic cells. The stage is prepared to gather more insights in their precise role in Pol I transcription both *in vitro* and *in vivo*.

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References

Albert, B., Leger-Silvestre, I., Normand, C., Ostermaier, M.K., Perez-Fernandez, J., Panov, K.I., Zomerdijk, J.C., Schultz, P., and Gadal, O. (2011). RNA polymerase I-specific subunits promote polymerase clustering to enhance the rRNA gene transcription cycle. J. Cell Biol. 192: 277–293.

Anderson, S.J., Sikes, M.L., Zhang, Y., French, S.L., Salgia, S., Beyer, A.L., Nomura, M., and Schneider, D.A. (2011). The transcription elongation factor Spt5 influences transcription by RNA polymerase I positively and negatively. J. Biol. Chem. 286: 18816–18824.

Appling, F.D., Lucius, A.L., and Schneider, D.A. (2015). Transient-state kinetic analysis of the RNA polymerase I nucleotide incorporation mechanism. Biophys. J. 109: 2382–2393.

Appling, F.D., Schneider, D.A., and Lucius, A.L. (2017). Multisubunit RNA polymerase cleavage factors modulate the kinetics and energetics of nucleotide incorporation: an RNA polymerase I case study. Biochemistry 56: 5654–5662.

Appling, F.D., Scull, C.E., Lucius, A.L., and Schneider, D.A. (2018). The A12.2 subunit is an intrinsic destabilizer of the RNA polymerase I elongation complex. Biophys. J. 114: 2507–2515.

Aprikian, P., Moorefield, B., and Reeder, R.H. (2001). New model for the yeast RNA polymerase I transcription cycle. Mol. Cell. Biol. 21: 4847–4855.

Awrey, D.E., Weilbaecher, R.G., Hemming, S.A., Orlicky, S.M., Kane, C.M., and Edwards, A.M. (1997). Transcription Elongation through DNA Arrest Sites: a multistep process involving both RNA polymerase II subunit RPB9 and TFIIS. J. Biol. Chem. 272: 14747–14754.

Baudin, F., Murciano, B., Fung, H.K.H., Fromm, S.A., Mattei, S., Mahamid, J., and Müller, C.W. (2022). Mechanism of RNA polymerase I selection by transcription factor UAF. Sci. Adv. 8: eabn5725.

Beckouet, F., Labarre-Mariotte, S., Albert, B., Imazawa, Y., Werner, M., Gadal, O., Nogi, Y., and Thuriaux, P. (2008). Two RNA polymerase I subunits control the binding and release of Rrn3 during transcription. Mol. Cell. Biol. 28: 1596–1605.

Belotserkovskaya, R., Oh, S., Bondarenko, V.A., Orphanides, G., Studitsky, V.M., and Reinberg, D. (2003). FACT facilitates transcription-dependent nucleosome alteration. Science 301: 1090–1093.

Bier, M., Fath, S., and Tschochner, H. (2004). The composition of the RNA polymerase I transcription machinery switches from initiation to elongation mode. FEBS Lett. 564: 41–46.

Birch, J.L., Tan, B.C., Panov, K.I., Panova, T.B., Andersen, J.S., Owen-Hughes, T.A., Russell, J., Lee, S.C., and Zomerdijk, J.C. (2009). FACT facilitates chromatin transcription by RNA polymerases I and III. EMBO J. 28: 854–865.

Bischler, N., Brino, L., Carles, C., Riva, M., Tschochner, H., Mallouh, V., and Schultz, P. (2002). Localization of the yeast RNA polymerase I-specific subunits. EMBO J. 21: 4136–4144.

Blattner, C., Jennebach, S., Herzog, F., Mayer, A., Cheung, A.C., Witte, G., Lorenzen, K., Hopfner, K.P., Heck, A.J., Aebersold, R., et al. (2011). Molecular basis of Rrn3-regulated RNA polymerase I initiation and cell growth. Genes Dev. 25: 2093–2105.

Braglia, P., Heindl, K., Schleiffer, A., Martinez, J., and Proudfoot, N.J. (2010a). Role of the RNA/DNA kinase Grc3 in transcription termination by RNA polymerase I. EMBO Rep. 11: 758–764.

Braglia, P., Kawauchi, J., and Proudfoot, N.J. (2011). Co-transcriptional RNA cleavage provides a failsafe termination mechanism for yeast RNA polymerase I. Nucleic Acids Res. 39: 1439–48.

Brueckner, F., Armache, K.J., Cheung, A., Damsma, G.E., Kettenberger, H., Lehmann, E., Sydow, J., and Cramer, P. (2009a). Structure-function studies of the RNA polymerase II elongation complex. Acta Crystallogr. D Biol. Crystallogr. 65: 112–120.

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

Chédin, S., Riva, M., Schultz, P., Sentenac, A., and Carles, C. (1998). The RNA cleavage activity of RNA polymerase III is mediated by an essential TFIIS-like subunit and is important for transcription termination. Genes Dev. 12: 3857–3871.

Chen, S., Seiler, J., Santiago-Reichelt, M., Felbel, K., Grummt, I., and Voit, R. (2013). Repression of RNA polymerase I upon stress is caused by inhibition of RNA-dependent deacetylation of PAF53 by SIRT7. Mol. Cell 52: 303–313.

Chernoff, Y.O., Vincent, A., and Liebman, S.W. (1994). Mutations in eukaryotic 18S ribosomal RNA affect translational fidelity and resistance to aminoglycoside antibiotics. EMBO J. 13: 906–913.

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

Clarke, A.M., Engel, K.L., Giles, K.E., Petit, C.M., and Schneider, D.A. (2018). NETSeq reveals heterogeneous nucleotide incorporation by RNA polymerase I. Proc. Natl. Acad. Sci. U.S.A. 115: E11633–E11641.

Clarke, A.M., Huffines, A.K., Edwards, Y.J.K., Petit, C.M., and Schneider, D.A. (2021). Defining the influence of the A12.2 subunit on transcription elongation and termination by RNA polymerase I *in vivo*. Genes (Basel) 12: 1939.

Claypool, J.A., French, S.L., Johzuka, K., Eliason, K., Vu, L., Dodd, J.A., Beyer, A.L., and Nomura, M. (2004). Tor pathway regulates Rrn3p-dependent recruitment of yeast RNA polymerase I to the promoter but does not participate in alteration of the number of active genes. Mol. Biol. Cell 15: 946–956.

Cole, S.E., LaRiviere, F.J., Merrikh, C.N., and Moore, M.J. (2009). A convergence of rRNA and mRNA quality control pathways revealed by mechanistic analysis of nonfunctional rRNA decay. Mol. Cell 34: 440–450.

Conconi, A., Widmer, R.M., Koller, T., and Sogo, J.M. (1989). Two different chromatin structures coexist in ribosomal RNA genes throughout the cell cycle. Cell 57: 753–761.

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.

Crickard, J.B., Lee, J., Lee, T.H., and Reese, J.C. (2017). The elongation factor Spt4/5 regulates RNA polymerase II transcription through the nucleosome. Nucleic Acids Res. 45: 6362–6374.

Daiß, J.L., Pilsl, M., Straub, K., Bleckmann, A., Höcherl, M., Heiss, F.B., Abascal-Palacios, G., Ramsay, E.P., Tlučková, K., Mars, J.-C., et al. (2022). The human RNA polymerase I structure reveals an HMG-like docking domain specific to metazoans. Life Sci Alliance 5: e202201568.

Dammann, R., Lucchini, R., Koller, T., and Sogo, J.M. (1995). Transcription in the yeast rRNA gene locus: distribution of the active gene copies and chromatin structure of their flanking regulatory sequences. Mol. Cell. Biol. 15: 5294–5303.

Darriere, T., Pilsl, M., Sarthou, M.K., Chauvier, A., Genty, T., Audibert, S., Dez, C., Leger-Silvestre, I., Normand, C., Henras, A.K., et al. (2019). Genetic analyses led to the discovery of a super-active mutant of the RNA polymerase I. PLoS Genet. 15: e1008157.

Depken, M., Galburt, E.A., and Grill, S.W. (2009). The origin of short transcriptional pauses. Biophys. J. 96: 2189–2193.

Desmoucelles, C., Pinson, B., Saint-Marc, C., and Daignan-Fornier, B. (2002). Screening the yeast "disruptome" for mutants affecting resistance to the immunosuppressive drug, mycophenolic acid. J. Biol. Chem. 277: 27036–27044.

Dez, C., Houseley, J., and Tollervey, D. (2006). Surveillance of nuclear-restricted pre-ribosomes within a subnucleolar region of *Saccharomyces cerevisiae*. EMBO J. 25: 1534–1546.

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.

El Hage, A., Koper, M., Kufel, J., and Tollervey, D. (2008). Efficient termination of transcription by RNA polymerase I requires the 5' exonuclease Rat1 in yeast. Genes Dev. 22: 1069–1081.

Elela, S.A., Igel, H., and Ares, M. (1996). RNase III cleaves eukaryotic preribosomal RNA at a U3 snoRNP-dependent site. Cell 85: 115–124.

Emsley, P. and Cowtan, K. (2004). Coot: model-building tools for molecular graphics. Acta Crystallogr. D Biol. Crystallogr. 60: 2126–2132.

Engel, C., Gubbey, T., Neyer, S., Sainsbury, S., Oberthuer, C., Baejen, C., Bernecky, C., and Cramer, P. (2017). Structural basis of RNA polymerase I transcription initiation. Cell 169: 120.e22–131.e22.

Engel, C., Neyer, S., and Cramer, P. (2018). Distinct mechanisms of transcription initiation by RNA polymerases I and II. Annu. Rev. Biophys. 47: 425–446.

Engel, C., Plitzko, J., and Cramer, P. (2016). RNA polymerase I-Rrn3 complex at 4.8 A resolution. Nat. Commun. 7: 12129.

Engel, C., Sainsbury, S., Cheung, A.C., Kostrewa, D., and Cramer, P. (2013). RNA polymerase I structure and transcription regulation. Nature 502: 650–655.

Erie, D.A., Hajiseyedjavadi, O., Young, M.C., and von Hippel, P.H. (1993). Multiple RNA polymerase conformations and GreA: control of the fidelity of transcription. Science 262: 867–873.

Fath, S., Milkereit, P., Peyroche, G., Riva, M., Carles, C., and Tschochner, H. (2001). Differential roles of phosphorylation in the formation of transcriptional active RNA polymerase I. Proc. Natl. Acad. Sci. U.S.A. 98: 14334–14339.

Feaver, W.J., Henry, N.L., Bushnell, D.A., Sayre, M.H., Brickner, J.H., Gileadi, O., and Kornberg, R.D. (1994). Yeast TFIIE. Cloning, expression, and homology to vertebrate proteins. J. Biol. Chem. 269: 27549–27553.

Fernandez-Tornero, C., Moreno-Morcillo, M., Rashid, U.J., Taylor, N.M., Ruiz, F.M., Gruene, T., Legrand, P., Steuerwald, U., and Muller, C.W. (2013). Crystal structure of the 14-subunit RNA polymerase I. Nature 502: 644–649.

French, S.L., Osheim, Y.N., Schneider, D.A., Sikes, M.L., Fernandez, C.F., Copela, L.A., Misra, V.A., Nomura, M., Wolin, S.L., and Beyer, A.L. (2008). Visual analysis of the yeast 5S rRNA gene transcriptome: regulation and role of La protein. Mol. Cell. Biol. 28: 4576–4587.

Gadal, O., Mariotte-Labarre, S., Chedin, S., Quemeneur, E., Carles, C., Sentenac, A., and Thuriaux, P. (1997). A34.5, a nonessential component of yeast RNA polymerase I, cooperates with subunit A14 and DNA topoisomerase I to produce a functional rRNA synthesis machine. Mol. Cell. Biol. 17: 1787–1795.

Gadal, O., Labarre, S., Boschiero, C., and Thuriaux, P. (2002). Hmo1, an HMG-box protein, belongs to the yeast ribosomal DNA transcription system. EMBO J. 21: S. 5498–S. 5507.

Galburt, E.A., Grill, S.W., Wiedmann, A., Lubkowska, L., Choy, J., Nogales, E., Kashlev, M., and Bustamante, C. (2007). Backtracking determines the force sensitivity of RNAP II in a factor-dependent manner. Nature 446: 820–823.

Gaykalova, D.A., Kulaeva, O.I., Volokh, O., Shaytan, A.K., Hsieh, F.K., Kirpichnikov, M.P., Sokolova, O.S., and Studitsky, V.M. (2015). Structural analysis of nucleosomal barrier to transcription. Proc. Natl. Acad. Sci. U.S.A. 112: E5787–E5795.

Geiger, S.R., Lorenzen, K., Schreieck, A., Hanecker, P., Kostrewa, D., Heck, A.J., and Cramer, P. (2010). RNA polymerase I contains a TFIIF-related DNA-binding subcomplex. Mol. Cell 39: 583–594.

Gerber, J., Reiter, A., Steinbauer, R., Jakob, S., Kuhn, C.D., Cramer, P., Griesenbeck, J., Milkereit, P., and Tschochner, H. (2008). Site specific phosphorylation of yeast RNA polymerase I. Nucleic Acids Res. 36: 793–802.

Girbig, M., Misiaszek, A.D., and Müller, C.W. (2022). Structural insights into nuclear transcription by eukaryotic DNA-dependent RNA polymerases. Nat. Rev. Mol. Cell Biol. 23: 603–622.

Girke, P. and Seufert, W. (2019). Compositional reorganization of the nucleolus in budding yeast mitosis. Mol. Biol. Cell 30: 591–606.

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 Å resolution. Science 292: 1876–1882.

Goetze, H., Wittner, M., Hamperl, S., Hondele, M., Merz, K., Stoeckl, U., and Griesenbeck, J. (2010). Alternative chromatin structures of the 35S rRNA genes in *Saccharomyces cerevisiae* provide a molecular basis for the selective recruitment of RNA polymerases I and II. Mol. Cell. Biol. 30: 2028–2045.

Gout, J.F., Li, W., Fritsch, C., Li, A., Haroon, S., Singh, L., Hua, D., Fazelinia, H., Smith, Z., Seeholzer, S., et al. (2017). The landscape of transcription errors in eukaryotic cells. Sci. Adv. 3: e1701484.

Green, R., Samaha, R.R., and Noller, H.F. (1997). Mutations at nucleotides G2251 and U2585 of 23 S rRNA perturb the peptidyl transferase center of the ribosome. J. Mol. Biol. 266: 40–50.

Griesenbeck, J., Tschochner, H., and Grohmann, D. (2017). Structure and function of RNA polymerases and the transcription machineries. Subcell. Biochem. 83: 225–270.

Grohmann, D., Nagy, J., Chakraborty, A., Klose, D., Fielden, D., Ebright, R.H., Michaelis, J., and Werner, F. (2011). The initiation factor TFE and the elongation factor Spt4/5 compete for the RNAP clamp during transcription initiation and elongation. Mol. Cell 43: 263–274.

Guglielmi, B., Soutourina, J., Esnault, C., and Werner, M. (2007). TFIIS elongation factor and Mediator act in conjunction during transcription initiation *in vivo*. Proc. Natl. Acad. Sci. U.S.A. 104: 16062–16067.

Han, Y., Yan, C., Nguyen, T.H.D., Jackobel, A.J., Ivanov, I., Knutson, B.A., and He, Y. (2017). Structural mechanism of ATP-independent transcription initiation by RNA polymerase I. Elife 6, https://doi.org/10.7554/eLife.27414.

Hanada, K., Song, C.Z., Yamamoto, K., Yano, K., Maeda, Y., Yamaguchi, K., and Muramatsu, M. (1996). RNA polymerase I associated factor 53 binds to the nucleolar transcription factor UBF and functions in specific rDNA transcription. EMBO J. 15: 2217–2226.

Hannan, K.M., Rothblum, L.I., and Jefferson, L.S. (1998). Regulation of ribosomal DNA transcription by insulin. Am. J. Physiol. 275: C130–C138.

Hannig, K., Babl, V., Hergert, K., Maier, A., Pilsl, M., Schächner, C., Stöckl, U., Milkereit, P., Tschochner, H., Seufert, W., et al. (2019). The C-terminal region of Net1 is an activator of RNA polymerase I transcription with conserved features from yeast to human. PLoS Genet. 15: e1008006.

Hausner, W., Lange, U., and Musfeldt, M. (2000). Transcription factor S, a cleavage induction factor of the archaeal RNA polymerase. J. Biol. Chem. 275: 12393–12399.

Heiss, F.B., Daiß, J.L., Becker, P., and Engel, C. (2021). Conserved strategies of RNA polymerase I hibernation and activation. Nat. Commun. 12: 758.

Helbo, A.S., Lay, F.D., Jones, P.A., Liang, G., and Grønbæk, K. (2017). Nucleosome positioning and NDR structure at RNA polymerase III promoters. Sci. Rep. 7: 41947.

Hemming, S.A., Jansma, D.B., Macgregor, P.F., Goryachev, A., Friesen, J.D., and Edwards, A.M. (2000). RNA polymerase II subunit Rpb9 regulates transcription elongation in vivo. J. Biol. Chem. 275: 35506–35511.

Henry, N.L., Sayre, M.H., and Kornberg, R.D. (1992). Purification and characterization of yeast RNA polymerase II general initiation factor g. J. Biol. Chem. 267: 23388–23392.

Herdman, C., Mars, J.C., Stefanovsky, V.Y., Tremblay, M.G., Sabourin-Felix, M., Lindsay, H., Robinson, M.D., and Moss, T. (2017). A unique enhancer boundary complex on the mouse ribosomal RNA genes persists after loss of Rrn3 or UBF and the inactivation of RNA polymerase I transcription. PLoS Genet. 13: e1006899.

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

Hoffmann, N.A., Jakobi, A.J., Moreno-Morcillo, M., Glatt, S., Kosinski, J., Hagen, W.J., Sachse, C., and Muller, C.W. (2015). Molecular structures of unbound and transcribing RNA polymerase III. Nature 528: 231–236.

Hontz, R.D., French, S.L., Oakes, M.L., Tongaonkar, P., Nomura, M., Beyer, A.L., and Smith, J.S. (2008). Transcription of multiple yeast ribosomal DNA genes requires targeting of UAF to the promoter by Uaf30. Mol. Cell. Biol. 28: 6709–6719.

Hori, Y., Engel, C., and Kobayashi, T. (2023). Regulation of ribosomal RNA gene copy number, transcription and nucleolus organization in eukaryotes. Nat. Rev. Mol. Cell Biol 24: 414–429.

Houseley, J. and Tollervey, D. (2009). The many pathways of RNA degradation. Cell 136: 763–776.

Hsieh, F.K., Kulaeva, O.I., Patel, S.S., Dyer, P.N., Luger, K., Reinberg, D., and Studitsky, V.M. (2013). Histone chaperone FACT action during transcription through chromatin by RNA polymerase II. Proc. Natl. Acad. Sci. U.S.A. 110: 7654–7659.

Hubert, J.C., Guyonvarch, A., Kammerer, B., Exinger, F., Liljelund, P., and Lacroute, F. (1983). Complete sequence of a eukaryotic regulatory gene. EMBO J. 2: 2071–2073.

Huet, J., Buhler, J.M., Sentenac, A., and Fromageot, P. (1975). Dissociation of two polypeptide chains from yeast RNA polymerase A. Proc. Natl. Acad. Sci. U.S.A. 72: 3034–3038.

Huet, J., Dezélée, S., Iborra, F., Buhler, J.M., Sentenac, A., and Fromageot, P. (1976). Further characterization of yeast RNA polymerases. Effect of subunits removal. Biochimie 58: 71–80.

Huffines, A.K., Edwards, Y.J.K., and Schneider, D.A. (2021). Spt4 promotes pol I processivity and transcription elongation. Genes 12: 413.

Izban, M.G. and Luse, D.S. (1993). The increment of SII-facilitated transcript cleavage varies dramatically between elongation competent and incompetent RNA polymerase II ternary complexes. J. Biol. Chem. 268: 12874–12885.

Izban, M.G. and Luse, D.S. (1992). The RNA polymerase II ternary complex cleaves the nascent transcript in a 3′→5′ direction in the presence of elongation factor SII. Genes Dev. 6: 1342–1356.

Jaiswal, R., Choudhury, M., Zaman, S., Singh, S., Santosh, V., Bastia, D., and Escalante, C.R. (2016). Functional architecture of the Reb1-Ter complex of *Schizosaccharomyces pombe*. Proc. Natl. Acad. Sci. U.S.A. 113: E2267–E2276.

Jennebach, S., Herzog, F., Aebersold, R., and Cramer, P. (2012). Crosslinking-MS analysis reveals RNA polymerase I domain architecture and basis of rRNA cleavage. Nucleic Acids Res. 40: 5591–5601.

Jeon, C. and Agarwal, K. (1996). Fidelity of RNA polymerase II transcription controlled by elongation factor TFIIS. Proc. Natl. Acad. Sci. U.S.A. 93: 13677–13682.

Jeon, C., Yoon, H., and Agarwal, K. (1994). The transcription factor TFIIS zinc ribbon dipeptide Asp-Glu is critical for stimulation of elongation and RNA cleavage by RNA polymerase II. Proc. Natl. Acad. Sci. U.S.A. 91: 9106–9110.

Kawauchi, J., Mischo, H., Braglia, P., Rondon, A., and Proudfoot, N.J. (2008). Budding yeast RNA polymerases I and II employ parallel mechanisms of transcriptional termination. Genes Dev. 22: 1082–1092.

Keener, J., Dodd, J.A., Lalo, D., and Nomura, M. (1997). Histones H3 and H4 are components of upstream activation factor required for the high-level transcription of yeast rDNA by RNA polymerase I. Proc. Natl. Acad. Sci. U.S.A. 94: 13458–13462.

Keener, J., Josaitis, C., Dodd, J., and Nomura, M. (1998). Reconstitution of yeast RNA polymerase I transcription *in vitro* from purified factors. J. Biol. Chem. 273: 33795–33802.

Kessler, A.C. and Maraia, R.J. (2021). The nuclear and cytoplasmic activities of RNA polymerase III, and an evolving transcriptome for surveillance. Nucleic Acids Res. 49: 12017–12034.

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.

Keys, D.A., Lee, B.S., Dodd, J.A., Nguyen, T.T., Vu, L., Fantino, E., Burson, L.M., Nogi, Y., and Nomura, M. (1996). Multiprotein transcription factor UAF interacts with the upstream element of the yeast RNA polymerase I promoter and forms a stable preinitiation complex. Genes Dev. 10: 887–903.

Keys, D.A., Vu, L., Steffan, J.S., Dodd, J.A., Yamamoto, R.T., Nogi, Y., and Nomura, M. (1994). RRN6 and RRN7 encode subunits of a multiprotein complex essential for the initiation of rDNA transcription by RNA polymerase I in *Saccharomyces cerevisiae*. Genes Dev. 8: 2349–2362.

Khatter, H., Vorlander, M.K., and Muller, C.W. (2017). RNA polymerase I and III: similar yet unique. Curr. Opin. Struct. Biol. 47: 88–94.

Kim, B., Nesvizhskii, A.I., Rani, P.G., Hahn, S., Aebersold, R., and Ranish, J.A. (2007). The transcription elongation factor TFIIS is a component of RNA polymerase II preinitiation complexes. Proc. Natl. Acad. Sci. U.S.A. 104: 16068–16073.

Kim, J., Guermah, M., and Roeder, R.G. (2010). The human PAF1 complex acts in chromatin transcription elongation both independently and cooperatively with SII/TFIIS. Cell 140: 491–503.

Kim, M., Krogan, N.J., Vasiljeva, L., Rando, O.J., Nedea, E., Greenblatt, J.F., and Buratowski, S. (2004). The yeast Rat1 exonuclease promotes transcription termination by RNA polymerase II. Nature 432: 517–522.

Knippa, K. and Peterson, D.O. (2013). Fidelity of RNA polymerase II transcription: role of Rpb9 [corrected] in error detection and proofreading. Biochemistry 52: 7807–7817.

Knutson, B.A., McNamar, R., and Rothblum, L.I. (2020). Dynamics of the RNA polymerase I TFIIF/TFIIE-like subcomplex: a mini-review. Biochem. Soc. Trans. 48: 1917–1927.

Komissarova, N. and Kashlev, M. (1997). RNA polymerase switches between inactivated and activated states by translocating back and forth along the DNA and the RNA. J. Biol. Chem. 272: 15329–15338.

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: e1000102.

Kostrewa, D., Kuhn, C.D., Engel, C., and Cramer, P. (2015). An alternative RNA polymerase I structure reveals a dimer hinge. Acta Crystallogr. D Biol. Crystallogr. 71: 1850–1855.

Kuhn, C.D., Geiger, S.R., Baumli, S., Gartmann, M., Gerber, J., Jennebach, S., Mielke, T., Tschochner, H., Beckmann, R., and Cramer, P. (2007). Functional architecture of RNA polymerase I. Cell 131: 1260–1272.

Laferte, A., Favry, E., Sentenac, A., Riva, M., Carles, C., and Chedin, S. (2006). The transcriptional activity of RNA polymerase I is a key determinant for the level of all ribosome components. Genes Dev. 20: 2030–2040.

Laird, C.D. and Chooi, W.Y. (1976). Morphology of transcription units in *Drosophila melanogaster*. Chromosoma 58, 193–218.

Lalo, D., Steffan, J.S., Dodd, J.A., and Nomura, M. (1996). RRN11 encodes the third subunit of the complex containing Rrn6p and Rrn7p that is essential for the initiation of rDNA transcription by yeast RNA polymerase I. J. Biol. Chem. 271: 21062–21067.

Lang, W.H., Morrow, B.E., Ju, Q., Warner, J.R., and Reeder, R.H. (1994). A model for transcription termination by RNA polymerase I. Cell 79: 527–534.

Lang, W.H. and Reeder, R.H. (1995). Transcription termination of RNA polymerase I due to a T-rich element interacting with Reb1p. Proc. Natl. Acad. Sci. U.S.A. 92: 9781–9785.

Lang, W.H. and Reeder, R.H. (1993). The REB1 site is an essential component of a terminator for RNA polymerase I in Saccharomyces cerevisiae. Mol. Cell. Biol. 13: 649–658.

LaRiviere, F.J., Cole, S.E., Ferullo, D.J., and Moore, M.J. (2006). A late-acting quality control process for mature eukaryotic rRNAs. Mol. Cell 24: 619–626.

Li, H., Tsang, C.K., Watkins, M., Bertram, P.G., and Zheng, X.F. (2006). Nutrient regulates Tor1 nuclear localization and association with rDNA promoter. Nature 442: 1058–1061.

Liebman, S.W., Chernoff, Y.O., and Liu, R. (1995). The accuracy center of a eukaryotic ribosome. Biochem. Cell Biol. 73: 1141–1149.

Liljelund, P., Mariotte, S., Buhler, J.M., and Sentenac, A. (1992). Characterization and mutagenesis of the gene encoding the A49 subunit of RNA polymerase A in *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. U.S.A. 89: 9302–9305.

Lin, C.W., Moorefield, B., Payne, J., Aprikian, P., Mitomo, K., and Reeder, R.H. (1996). A novel 66-kilodalton protein complexes with Rrn6, Rrn7, and TATA-binding protein to promote polymerase I transcription initiation in *Saccharomyces cerevisiae*. Mol. Cell. Biol. 16: 6436–6443.

Lisica, A., Engel, C., Jahnel, M., Roldan, E., Galburt, E.A., Cramer, P., and Grill, S.W. (2016). Mechanisms of backtrack recovery by RNA polymerases I and II. Proc. Natl. Acad. Sci. U.S.A. 113: 2946–2951.

Luo, W., Johnson, A.W., and Bentley, D.L. (2006). The role of Rat1 in coupling mRNA 3′-end processing to transcription termination: implications for a unified allosteric-torpedo model. Genes Dev. 20: 954–965.

Luse, D.S. and Studitsky, V.M. (2011). The mechanism of nucleosome traversal by RNA polymerase II: roles for template uncoiling and transcript elongation factors. RNA Biol. 8: 581–585.

Malagon, F., Tong, A.H., Shafer, B.K., and Strathern, J.N. (2004). Genetic interactions of DST1 in *Saccharomyces cerevisiae* suggest a role of TFIIS in the initiation-elongation transition. Genetics 166: 1215–1227.

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.

Mayer, A., Lidschreiber, M., Siebert, M., Leike, K., Soding, J., and Cramer, P. (2010). Uniform transitions of the general RNA polymerase II transcription complex. Nat. Struct. Mol. Biol. 17: 1272–1278.

McCusker, J.H., Yamagishi, M., Kolb, J.M., and Nomura, M. (1991). Suppressor analysis of temperature-sensitive RNA polymerase I mutations in Saccharomyces cerevisiae: suppression of mutations in a zinc-binding motif by transposed mutant genes. Mol. Cell. Biol. 11: 746–753.

McKune, K., Moore, P.A., Hull, M.W., and Woychik, N.A. (1995). Six human RNA polymerase subunits functionally substitute for their yeast counterparts. Mol. Cell. Biol. 15: 6895–6900.

Merkl, P., Perez-Fernandez, J., Pilsl, M., Reiter, A., Williams, L., Gerber, J., Bohm, M., Deutzmann, R., Griesenbeck, J., Milkereit, P., et al. (2014). Binding of the termination factor nsi1 to its cognate DNA site is sufficient to terminate RNA polymerase I transcription in vitro and to induce termination *in vivo*. Mol. Cell. Biol. 34: 3817–3827.

Merkl, P.E., Pilsl, M., Fremter, T., Schwank, K., Engel, C., Längst, G., Milkereit, P., Griesenbeck, J., and Tschochner, H. (2020). RNA

polymerase I (Pol I) passage through nucleosomes depends on Pol I subunits binding its lobe structure. J. Biol. Chem. 295: 4782–4795.

Merz, K., Hondele, M., Goetze, H., Gmelch, K., Stoeckl, U., and Griesenbeck, J. (2008). Actively transcribed rRNA genes in S. cerevisiae are organized in a specialized chromatin associated with the high-mobility group protein Hmo1 and are largely devoid of histone molecules. Genes Dev. 22: 1190–1204.

Milkereit, P., Schultz, P., and Tschochner, H. (1997). Resolution of RNA polymerase I into dimers and monomers and their function in transcription. Biol. Chem. 378: 1433–1443.

Milkereit, P. and Tschochner, H. (1998). A specialized form of RNA polymerase I, essential for initiation and growth dependent regulation of rRNA synthesis, is disrupted during transcription. EMBO J. 17: 3692–3703.

Misiaszek, A.D., Girbig, M., Grötsch, H., Baudin, F., Murciano, B., Lafita, A., and Müller, C.W. (2021). Cryo-EM structures of human RNA polymerase I. Nat. Struct. Mol. Biol. 28: 997–1008.

Moir, R.D. and Willis, I.M. (2013). Regulation of pol III transcription by nutrient and stress signaling pathways. Biochim. Biophys. Acta 1829: 361–375.

Nemeth, A., Perez-Fernandez, J., Merkl, P., Hamperl, S., Gerber, J., Griesenbeck, J., and Tschochner, H. (2013). RNA polymerase I termination: where is the end? Biochim. Biophys. Acta 1829: 306–317.

Nesser, N.K., Peterson, D.O., and Hawley, D.K. (2006). RNA polymerase II subunit Rpb9 is important for transcriptional fidelity *in vivo*. Proc. Natl. Acad. Sci. U.S.A. 103: 3268–3273.

Neyer, S., Kunz, M., Geiss, C., Hantsche, M., Hodirnau, V.V., Seybert, A., Engel, C., Scheffer, M.P., Cramer, P., and Frangakis, A.S. (2016). Structure of RNA polymerase I transcribing ribosomal DNA genes. Nature 540: 607–610.

Nogi, Y., Vu, L., and Nomura, M. (1991). An approach for isolation of mutants defective in 35S ribosomal RNA synthesis in Saccharomyces cerevisiae. Proc. Natl. Acad. Sci. U.S.A. 88: 7026–7030.

Nogi, Y., Yano, R., Dodd, J., Carles, C., and Nomura, M. (1993). Gene RRN4 in *Saccharomyces cerevisiae* encodes the A12.2 subunit of RNA polymerase I and is essential only at high temperatures. Mol. Cell. Biol. 13: 114–122.

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.

Oakes, M., Nogi, Y., Clark, M.W., and Nomura, M. (1993). Structural alterations of the nucleolus in mutants of *Saccharomyces cerevisiae* defective in RNA polymerase I. Mol. Cell. Biol. 13: 2441–2455.

Osheim, Y.N., French, S.L., Keck, K.M., Champion, E.A., Spasov, K., Dragon, F., Baserga, S.J., and Beyer, A.L. (2004). Pre-18S ribosomal RNA is structurally compacted into the SSU processome prior to being cleaved from nascent transcripts in *Saccharomyces cerevisiae*. Mol. Cell 16: 943–954.

Parnell, T.J., Huff, J.T., and Cairns, B.R. (2008). RSC regulates nucleosome positioning at Pol II genes and density at Pol III genes. EMBO J. 27: 100–110.

Penrod, Y., Rothblum, K., Cavanaugh, A., and Rothblum, L.I. (2015). Regulation of the association of the PAF53/PAF49 heterodimer with RNA polymerase I. Gene 556: 61–67.

Penrod, Y., Rothblum, K., and Rothblum, L.I. (2012). Characterization of the interactions of mammalian RNA polymerase I associated proteins PAF53 and PAF49. Biochemistry 51: 6519–6526.

Philippi, A., Steinbauer, R., Reiter, A., Fath, S., Leger-Silvestre, I., Milkereit, P., Griesenbeck, J., and Tschochner, H. (2010). TOR-dependent reduction in the expression level of Rrn3p lowers the activity of the yeast RNA Pol I machinery, but does not account for the strong inhibition of rRNA production. Nucleic Acids Res. 38: 5315–5326.

Pilsl, M., Crucifix, C., Papai, G., Krupp, F., Steinbauer, R., Griesenbeck, J., Milkereit, P., Tschochner, H., and Schultz, P. (2016). Structure of the initiation-competent RNA polymerase I and its implication for transcription. Nat. Commun. 7: 12126.

Pilsl, M. and Engel, C. (2020). Structural basis of RNA polymerase I pre-initiation complex formation and promoter melting. Nat. Commun. 11: 1206.

Pilsl, M. and Engel, C. (2022). Structural studies of eukaryotic RNA polymerase i using cryo-electron microscopy. Methods Mol. Biol. 2533: 71–80.

Powers, T. and Noller, H.F. (1990). Dominant lethal mutations in a conserved loop in 16S rRNA. Proc. Natl. Acad. Sci. U.S.A. 87: 1042–1046.

Prather, D.M., Larschan, E., and Winston, F. (2005). Evidence that the Elongation Factor TFIIS Plays a Role in Transcription Initiation at GAL1 in *Saccharomyces cerevisiae*. Mol. Cell. Biol. 25: 2650–2659.

Prescott, E.M., Osheim, Y.N., Jones, H.S., Alen, C.M., Roan, J.G., Reeder, R.H., Beyer, A.L., and Proudfoot, N.J. (2004). Transcriptional termination by RNA polymerase I requires the small subunit Rpa12p. Proc. Natl. Acad. Sci. U.S.A. 101: 6068–6073.

Raué, H.A. and Planta, R.J. (1995). The pathway to maturity: processing of ribosomal RNA in *Saccharomyces cerevisiae*. Gene Expr 5: 71–77.

Reeder, R.H., Guevara, P., and Roan, J.G. (1999). *Saccharomyces cerevisiae* RNA polymerase I terminates transcription at the Reb1 terminator *in vivo*. Mol. Cell. Biol. 19: 7369–7376.

Reines, D. (1992). Elongation factor-dependent transcript shortening by template-engaged RNA polymerase II. Elongation factor-dependent transcript shortening by template-engaged RNA polymerase II. J. Biol. Chem. 267: 3795–3800.

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.

Reiter, A., Hamperl, S., Seitz, H., Merkl, P., Perez-Fernandez, J., Williams, L., Gerber, J., Nemeth, A., Leger, I., Gadal, O., et al. (2012). The Reb1-homologue Ydr026c/Nsi1 is required for efficient RNA polymerase I termination in yeast. EMBO J 31: 3480–3493.

Reiter, A., Steinbauer, R., Philippi, A., Gerber, J., Tschochner, H., Milkereit, P., and Griesenbeck, J. (2011). Reduction in ribosomal protein synthesis is sufficient to explain major effects on ribosome production after short-term TOR inactivation in *Saccharomyces cerevisiae*. Mol. Cell. Biol. 31: 803–817.

Rossi, M.J., Kuntala, P.K., Lai, W.K.M., Yamada, N., Badjatia, N., Mittal, C., Kuzu, G., Bocklund, K., Farrell, N.P., Blanda, T.R., et al. (2021). A high-resolution protein architecture of the budding yeast genome. Nature 592: 309–314.

Ruan, W., Lehmann, E., Thomm, M., Kostrewa, D., and Cramer, P. (2011). Evolution of two modes of intrinsic RNA polymerase transcript cleavage. J. Biol. Chem. 286: 18701–18707.

Sadian, Y., Baudin, F., Tafur, L., Murciano, B., Wetzel, R., Weis, F., and Müller, C.W. (2019). Molecular insight into RNA polymerase I promoter recognition and promoter melting. Nat. Commun. 10: 5543.

Sadian, Y., Tafur, L., Kosinski, J., Jakobi, A.J., Wetzel, R., Buczak, K., Hagen, W.J., Beck, M., Sachse, C., and Muller, C.W. (2017). Structural insights into transcription initiation by yeast RNA polymerase I. Embo. J. 36: 2698–2709.

Sadowski, I., Breitkreutz, B.-J., Stark, C., Su, T.-C., Dahabieh, M., Raithatha, S., Bernhard, W., Oughtred, R., Dolinski, K., Barreto, K., et al. (2013). The

PhosphoGRID *Saccharomyces cerevisiae* protein phosphorylation site database: version 2.0 update. Database 2013: bat026.

Schneider, D.A., French, S.L., Osheim, Y.N., Bailey, A.O., Vu, L., Dodd, J., Yates, J.R., Beyer, A.L., and Nomura, M. (2006). RNA polymerase II elongation factors Spt4p and Spt5p play roles in transcription elongation by RNA polymerase I and rRNA processing. Proc. Natl. Acad. Sci. U.S.A. 103: 12707–12712.

Schwank, K., Schmid, C., Fremter, T., Milkereit, P., Griesenbeck, J., and Tschochner, H. (2022). RNA polymerase I (Pol I) lobe-binding subunit Rpa12.2 promotes RNA cleavage and proofreading. J. Biol. Chem. 298: 101862.

Scull, C.E., Clarke, A.M., Lucius, A.L., and Schneider, D.A. (2020). Downstream sequence-dependent RNA cleavage and pausing by RNA polymerase I. J. Biol. Chem. 295: 1288–1299.

Scull, C.E., Lucius, A.L., and Schneider, D.A. (2021). The N-terminal domain of the A12.2 subunit stimulates RNA polymerase I transcription elongation. Biophys. J. 120: 1883–1893.

Sentenac, A. (1985). Eukaryotic RNA polymerases. CRC Crit. Rev. Biochem 18: 31–90.

Shou, W., Sakamoto, K.M., Keener, J., Morimoto, K.W., Traverso, E.E., Azzam, R., Hoppe, G.J., Feldman, R.M., DeModena, J., Moazed, D., et al. (2001). Net1 stimulates RNA polymerase I transcription and regulates nucleolar structure independently of controlling mitotic exit. Mol. Cell 8: 45–55.

Siddiqi, I.N., Dodd, J.A., Vu, L., Eliason, K., Oakes, M.L., Keener, J., Moore, R., Young, M.K., and Nomura, M. (2001). Transcription of chromosomal rRNA genes by both RNA polymerase I and II in yeast uaf30 mutants lacking the 30 kDa subunit of transcription factor UAF. EMBO J. 20: 4512–4521.

Steffan, J.S., Keys, D.A., Dodd, J.A., and Nomura, M. (1996). The role of TBP in rDNA transcription by RNA polymerase I in *Saccharomyces cerevisiae*: TBP is required for upstream activation factor dependent recruitment of core factor. Genes Dev. 10: 2551–2563.

Studitsky, V.M., Clark, D.J., and Felsenfeld, G. (1995). Overcoming a nucleosomal barrier to transcription. Cell. Oct 83: 19–27.

Studitsky, V.M., Kassavetis, G.A., Geiduschek, E.P., and Felsenfeld, G. (1997). Mechanism of transcription through the nucleosome by eukaryotic RNA polymerase. Science 278: 1960–1963.

Sydow, J.F., Brueckner, F., Cheung, A.C., 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.

Tafur, L., Sadian, Y., Hanske, J., Wetzel, R., Weis, F., and Muller, C.W. (2019). The cryo-EM structure of a 12-subunit variant of RNA polymerase I reveals dissociation of the A49-A34.5 heterodimer and rearrangement of subunit A12.2. eLife 8: e101862.

Tafur, L., Sadian, Y., Hoffmann, N.A., Jakobi, A.J., Wetzel, R., Hagen, W.J.H., Sachse, C., and Muller, C.W. (2016). Molecular Structures of Transcribing RNA Polymerase I. Mol. Cell 64: 1135–1143.

Tan, L., Wiesler, S., Trzaska, D., Carney, H.C., and Weinzierl, R.O.J. (2008). Bridge helix and trigger loop perturbations generate superactive RNA polymerases. J Biol 7: 40.

Thomas, M.J., Platas, A.A., and Hawley, D.K. (1998). Transcriptional fidelity and proofreading by RNA polymerase II. Cell 93: 627–637.

Torreira, E., Louro, J.A., Pazos, I., Gonzalez-Polo, N., Gil-Carton, D., Duran, A.G., Tosi, S., Gallego, O., Calvo, O., and Fernandez-Tornero, C. (2017). The dynamic assembly of distinct RNA polymerase I complexes modulates rDNA transcription. eLife 6: e20832.

Van Mullem, V., Landrieux, E., Vandenhaute, J., and Thuriaux, P. (2002). Rpa12p, a conserved RNA polymerase I subunit with two functional domains. Mol. Microbiol. 43: 1105–1113.

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

Viktorovskaya, O.V., Appling, F.D., and Schneider, D.A. (2011). Yeast transcription elongation factor Spt5 associates with RNA polymerase I and RNA polymerase II directly. J. Biol. Chem. 286: 18825–18833.

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 Ångstrom resolution. Science 324: 1203–1206.

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, Q., Daiß, J.L., Xu, Y., and Engel, C. (2022). Snapshots of RNA polymerase III in action – a mini review. Gene 821: 146282.

Weinzierl, R.O.J. (2011). The Bridge Helix of RNA polymerase acts as a central nanomechanical switchboard for coordinating catalysis and substrate movement. Archaea 2011: 608385.

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

West, S., Gromak, N., and Proudfoot, N.J. (2004). Human 5′ → 3′ exonuclease Xrn2 promotes transcription termination at co-transcriptional cleavage sites. Nature 432, 522–525.

Westover, K.D., Bushnell, D.A., and Kornberg, R.D. (2004). Structural basis of transcription: nucleotide selection by rotation in the RNA polymerase II active center. Cell 119: 481–489.

Wittner, M., Hamperl, S., Stockl, U., Seufert, W., Tschochner, H., Milkereit, P., and Griesenbeck, J. (2011). Establishment and maintenance of alternative chromatin states at a multicopy gene locus. Cell 145: 543–554.

Yamamoto, R.T., Nogi, Y., Dodd, J.A., and Nomura, M. (1996). RRN3 gene of Saccharomyces cerevisiae encodes an essential RNA polymerase I transcription factor which interacts with the polymerase independently of DNA template. EMBO J. 15: 3964–3973.

Yoshizawa, S., Fourmy, D., and Puglisi, J.D. (1999). Recognition of the codon-anticodon helix by ribosomal RNA. Science 285: 1722–1725.

Youngman, E.M., Brunelle, J.L., Kochaniak, A.B., and Green, R. (2004). The active site of the ribosome is composed of two layers of conserved nucleotides with distinct roles in peptide bond formation and peptide release. Cell 117: 589–599.

Zhang, G., 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 Å resolution. Cell 98: 811–824.

Zhang, Y., Sikes, M.L., Beyer, A.L., and Schneider, D.A. (2009). The Paf1 complex is required for efficient transcription elongation by RNA polymerase I. Proc. Natl. Acad. Sci. U.S.A. 106: 2153–2158.

Zhang, J., Palangat, M., and Landick, R. (2010). Role of the RNA polymerase trigger loop in catalysis and pausing. Nat. Struct. Mol. Biol. 17: 99–104.

Zhang, Y., Smith, A.D., Renfrow M.B., and Schneider D.A. (2010). The RNA polymerase-associated factor 1 complex (Paf1C) directly increases the elongation rate of RNA polymerase I and is required for efficient regulation of rRNA synthesis. J. Biol. Chem. 285: 14152–14159.