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Snapshots of RNA polymerase III in action – A mini review

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ABSTRACT

RNA polymerase (Pol) III is responsible for the transcription of tRNAs, 5S rRNA, U6 snRNA, and other non-coding RNAs. Transcription factors such as TFIIIA, -B, -C, SNAPc, and Maf1 are required for promoter recognition, promoter opening, and Pol III activity regulation. Recent developments in cryo-electron microscopy and advanced purification approaches for endogenous multi-subunit complexes accelerated structural studies resulting in detailed structural insights which allowed an in-depth understanding of the molecular mechanisms underlying Pol III transcription. Here, we summarize structural data on Pol III and its regulating factors providing a three-dimensional framework to guide further analysis of RNA polymerase III.

1. Introduction

Transcription of cellular genomes is the first step of gene expression, which is carried out by DNA-dependent RNA polymerases (Pols) (Roeder and Rutter, 1969). In eukaryotic cells, Pol I is located in nucleoli, while Pol II and Pol III are located in the nucleoplasm. Pol I transcribes the ribosomal RNA (rRNA) precursor comprising 28S, 5.8S, and 18S ribosomal RNAs, ensuring synthesis of equimolar amounts of these three rRNAs (Moss et al., 2007; Engel et al., 2018). Pol II transcribes all protein-coding messenger RNAs (mRNAs) and many small, regulatory RNAs (Sainsbury et al., 2015). Pol III synthesizes abundant non-coding RNAs in eukaryotic cells, including transfer RNAs (tRNAs), the 5S rRNA, the U6 spliceosomal RNA, and others (Roeder and Rutter, 1969; Dieci et al., 2007). These short, Pol III-dependent transcripts are essential for precise gene transcription and protein synthesis.

Pol III is highly conserved throughout eukaryotic organisms and in comparison to Pol I and II (Vannini and Cramer, 2012). It is the largest RNA polymerase and comprises 17 subunits with a combined molecular weight of 0.7 MDa (Geiduschek and Kassavetis, 2001). In comparison, Pol I and Pol II carry 13 and 12 subunits, respectively (Armache et al., 2005; Daiß et al., 2021; Misiaszek et al., 2021; Zhao et al., 2021). However, each Pol needs accessory factors to initiate and regulate the transcription. Pol III recognizes less diverse promoter types compared to Pol II (Kessler and Maraia, 2021). Pol III promoters are classified in three types: Type I, present in the 5S rRNA genes, containing an internal control region (ICR) which is recognized by TFIIIA and multi-subunit TFIIIC complex; Type II, present in tRNA genes, including the highly conserved A and B boxes which are recognized by TFIIIC directly; and Type III, present in a small collection of genes with various functions, such as U6 snRNA, MRP RNA, and 7SK RNA genes (Arimbasseri and Maraia, 2016). At Type I and II promoters, the TFIIIB complex is recruited by TFIIIC onto the promoter region (Vannini, 2013; Arimbasseri and Maraia, 2016). Type III promoters only exist in vertebrates and contain a TATA box and a proximal sequence element (PSE), that is situated upstream of the transcription start site (TSS) and the TATA box. While the TATA-box is recognized by TFIIIB, the PSE is bound by multi-

Abbreviations: cryo-EM, cryo-electron microscopy; dsDNA, double-stranded DNA; EMSA, electrophoretic mobility shift assay; HL, hypomyelinated leukodystrophy; HPD, histidine phosphatase domain; ICR, internal control region; mRNA, messenger RNA; NMR, nuclear magnetic resonance spectroscopy; Pol, DNA-directed RNA polymerase; PSE, proximal sequence element; RPA, RNA polymerases I subunit; RPABC, RNA polymerases I, II and III subunit; RPAC, RNA polymerases I and III subunit; RPB, RNA polymerase II subunit; RPC, RNA polymerase III subunit; rRNA, Ribosomal RNA; rRNA, ribosomal RNA; SNAPc, snRNA-activating protein complex; snRNA, Small nuclear RNA; τ (tau), General transcription factor 3C polypeptide; TBP, TATA-box-binding protein; TCS, Treacher-Collins-syndrome; TF, Transcription factor; tRNA, transfer RNA; TSS, Transcription start site; VZV, Varicella zoster virus; WHD, winged helix domains.

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subunit 'snRNA-activating protein complex' (SNAPc, Fig. 1) (Schramm and Hernandez, 2002; Orioli et al., 2012; Vannini, 2013). Independent of the promoter class, Pol III activity is globally regulated by the repressor Maf1 (Vannini et al., 2010; Boguta, 2013).

Although the first yeast Pol II structure was solved using X-ray crystallography in 2001 at 2.8 Å (Cramer et al., 2001), it took a decade until the near-atomic resolution structure of Pol I was determined also using X-ray crystallography (Engel et al., 2013; Fernandez-Tornero et al., 2013). In contrast, the first Pol III structure was solved by cryo-electron microscopy (cryo-EM) (Hoffmann et al., 2015). Later on, cryo-EM reconstructions of yeast Pol III were determined in complex with TFIIIB and a DNA-RNA scaffold mimicking a transcription initiation intermediate (Abascal-Palacios et al., 2018; Han et al., 2018; Vorlander et al., 2018) (Fig. 2). Recently, four teams independently determined reconstructions of human Pol III in both apo and elongating state (Ramsay et al., 2020; Girbig et al., 2021; Li et al., 2021; Wang et al.,

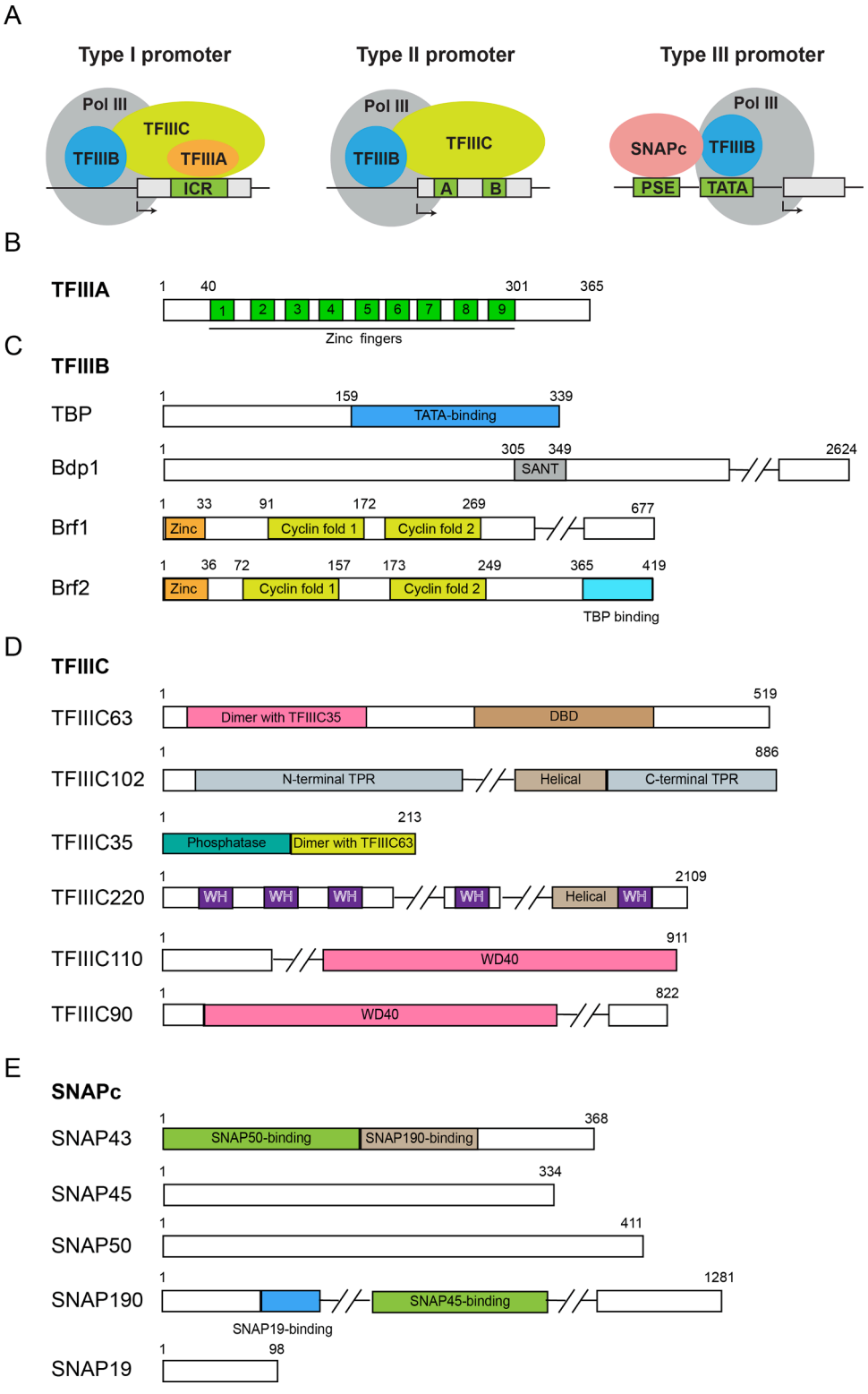

Fig. 1. Schematic overview of Pol III promoters and domain organization of general transcriptional factors from *H. sapiens*. A. The composition of initiation complexes formed on type I, II, and III promoters are schematically illustrated, a black arrow marks the transcription start site; B. Schematic domain organization of TFIIIA; C. Schematic domain organization of TFIIIB subunits TBP, Bdp1, and Brf1/Brf2; D. Schematic domain organization of TFIIIC subunits TFIIIC63, TFIIIC102, TFIIIC35, TFIIIC220, TFIIIC110, and TFIIIC90; E. Schematic domain organization of SNAPc subunits SNAP43, SNAP45, SNAP50, SNAP190, and SNAP19. Functional domains are colored with names indicated. Abbreviations: ICR, internal control region; PSE, proximal sequence element; TPR, tetratricopeptide repeats; DBD, DNA-binding domain; WH, winged helix.

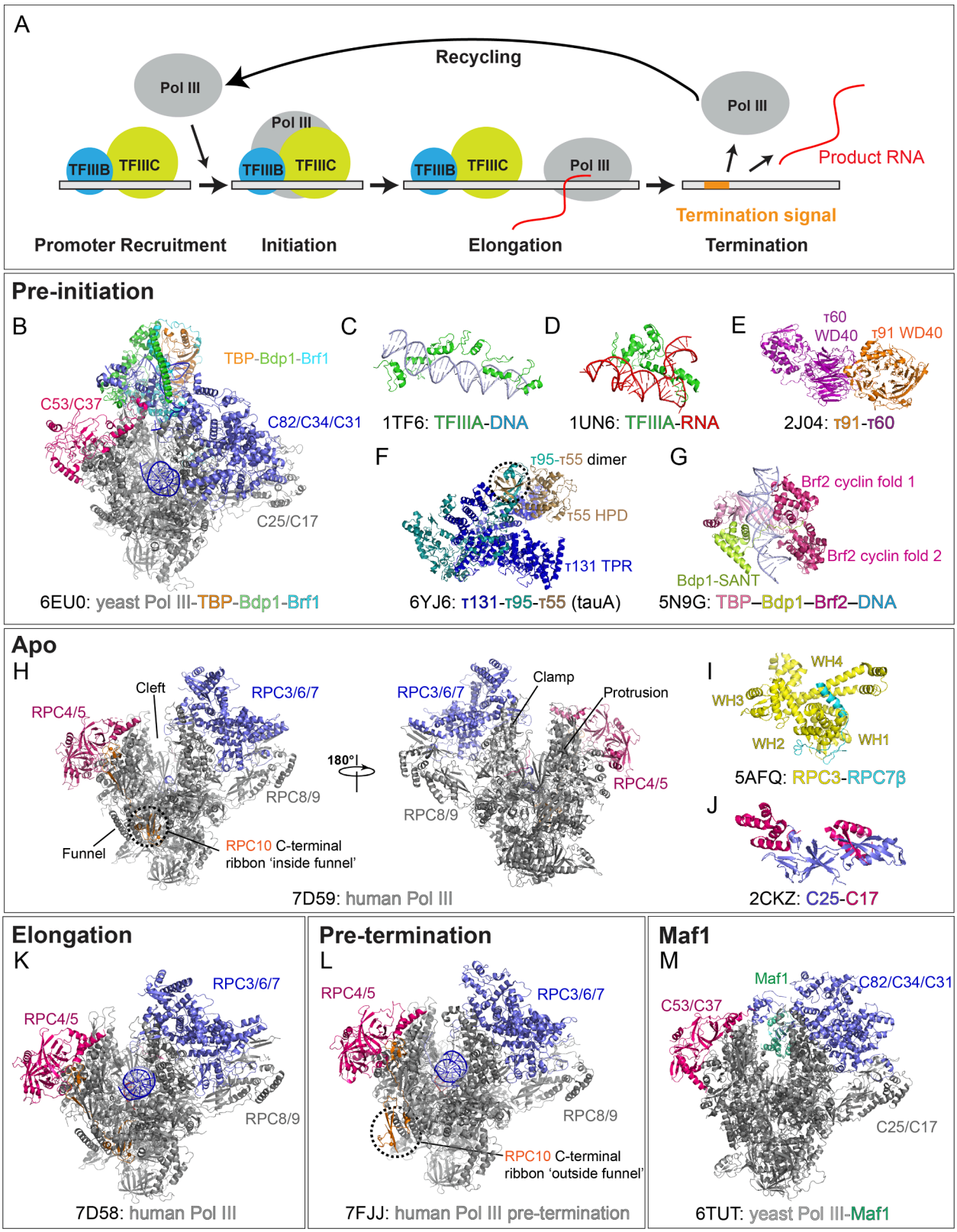

Fig. 2. Overview of transcription cycle and selected three-dimensional structures of Pol III and its transcription factors. A. Schematic depiction of Pol III transcription cycle phases, including initiation, elongation, termination, and recycling; B. Yeast Pol III in pre-initiation state; C. *Xenopus leavis* TFIIIA in complex with 5S rDNA; D. *Xenopus laevis* TFIIIA in complex with 5S rRNA; E. τ91-τ60 subcomplex of yeast TFIIIC; F. The τA subcomplex of yeast TFIIIC; G. Human TBP-Bdp1-Brf2 in complex with DNA; H. Human Pol III in apo state; I. Human RPC3-RPC7β; J. Yeast stalk C25-C17; K. Human Pol III in elongation state; L. Human Pol III in pre-termination state; M. Yeast Pol III in complex with Maf1.

2021) (Fig. 2). These structures provided important details to elucidate the basic mechanisms of Pol III transcription. Here, we summarize the results of these and other studies on Pol III and its transcription factors from a structural perspective to outline their impact on our understanding of structure–function relationships in this transcription system.

2. RNA polymerase III

Yeast and human Pol III both consist of a ten-subunit core, of which the five subunits RPABC1-5 (RPB5, 6, 8, 12, and 10 in yeast) are common among Pols I, II, and III (Cramer et al., 2008). RPAC1 and RPAC2 (AC40 and AC19 in yeast) are shared between Pol I and Pol III (Vannini and Cramer, 2012; Engel et al., 2018). In addition, Pol III has 10 unique subunits, named from RPC1 to RPC10 (Hoffmann et al., 2015), whose structure and function shall be discussed in more detail.

The two largest subunits RPC1 and RPC2 are similar to RPB1 and RPB2 in Pol II and form the majority of the 10-subunit core. A peripheral stalk sub-complex is composed of subunits RPC8 and RPC9 subcomplex and resembles RPA43 (A14/43) in Pol I and Rpb4/7 in Pol II (Armache et al., 2005; Meka et al., 2005; Jasiak et al., 2006; Kuhn et al., 2007). The stalk is important for transcription initiation and electrophoretic mobility shift assays (EMSAs) showed that the Pol III stalk binds to tRNA with high affinity, suggesting that the Pol III stalk might bind newly synthesized RNA products emerging from the adjacent RNA-exit channel (Jasiak et al., 2006).

Subunits RPC3 and RPC6, are distantly related to the Pol II initiation factors TFIIEα and TFIIEβ, respectively (Vannini and Cramer, 2012). Together with RPC7, they form a heterotrimer, which is required for promoter DNA opening (Werner et al., 1992; Thuillier et al., 1995; Brun et al., 1997; Wang and Roeder, 1997; Carter and Drouin, 2010) and open promoter complex stabilization (Fig. 2H). Human Pol III subunit RPC6 contains an iron-sulphur cluster-binding motif which is not present in its *S. cerevisiae* counterpart C34. RPC3 comprises four tandem extended winged helix domains (WHD) and shows helicase activity (Lefevre et al., 2011; Ayoubi et al., 2019). The central part of subunit RPC7 is sufficient for RPC3 interaction (Boissier et al., 2015). In human cells, RPC7 has two isoforms, RPC7α and RPC7β, the former of which is upregulated in cancer cells (Renaud et al., 2014). The C-terminus of both RPC7 isoforms is identical and contains negatively charged as well as aromatic residues. This RPC7 C-terminus can occupy the apo Pol III active center cleft, apparently preventing unspecific transcription (Wang et al., 2021). Genetic studies in yeast indicated that these conserved C-terminal RPC7-residues are indispensable for Pol III transcription since their deletion or mutations was lethal (Thuillier et al., 1995; Wang et al., 2021). Moreover, comparative modelling revealed that binding of the RPC7 C-terminal tail is incompatible with recruitment of the Brf1 Zn-ribbon domain (TFIIIB), indicating a role in promoter-dependent initiation (Wang et al., 2021). The N-terminus of RPC7α interacts with the clamp coiled-coil region of the largest Pol III subunit RPC1, clashing with, and thus preventing, Maf1 recruitment and consequently Pol III regulation (Vorlander et al., 2020a; Girbig et al., 2021; Li et al., 2021; Wang et al., 2021). Additionally, RPC7 also contacts the Pol III stalk module, likely coordinating the conformational transition between apo and elongating states (Girbig et al., 2021; Li et al., 2021; Wang et al., 2021).

The RPC4/RPC5 heterodimer is closely related to its Pol II counterpart TFIIF and is located opposite of the stalk within the Pol III structure (Fernandez-Tornero et al., 2007). This heterodimer is indispensable for accurate transcription initiation and termination (Kassavetis et al., 2010; Arimbasseri et al., 2014; Arimbasseri and Maraia, 2015) and the C-terminal extension of RPC5 contains two tandem WHDs which are connected by an extended linker. This C-terminal RPC5-extension apparently stabilizes the enzyme itself (Ramsay et al., 2020). Notably, RPC10 (C11 in yeast) has a significant homology to subunit RPA12 (A12.2) in Pol I (Werner et al., 2009). Both, RPC10 and RPA12, are orthologous to the N-terminal ribbon domain of Pol II subunit RPB9 and the C-terminal ribbon domain of Pol II transcription elongation factor TFIIS (Jennebach et al., 2012; Engel et al., 2013; Fernandez-Tornero et al., 2013). The N-terminal ribbon of RPC10 has an essential role in transcription termination (Chedin et al., 1998; Landrieux et al., 2006; Mishra et al., 2021) and the linker region to the C-terminal domain functions in Pol III reinitiation-recycling (Mishra et al., 2021). The incorporation of a TFIIS-like domain results in intrinsic Pol III RNA cleavage activity to ensure transcriptional fidelity and proofreading during transcription (Whitehall et al., 1994; Alic et al., 2007). One Pol III study presented structures of RPC10 in two distinct conformations: 'inside funnel' and 'outside funnel'. In the 'inside' conformation the RPC10 C-terminal hairpin docks to the funnel similar to TFIIS (Fig. 2H and 2 K), whereas, it contacts the RPC10 linker and the RPC1 jaw domain in the passive 'outside' conformation (Girbig et al., 2021). Another study observed the RPC10 C-terminus 'inside the funnel' in a backtracked state, even though the density is less defined due to conformational flexibility (Li et al., 2021). Interestingly, the C-terminal domain of the related Pol I subunit A12.2 is also found inside the funnel during initiation (Sadian et al., 2017) or elongation (Tafur et al., 2019) in some cases, while it is flexible in other initiation (Engel et al., 2017; Han et al., 2017; Sadian et al., 2019; Pilsl and Engel, 2020) and elongation states (Neyer et al., 2016; Tafur et al., 2016), pointing towards similar roles of this built-in transcription factor in both transcription systems.

Additional work presenting the structure of human Pol III in a pre-termination state has been reported recently (Hou et al., 2021). In this state, a compacted non-template strand exit tunnel was found to trap the poly-dT termination sequence (Hou et al., 2021). The N-terminal ribbon of RPC10 might enhance the binding of RPC4-RPC5 to the polymerase core, while the C-terminal ribbon of RPC10 remains outside the funnel (Fig. 2L) (Hou et al., 2021). These observations further strengthen the multiple, diverse roles of built-in transcription factor subunit RPC10 during all phases of the Pol III transcription cycle.

The structural information of human Pol III furthermore allowed a detailed mapping of known mutations relevant to human disease (Ramsay et al., 2020; Girbig et al., 2021; Li et al., 2021). These mutations may result in acute severe response to Varicella zoster virus (VZV) infection (Ogunjimi et al., 2017; Carter-Timofte et al., 2018; Carter-Timofte et al., 2019) or neurodevelopmental pathologies such as hypomyelinated leukodystrophy (HL) (Daoud et al., 2013; Thiffault et al., 2015; Dorboz et al., 2018; Gauquelin et al., 2019), Treacher-Collins syndrome (TCS) (Dauwerse et al., 2011; Schaefer et al., 2014; Walker-Kopp et al., 2017) or Wiedemann-Rautenstrauch syndrome (WRS) (Paolacci et al., 2018; Wambach et al., 2018). Most of the mutations causing an acute severe response to VZV infection are found surface-exposed at the enzyme periphery, often in proximity to DNA binding regions (Ramsay et al., 2020; Girbig et al., 2021). These mutations seem to affect only non-specifically initiated transcription in the cytosol (Girbig et al., 2021). Effects of these mutations are apparently buffered by Pol III transcription factors during specific transcription initiation in the nucleus, explaining their defined occurence during acute viral infection (Ramsay et al., 2020; Girbig et al., 2021). In contrast, reported mutations resulting in central nervous system disorders tend to cluster in specific hotspots at subunit-subunit interfaces or effect amino acids which seem to be important for subunit stability itself (Ramsay et al., 2020; Girbig et al., 2021; Li et al., 2021). Disrupting inter- and intramolecular interactions may impair Pol III integrity and thereby enzyme function, as was indicated from studies in yeast (Walker-Kopp et al., 2017).

Pol III deregulation is observed in human cells during cancer development, usually associated with up-regulation of Pol III transcription (White, 2004; Yeganeh and Hernandez, 2020). Consequently, increased expression levels of Pol III, TFIIIB, and TFIIIC subunits, and increased amounts of Pol III transcripts such as pre-tRNA and 5S rRNA were detected in different types of transformed cells and tumor cells (Yeganeh and Hernandez, 2020). Structural evidence indicates that impaired negative regulation by preventing Maf1 association with Pol III containing the RPC7α isoform (see Maf1 chapter below) may play

a role in the process (Girbig et al., 2021), even though many details remain elusive at the moment.

3. General Pol III transcription factors

3.1. TFIIIA

TFIIIA was the first transcription factor identified in eukaryotes (Engelke et al., 1980). It binds the ICR within the 5S rRNA gene, which is considered the first step in the 5S rRNA transcription by RNA polymerase III. TFIIIA is composed of nine Cys2His2-type zinc-finger motifs (Fig. 1B) of which the first three are sufficient for high-affinity interaction with the 5S rRNA gene (Liao et al., 1992) (Fig. 2C and D). Both, nuclear magnetic resonance (NMR) and crystal structures showed how TFIIIA recognizes promoter DNA but also the transcribed 5S rRNA product itself (Fig. 2C and D) (Foster et al., 1997; Lu et al., 2003; Lu and Klug, 2007). This ability to bind both, template DNA and product RNA, reflects the multiple roles of TFIIIA in 5S rRNA gene expression, including 5S rRNA cytoplasmic export (Vannini, 2013).

3.2. TFIIIB

TFIIIB is a hetero-trimeric complex consisting of the TATA-box binding protein (TBP), B-related factor 1 (Brf1), and the B double prime (Bdp1) subunit (Fig. 1C and Table 1). TBP is shared between the Pol I, II, and III transcription systems (Kramm et al., 2019). Brf1 contains an N-terminal Zn-ribbon domain and two consecutive cyclin folds, resembling transcription initiation factor TFIIB and core factor subunit Rrn7 in Pol II and I initiation, respecitvely (Knutson and Hahn, 2011; Naidu et al., 2011). In vertebrates, Brf2 can assemble a TFIIIB version together with Bdp1 and TBP instead of Brf1 to regulate transcription from Type III promoters such as the human U6 snRNA. Intriguingly, Brf2 is also a redox-sensor capable of modulating cellular responses to oxidative stress (Gouge et al., 2015). Bdp1 contains a conserved SANT domain, binding the major groove of double-stranded DNA (dsDNA) and interacting with the TBP-Brf2 complex. In addition, a linker N-terminal of the SANT domain interacts with the minor groove of DNA, further contributing to the stability of the TFIIIB-DNA complex (Gouge et al., 2017) (Fig. 2G and Table 2). In comparison to yeast Bdp1, human Bdp1 contains a C-terminal extension, which is dispensable for initiating transcription *in vitro* (Hu et al., 2004).

Three cryo-EM reconstructions of yeast Pol III-TFIIIB pre-initiation complexes revealed that TFIIIB forms a compact core and two arm modules (Fig. 2B) (Abascal-Palacios et al., 2018; Han et al., 2018; Vorlander et al., 2018). Specifically, the compact core of TFIIIB fixates the TATA box region. One arm module formed by the Bdp1 tether domain (residues 360–398) directly contacts the Pol III protrusion domain, the WHD1 and WHD2 of RPC6 (C34), as well as the 'initiation/termination loop' of RPC5 (C37). The second arm domain consists of a Bdp1-Brf1 coiled-coil and interacts with the WHD2 and WHD3 of RPC6 (C34) (Abascal-Palacios et al., 2018; Han et al., 2018; Vorlander et al., 2018). These structures of Pol III pre-initiation complexes allowed insight into the mechanisms of Pol III promoter melting mechanisms. The closed dsDNA is bent by the Pol III clamp and the cleft loop of RPC3 (C82).

Table 1
Subunit composition of RNA polymerase Pol III in yeast (*S. cerevisiae*) and human.

Pol III		Yeast		Human
Core		RPC160/C160		RPC1/RPC155
		RPC128/C128		RPC2
		RPC40/AC40		RPAC1
		RPC19/AC19		RPAC2
		RPC11/C11		RPC10/hRPC11
		RPB5		RPABC1
		RPB6		RPABC2
		RPB8		RPABC3
		RPB10		RPABC5
		RPB12		RPABC4
Stalk		RPC17/C17		RPC9
		RPC25/C25		RPC8
Trimer		RPC82/C82		RPC3/RPC62
		RPC34/C34		RPC6/RPC39
		RPC31/C31		RPC7α/RPC32α
		(NA*)		(RPC7β/RPC32β)
Dimer		RPC37/C37		RPC5
		RPC53/C53		RPC4
General Transcription Factors				
TFIIIA		TFIIIA		TFIIIA
TFIIIB		TBP		TBP
		Bdp1		Bdp1
		Brf1		Brf1
		(-)		(Brf2)
TFIIIC			TFC1/τ95	TFIIIC63
	τA		TFC4/τ131	TFIIIC102
			TFC7/τ55	TFIIIC35
			TFC3/τ138	TFIIIC220
	τB		TFC6/τ91	TFIIIC110
			TFC8/τ60	TFIIIC90
SNAPc		–		SNPC1/SNAP43
		–		SNPC2/SNAP45
		–		SNPC3/SNAP50
		–		SNPC4/SNAP190
		–		SNPC5/SNAP19

Table 2
Summary of structural studies of Pol III and its transcription factors.

Year	Selected Pol III and its transcription factors	PDB codes	References
1997	NMR structure of Xenopus TFIIIA (zinc finger 1–3) in complex with DNA	1TF3	(Foster et al., 1997)
1998	Crystal structure of Xenopus TFIIIA (zinc finger 1–6) in complex with DNA	1TF6	(Nolte et al., 1998)
2003	Crystal structure of a yeast Brf1-TBP-DNA ternary complex	1NGM	(Juo et al., 2003)
2003	Crystal structure of Xenopus TFIIIA (zinc finger 4–6) in complex with 5S RNA	1UN6	(Lu et al., 2003)
2006	NMR structure of Xenopus TFIIIA (zinc finger 4–6) in complex with 5S RNA	2HGH	(Lee et al., 2006)
2007	Crystal structure of Xenopus TFIIIA (zinc finger 4–6)	2J7J	(Lu and Klug, 2007)
2006	Crystal structure of yeast C17-C25 subcomplex	2CKZ	(Jasiak et al., 2006)
2006	Crystal structure of τ91 and τ60 subcomplex	2J04	(Mylona et al., 2006)
2010	Crystal structure of human Maf1	3NR5	(Vannini et al., 2010)
2010	Cryo-EM structure of yeast Pol III	–	(Vannini et al., 2010)
2011	Crystal structure of human RPC3	2XUB	(Lefevre et al., 2011)
2015	Crystal structure of human RPC3-RPC7β	5AFQ	(Boissier et al., 2015)
2015	Cryo-EM structure of yeast Pol III in apo state	5FJ9, 5FJA	(Hoffmann et al., 2015)
2015	Cryo-EM structure of yeast Pol III elongation complex	5FJ8	(Hoffmann et al., 2015)
2015	Crystal structure of N-terminal TPR repeats of τ131	5AIO	(Male et al., 2015)
2017	Crystal structure of Brf2-TBP-Bdp1-DNA complex	5N9G	(Gouge et al., 2017)
2018	Cryo-EM structure of yeast Pol III pre-initiation complex	6EU0,6EU1, 6EU2, 6EU3, 6F40, 6F41, 6F42, 6F44 6CNB, 6CNC, 6CND, 6CNF	(Vorlander et al., 2018), (Abascal-Palacios et al., 2018), (Han et al., 2018)
2020	Cryo-EM structure of yeast Pol III in complex with Maf1	6TUT	(Vorlander et al., 2020a)
2020	Cryo-EM structure of yeast τA subcomplex of TFIIIC	6YJ6	(Vorlander et al., 2020b)
2020	Cryo-EM structure of human Pol III in apo state	7AST	(Ramsay et al., 2020)
2021	Cryo-EM structure of human Pol III in apo state	7D59, 7A6H	(Girbig et al., 2021), (Wang et al., 2021)
2021	Cryo-EM structure of human Pol III elongation complex	7D58, 7DU2, 7AE1, 7AE3	(Girbig et al., 2021), (Wang et al., 2021), (Li et al., 2021)
2021	Cryo-EM structure of human Pol III in backtracked state	7DN3	(Li et al., 2021)
2021	Cryo-EM structure of human Pol III in pre-termination state	7FJJ	(Hou et al., 2021)

Opening of the Pol III cleft causes a closed DNA movement between the clamp and lobe domains. Next, previsouly flexible WHD1 and WHD2 of RPC6 (C34) apparently become ordered and interact with the Bdp1 tether domain, as well as the 'initiation/termination loop' of RPC5 (C37). This may stabilize dsDNA on the upstream face of Pol III prior to cleft contraction and movement of the RPC3 (C82) cleft loop, supporting DNA-melting and template strand loading into the active site (Abascal-Palacios et al., 2018; Han et al., 2018; Vorlander et al., 2018).

TFIIIB apparently also plays a role in the retrotransposon intergration: recent work presented the cryo-EM reconstruction of the yeast TFIIIB on a tRNA gene promoter in complex with the retrotransposon Ty3, that integrates upstream of Pol III gene promoters (Abascal-Palacios et al., 2021). This structure shows that the architecture of TFIIIB is maintained in both processes and demonstrates that the specific recruitment of the Ty3 integration machinery requires interaction with the Brf1-TBP interface that is also responsible for Pol III recruitment. Thus, Ty3 intregration and Pol III transcription initiation are mutually exclusive (Abascal-Palacios et al., 2021).

3.3. TFIIIC

TFIIIC comprises six subunits in yeast and humans as summarized in Fig. 1D and Table 1. The τA assembly is formed by subunits τ95, τ131, and τ55, and binds to the A box of Pol III type II promoters (Marzouki et al., 1986; Schramm and Hernandez, 2002). The τB sub-complex is formed by τ138, τ91, and τ60 and strongly interacts with the B-box elements of Pol III promoters (Marzouki et al., 1986; Schramm and Hernandez, 2002). The A box is located 12–20 base pairs (bp) downstream of the TSS, followed by the B box in a distance of 30–60 bp (Ramsay and Vannini, 2018). To cope with of the various distances between boxes A and B, τA and τB sub-complexes are separated by a flexible linker region (Schramm and Hernandez, 2002). The τA subunit τ95 comprises a C-terminal DNA-binding domain and an N-terminal domain which dimerizes with τ55. Besides the dimerization domain, τ55 contains a histidine phosphatase domain (HPD) at its N-terminus (Fig. 2F). τ131 consists of helical TPR repeats that bind the HPD of τ55 and a 'triple β-barrel' which is formed by the dimerization domains of subunits τ55 and τ95 (Vorlander et al., 2020b). Subunit τ95 furthermore contains an acidic C-terminal element capable of autoinhibiting DNA binding (Vorlander et al., 2020b), and τ131 bridges between τA and τB subunit τ138. This τB subunit τ138 is the largest protein in TFIIIC, interacts with the two WD40-propeller-containing subunits τ91 and τ60 (Mylona et al., 2006; Male et al., 2015) (Fig. 2E), and comprises five WHDs of which one was crystallized (Male et al., 2015). The direct binding between τA and TFIIIB illustrates the role of TFIIIC in promoter recognition and transcription initiation regulation in synergy with TFIIIB and potentially other factors (Vorlander et al., 2020b).

3.4. MAF1

Single-subunit transcription factor Maf1 negatively regulates RNA polymerase III during the transcription of tRNA and 5S rRNA genes (Boguta et al., 1997), while a subset of 'housekeeping' tRNA genes is less sensitive to Maf1-mediated repression in either yeast or human (Turowski et al., 2016). It has been found that Maf1 can be phosphorylated by protein kinases PKA, Sch9, TORC1, and casein kinase II, which results in increased Pol III activity in either case (Moir et al., 2006; Lee et al., 2009; Graczyk et al., 2011). Phosphorylation by PKA inhibits Maf1 nuclear import and deletion of Sch9 downregulates Pol III transcription in a Maf1-dependent manner (Moir et al., 2006; Lee et al., 2009). On the other hand, Maf1 can be dephosphorylated by phosphatase type 2A (PP2A) resulting in Pol III repression through nuclear accumulation of Maf1 (Oficjalska-Pham et al., 2006). Structural investigation revealed that Maf1 binds yeast Pol III between the clamp, wall, and protrusion domains and the WH2 domain of subunit C34 (Vorlander et al., 2020a) (Fig. 2M). Due to their overlapping binding sites, Maf1 competes with TFIIIB resulting in repression of transcription initiation (Vorlander et al., 2020a). In yeast, the interaction between Maf1 and Pol III is strengthened by an aromatic stacking between the tryptophane residues W319 in Maf1 and W294 in C160 (Vorlander et al., 2020a). Notably, in human

Pol III the N-terminal part of RPC7α also interacts with the Pol III clamp domain through hydrophobic interaction, overlapping with Maf1 binding surface (Girbig et al., 2021; Li et al., 2021; Wang et al., 2021). Therefore, Maf1 binding is probably incompatible with the RPC7α isoform in human Pol III, further indicating that RPC7α might specifically protect human Pol III from Maf1-mediated inhibition in cancer cells (Girbig et al., 2021). This hypothesis is strengthend by the finding that RPC7α is enriched in embryonic stem cells and tumor cells (Haurie et al., 2010).

3.5. SNAPc

snRNA-activating protein complex (SNAPc) is composed of the five subunits SNAP43, SNAP45, SNAP50, SNAP190, and SNAP19 (see review (Schramm and Hernandez, 2002), Fig. 1E and Table 1). Unlike other Pol III transcription factors, SNAPc only exists in vertebrates and is active in transcription of U1/U6 snRNA, 7SK RNA and others by both, Pol II and Pol III (Schramm and Hernandez, 2002). SNAPc recognizes a proximal sequence element (PSE) within the human U6 snRNA promoter that cooperatively regulates transcription together with a TFIIIB form that contains Brf2, but not Brf1 (Schramm and Hernandez, 2002). The direct interaction between SNAPc and TFIIIB is mediated by a contact between SNAPc and the complete Bdp1 subunit of TFIIIB independent of promoter DNA (Gouge et al., 2017). However, the structure of SNAPc and its exact mechanism of action remain largely elusive except for an N-terminal peptide of SNAP190 which interacts with the Pol II activator Oct-1 (Hovde et al., 2002).

3.6. Perspective

To date, the structural basis of Pol III and its transcriptional machineries has been widely studied using different methods including NMR, X-ray crystallography, and cryo-EM, providing insights into the molecular mechanisms of Pol III transcription initiation, elongation and their regulation. Considering the overall high homology between yeast and human Pol III, and between Pol III and Pol II, many features are deductive. However, Pol III-specific regulatiory mechanisms diverge and are still not completely understood. As such, it remains unclear how the C-terminal acidic tail of subunit RPC7 (C31) may regulate transitions between Pol III transcription cycle phases. While the C-terminal domain of RPC5 is required for human Pol III integrity in general (Ramsay et al., 2020), further specific functions of the domain may come to light. The structure and function of specific Pol III initiation factors, such as SNAPc, the C-terminal domain of human Bdp1, and holo-TFIIIC are only partially described. Their structural and functional characterization and as well as their assembly with Pol III on different promoter types remain challenging to study and will be the subjects of future work. We speculate that the structures of the complete Pol III initiation complexes in yeast and human systems will be determined over the coming years, advancing our understanding of the underlying molecular mechanisms throughout organisms. Results will allow us to understand how selective recognition and Pol III transcription regulation are achieved and coordinated, and how the underlying mechanisms evolved throughout species.

Furthermore, cytosolic Pol III activities linked to viral defence may differ from those studied in the nucleus (Kessler and Maraia, 2021). It remains to be seen whether *in vitro* structure–function analysis is capable of capturing and understanding these differences in relation to the respective templates and products. This includes the question whether product RNA-binding to the Pol III stalk is of functional relevance or specifically required during the transcription of defined RNAs to support their folding, regulate re-initiation, or support the recruitment of processing factors. In addition, the process of transcription through nucleosome-containing templates mimicking the *in vivo* situation and the process of transcription termination are tough to study on a structural level due to their transient nature and the underlying flexibilities. Neverthelss, a first structure depicting human Pol III in a pre-termination state was published recently (Hou et al., 2021). The complete termination and re-initiation cycle of Pol III transcription, however, remains elusive at this point.

The recent advancements allowing a more complete understanding of transcription mechanisms and regulatory strategies employed *in vivo* must now be combined with the increasingly detailed mechanistic insights underlying disease-related Pol III dysfunction to translate into therapeutic strategies.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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