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Structural Biology of RNA Polymerase III: Subcomplex C17/25 X-Ray Structure and 11 Subunit Enzyme Model

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Summary

We obtained an 11 subunit model of RNA polymerase (Pol) III by combining a homology model of the nine subunit core enzyme with a new X-ray structure of the subcomplex C17/25. Compared to Pol II, Pol III shows a conserved active center for RNA synthesis but a structurally different upstream face for specific initiation complex assembly during promoter selection. The Pol III upstream face includes a HRDC domain in subunit C17 that is translated by 35 Å and rotated by 150° compared to its Pol II counterpart. The HRDC domain is essential in vivo, folds independently in vitro, and, unlike other HRDC domains, shows no indication of nucleic acid binding. Thus, the HRDC domain is a functional module that could account for the role of C17 in Pol III promoter-specific initiation. During elongation, C17/25 may bind Pol III transcripts emerging from the adjacent exit pore, because the subcomplex binds to tRNA in vitro.

Introduction

RNA synthesis in the eukaryote nucleus is carried out by the multisubunit RNA polymerases I, II, and III. Whereas Pol I and Pol II synthesize ribosomal and mainly messenger RNA, respectively, Pol III transcribes small RNAs, including transfer RNAs, 5S ribosomal RNA, and U6 small nuclear RNA. Structural studies have so far concentrated on Pol II (reviewed in Armache et al. [2005a], Asturias [2004], Boeger et al. [2005], Cramer [2004], and Hahn [2004]). X-ray structures are known of the ten subunit Pol II core and its complexes (Bushnell et al., 2002, 2004; Cramer et al., 2000; Cramer et al., 2001; Gnatt et al., 2001; Westover et al., 2004a, 2004b) and of the complete 12 subunit Pol II and its complexes (Armache et al., 2003, 2005b; Bushnell and Kornberg, 2003; Kettenberger et al., 2003, 2004, 2006). Structural information on the other nuclear RNA polymerases is limited to electron microscopic investigations of Pol I (Bischler et al., 2002; Schultz et al., 1993).

Here, we report structural information on Pol III, the largest nuclear RNA polymerase (for reviews see Chedin et al. [1998a], Geiduschek and Kassavetis [2001], and Schramm and Hernandez [2002]). Pol III has a total molecular weight of around 0.7 MDa and comprises 17 subunits (Table 1). Nine Pol III subunits form a structural core (Table 1). The two largest subunits, C160 and C128, show substantial homology to the Pol II subunits Rpb1 and Rpb2, respectively. Five Pol III subunits are identical in Pol I and Pol II (Rpb5, Rpb6, Rpb8, Rpb10, and Rpb12). The Pol III subunits AC40 and AC19 are identical in Pol I and homologous to the Pol II subunits Rpb3 and Rpb11, respectively. On the periphery of the core enzyme, Pol III contains eight additional subunits, which form three distinct subcomplexes. The subcomplex C82/34/31 (Wang and Roeder, 1997; Werner et al., 1992) and the subcomplex C53/37/11 (Hu et al., 2002; Landrieux et al., 2006) are Pol III specific, although subunit C11 shows limited homology to the Pol II subunit Rpb9 and to the Pol II elongation factor TFIIS (Chedin et al., 1998b; Kettenberger et al., 2003). Finally, the Pol III subcomplex C17/25 has been suggested to be the counterpart of subcomplexes Rpb4/7 in Pol II (Hu et al., 2002; Sadhale and Woychik, 1994; Siaut et al., 2003), Rpa14/43 in Pol I (Meka et al., 2003; Peyroche et al., 2002; Shpakovski and Shematorova, 1999), and RpoF/E in archaeal RNA polymerase (Todone et al., 2001), although the corresponding subunit sequences show only weak conservation in some regions.

We present here the crystal structure and a functional analysis of the yeast Pol III subcomplex C17/25 and describe an 11 subunit model of Pol III. The results provide structural insights into Pol III and reveal specific features of the enzyme that can account for functional differences between nuclear RNA polymerases.

Results

Model of the Pol III Core

Based on the Pol II structure, we constructed a homology model for the Pol III nine subunit core. Subunits Rpb4, Rpb7, and Rpb9 were deleted from the Pol II structure (Armache et al., 2005b) because their homologies to potential Pol III counterparts were too weak. The common subunits Rpb5, Rpb6, Rpb8, Rpb10, and Rpb12 were retained in the model. For the Pol II subunits Rpb1, Rpb2, Rpb3, and Rpb11, sequence alignments with their Pol III homologs were obtained with CLUSTAL W (Thompson et al., 1994) and were used for an initial homology modeling. Side chains in these four Pol II subunits were kept when identical in the Pol III homologs and otherwise replaced by the most common rotamer of the counterpart residues. The resulting nine subunit model was inspected residue by residue. In most regions, the model showed meaningful internal nonpolar contacts and salt bridges, confirming the alignments. A few regions, however, showed steric clashes or disallowed contacts, indicating misalignment of the corresponding sequence stretches. Manual realignment of these weakly conserved stretches (Figure S1 available in the Supplemental Data with this article online) led to a model with good internal packing throughout. The three-dimensional context provided by the Pol II structure allowed for confirmation of the model, including

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Table 1. RNA Polymerase Subunits

Polymerase Part	Pol III Subunit	Pol II Subunit	Subunit Type	Sequence Identity (%)	Conserved Fold (%)
Core	C160	Rpb1	Homolog	28.4	83.2
	C128	Rpb2	Homolog	35.8	87.2
	AC40	Rpb3	Homolog	25.8	60.2
	AC19	Rpb11	Homolog	20.8	81.6
	Rpb5	Rpb5	Common	100	100
	Rpb6	Rpb6	Common	100	100
	Rpb8	Rpb8	Common	100	100
	Rpb10	Rpb10	Common	100	100
	Rpb12	Rpb12	Common	100	100
Rpb4/7 subcomplex	C17	Rpb4	Homolog	7.2	50
	C25	Rpb7	Homolog	25.2	81.3
Upstream subcomplex	C82/34/31		Specific	–	–
Downstream subcomplex	C53/37/11[a]	Rpb9[a]	Unclear	–	–
Eleven subunit Pol III model	–	–	–	39.4	83.4

[a] Subunit C11 shows homology to Rpb9 and TFIIS. Rpb9 was previously defined as a part of the Pol II core.

interactions that involve subunit interfaces and regions that are distant in the sequences.

X-Ray Analysis of the Pol III Subcomplex C17/25

The Pol II subcomplex Rpb4/7 could not be used in the Pol III modeling because of weak or apparently lacking sequence conservation with its Pol III counterpart C17/25. We therefore determined the C17/25 structure independently by X-ray crystallography (Experimental Procedures). After coexpression of C17 and C25 in *E. coli*, a soluble C17/25 heterodimer could be purified and crystallized. Because molecular replacement with the structures of Rpb4/7 (Armache et al., 2005b) and the archaeal counterpart RpoF/E (Todone et al., 2001) failed, the crystals were phased de novo with selenomethionine labeling and single anomalous diffraction (Table 2). The structure was refined at 3.2 Å resolution to a free R factor of 30.7% and showed good stereochemistry (Table 2).

Overall C17/25 Structure

The structure of C25 resembles that of its counterparts Rpb7 and RpoE (Figure 1). The N-terminal "tip" domain of C25 shows a root-mean-square deviation (rmsd) in Cα atom positions of 4.2 Å and 1.6 Å in Rpb7 and RpoE, respectively, whereas the C-terminal OB domain is quite divergent. The relative position of the two C25 domains differs slightly from that observed in Rpb7 (Figure S2). C25 differs from Rpb7 mainly by the absence of the short helical turn K* in the tip domain and the presence of a flexible, nonconserved loop, B4-B5, that is 34 residues longer than in Rpb7 (Figure 1).

The structure of C17 reveals a compact N-terminal "tip-associated" domain, which packs mainly against the C25 tip domain and not between the tip and OB domains as in Rpb4/7 and RpoF/E (Figure 1B). The only contact between the C17 tip-associated domain and the C25 OB domain is formed between C17 helix H2 and C25 loop B2-B3. Consistently, a mutation at the B2-B3 loop (S100P) impairs C17 binding in vivo (Zaros and Thuriaux, 2005). The C17 tip-associated domain connects via a flexible linker to a C-terminal HRDC domain, a fold that occurs in RecQ helicases and ribonucleases (Meka et al., 2003; Morozov et al., 1997). The C17 HRDC fold resembles the corresponding domains in Rpb4 and RpoF (rmsd in Cα positions of 2.07 and 4.5 Å, respectively, Figure S2), although the sequence conservation is very weak or absent (Figure 1A and Table 1).

The C17 HRDC Domain Adopts a Unique Position

Although the overall domain folds are conserved between C17/25 and Rpb4/7, the observed position of the C17 HRDC domain is very different from that in Rpb4 and RpoF (Figure 1B). Compared to Rpb4 or RpoF, the HRDC domain of C17 is translated by about 35 Å and rotated by about 150°. The C17 HRDC domain packs against the C25 OB domain, between the C2-C3 loop and the C terminus (Figures 1B and 2A). The HRDC-OB interface is complementary in shape and electrostatics (Figure 2A) and includes many hydrophobic residues, which are well conserved among several species (Figure S3) but are generally not conserved in the Pol I and Pol II counterparts (C25 residues F116, W130, M132, L138, and W211, and C17 residues M107, L121, and V124) (Figure 2B). Residues in the Rpb4 HRDC domain-Rpb7 interface are also conserved

Table 2. C17/25 X-Ray Diffraction Data and Refinement Statistics

Crystal	C17/25 SeMet	C17/25 Native
Data Collection[a]		
Space group	$P6_122$	$P6_122$
Wavelength (Å)	0.97932	0.97894
Unit cell axis (Å)	137.5, 240.6	138.2, 247.1
Resolution (Å)	50–3.5 (3.63–3.5)[b]	30–3.2 (3.31–3.2)
Completeness (%)	100	88.6 (91.4)
Unique reflections	17,684 (1,726)	21,061 (2,107)
Redundancy	14.4 (14.7)	3.9 (3.9)
R_{sym} (%)	10.3 (38.5)	9.1 (46.9)
$\langle I/\sigma I \rangle$	10.6 (8.3)	17.6 (2.6)
Refinement		
Amino acid residues		537
Rmsd bonds (Å)		0.007
Rmsd angles (°)		1.3
R_{cryst} (%)		23.6
R_{free} (%)		30.7

[a] Diffraction data were collected at beamline X06SA at the Swiss Light Source, Villigen, Switzerland.
[b] Numbers in parenthesis refer to the highest resolution shell.