Crystal structure of the C17/25 subcomplex from *Schizosaccharomyces pombe* RNA polymerase III

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Abstract: Eukaryotic RNA polymerase III (Pol III) is a multisubunit enzyme responsible for transcribing tRNA, 5S rRNA, and several small RNAs. Of the 17 subunits in Pol III, the C17 (Rpc17) and C25 (Rpc25) subunits form a stable subcomplex that protrudes from the core polymerase. In this study, we determined the crystal structure of the C17/25 subcomplex from *Schizosaccharomyces pombe*. The subcomplex adopts an elongated shape, and each subunit has two domains. The two subunits in the subcomplex are tightly packed and extensively interact, with a contact area of 2080 Å². The overall conformation of *S. pombe* C17/25 is considerably different from the previously reported structure of C17/25 from *Saccharomyces cerevisiae*, with respect to the position of the C17 HRDC domain, a helix bundle essential for cell viability. In contrast, the *S. pombe* C17/25 structure is quite similar to those of the Pol II and archaeal counterparts, Rpb4/7 and RpoE/F, respectively, despite the low sequence similarity. A phylogenetic comparison of the C17 subunits among eukaryotes revealed that they can be classified into three groups, according to the length of the interdomain linker. *S. pombe* C17, as well as Rpb4 and RpoF, belongs to the largest group, with the short linker. On the other hand, *S. cerevisiae* C17 belongs to the smallest group, with the long linker, which probably enables the subcomplex to assume the alternative conformation.

Keywords: X-ray crystallography; transcription; C17/25; Rpc17/25; Rpb4/7 family; RNA polymerase III

Introduction

Eukaryotes have three nuclear RNA polymerases, RNA polymerases I, II, and III (Pol I, Pol II, and Pol III). They are huge protein complexes with molecular masses over 500 kDa and consist of many subunits (14 for Pol I, 12 for Pol II, and 17 for Pol III). All three

of these polymerases have a homologous 10-subunit core, which is central to the catalytic function of DNA-dependent RNA synthesis. In contrast, the rest of the subunits are more divergent and located peripherally to the catalytic core. Many studies have shown that these additional subunits are important for more specific functions, such as initiation, termination, and regulation of transcription.^{1,2}

Pol III transcribes tRNA, 5S rRNA, and several other noncoding RNAs.³ As its products are often needed in large amounts, Pol III exhibits some interesting features for efficient transcription, such as facilitated recycling.⁴ Transcription initiation by Pol III is governed by a distinct set of transcription factors, including TFIIB and TFIIC.⁵ Efficient termination by a poly-U stretch is another unique property of Pol

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III transcription.⁵ Pol III is the largest and most complex enzyme of all known polymerases. Besides the 10-subunit core, it has seven additional subunits, which can be divided into three stable subcomplexes.⁶ The C53/37 subcomplex is a distant homolog of TFIIF, one of the Pol II transcription factors, and participates in transcription initiation, elongation, and termination.^{7–10} The C82/34/31 subcomplex is involved in transcription initiation and Maf1-mediated repression.^{11–14} The C17/25 subcomplex is also implicated in transcription initiation, by interacting with TFIIB.¹⁵

Among the three subcomplexes of Pol III, the C17/25 (Rpc17/25) heterodimer has counterparts in other polymerases (A14/43 in Pol I, Rpb4/7 in Pol II, and RpoE/F in the archaeal RNA polymerase). Although the paralogs of the C25 subunit (A43, Rpb7, and RpoE) share high homology, the sequence similarities between C17 and its counterparts (A14, Rpb4, and RpoF) are often low and variable.^{16,17} Unlike A14,¹⁸ Rpb4,¹⁹ and RpoF,²⁰ the C17 subunit is essential for viability in the budding yeast *Saccharomyces cerevisiae*.¹⁵ This subunit interacts with Brf1, one of the components of TFIIB, and is required for proper initiation from Pol III-specific promoters.^{15,17} The C25 subunit is responsible for the binding of the subcomplex to the polymerase core. Furthermore, this subcomplex also has nucleic acid-binding ability, which may assist in the folding of the nascent transcript.²¹ The crystal structures of Pol II Rpb4/7 and archaeal RpoE/F from various organisms have been determined and revealed that they all share a similar stalk-like structure that protrudes from the catalytic core.^{22–28} However, structural studies on the C17/25 subcomplex have been limited to that from *S. cerevisiae*, and some conformational differences were noted between the crystal structure and the cryo-EM images.^{21,29}

In this study, we solved the crystal structure of the C17/25 subcomplex from the fission yeast *Schizosaccharomyces pombe*. The overall structure resembles those of the Pol II Rpb4/7 and archaeal RpoE/F complexes and is also similar to the conformation observed in cryo-EM images of *S. cerevisiae* Pol III.²⁹ However, the position and orientation of the HRDC domain of the C17 subunit are quite different, in comparison with those described for the previously reported crystal structure of *S. cerevisiae* C17/25. A sequence analysis of the C17 subunit, together with the results from previous studies, suggested that the conformation observed in this study is common among eukaryotes.

Results and Discussion

Overall structure

The crystal structure of the *S. pombe* C17/25 subcomplex was solved by molecular replacement and refined to an R_{work} of 19.0% at 2.19-Å resolution (Table I). The crystal contains one complex per asym-

Table I. Data Collection and Refinement Statistics

	Native
Data collection	
Space group	$P322_1$
Cell constant (Å)	$a = b = 85.4, c = 93.3$
Wavelength (Å)	1.0000
Resolution range ^a (Å)	34.4–2.19 (2.25–2.19)
Reflections	221,418
Completeness ^a (%)	99.9 (99.9)
R_{merge} ^{a,b} (%)	10.8 (66.9)
I/σ ^a	19.2 (4.0)
Redundancy ^a	10.7 (10.0)
Wilson B -factor (Å ²)	30.5
Structural refinement	
Total protein atoms	2582
Total solvent atoms	161
Resolution range ^a (Å)	34.4–2.19 (2.31–2.19)
R_{work} ^{a,c} (%)	19.0 (20.5)
R_{free} ^{a,c} (%)	23.7 (27.6)
Overall B -factors (Å ²)	
Average	36.3
Min	12.9
Max	109.7
RMSD from ideality	
Bond lengths (Å)	0.008
Bond angles (°)	1.0
MolProbity scores ^d	
All atom clash score	11.53 (85th percentile)
Bad rotamers (%)	0.1
Ramachandran outliers (%)	0.0
Ramachandran favored (%)	93.6
Molprobity score	2.00 (85th percentile)

^a Values in parentheses are for the highest resolution shell.

^b $R_{\text{merge}} = \sum |I_{\text{av}} - I_i| / \sum I_i$, where I_{av} is the average of all individual observations I_i .

^c $R_{\text{work/free}} = \sum |F_o - F_c| / \sum F_o$, where the crystallographic R -factor is calculated including and excluding refinement reflections. In each refinement, free reflections consist of 5% of the total number of reflections.

^d From MolProbity.

metric unit, and all amino acid residues were modeled except for the disordered residues, 162–181, of the C25 subunit. The overall structure of C17/25 adopts an elongated shape [Fig. 1(A)]. The C25 subunit consists of two domains, the tip domain and the oligonucleotide binding-fold (OB-fold) domain. The tip domain is composed of a ribonucleoprotein fold, which provides a binding surface for the Pol III core. The tip region of the domain is thought to interact with the Rpb6 subunit and the C-terminal region of the largest subunit.²¹ The C25 OB-fold domain forms a five-stranded closed β barrel, a motif that is often used for nucleic acid recognition.³⁰ The distal end of the OB-fold domain is capped by a lid, consisting of three antiparallel β strands. The C17 subunit also consists of two domains, the tip-associated domain and the HRDC domain. The tip-associated domain has two α helices and an isolated β strand and extensively interacts with the C25 tip domain. The C17 HRDC domain forms an α helix bundle and resides at the boundary between the two domains of the C25 subunit.

Figure 1. Crystal structure of *S. pombe* C17/25 and comparison with that of *S. cerevisiae* C17/25. (A) Overall structure of *S. pombe* C17/25. C17 is colored red and C25 is colored blue. The α -helices on the C17 subunit are numbered and labeled accordingly ($\alpha 1$ – $\alpha 6$). (B) Overall structure of *S. cerevisiae* C17/25 (Chain C/D of PDBID 2CKZ). (C) Main chain superposition of the C25 subunit, the HRDC domain of the C17 subunit, and the tip-associated domain of the C17 subunit. *S. pombe* C17/25 is colored purple and *S. cerevisiae* C17/25 is colored cyan. (D) Schematic diagram showing the approximate position of the C17/25 subcomplex on the polymerase core.

Position of the HRDC domain of C17

The C17 tip-associated domain, the C17 HRDC domain, and the C25 subunit superposed well with their counterparts in the *S. cerevisiae* C17/25 structure²¹ (C_{α} RMSDs of 0.72 Å for the tip-associated domain, 0.87 Å for the HRDC domain, and 1.05 Å for the C25 subunit). The only exception is an unconserved, short insertion between helices $\alpha 1$ and $\alpha 2$ in the C17 subunit [Fig. 1(C)]. Nevertheless, the observed position of the C17 HRDC domain differs between the two species [Fig. 1(A,B)]. The HRDC domain of *S. pombe* C17/25 lies at the boundary between the two domains of the C25 subunit. This conformation is designated as the Type I conformation in this report. In contrast, in the *S. cerevisiae* C17/25 structure, the HRDC domain is packed against the cleft formed between the lid and the C-terminus of the C25 OB domain (designated here as the Type II conformation). The conformational difference is substantial and can be approximately represented by a 70 Å translation and 140° rotation. The Type I conformation of *S. pombe* C17/25 is much more similar to those observed in Pol II Rpb4/7 and archaeal RpoE/F. Indeed, a comparison of the known crystal structures of Rpb4/7 family members showed

that only *S. cerevisiae* C17/25 displays the Type II conformation (Fig. 2).²¹ Pol II Rpb4/7 and archaeal RpoE/F, each from three distinct organisms, all assume the Type I conformation.^{22–28} In Pol I A14/43, the HRDC domain is missing and is replaced by the disordered tail.³¹

Molecular interactions around the C17 HRDC domain

The interaction between C17 and C25 is extensive, with a total contact area of 2080 Å². The contribution of the HRDC domain is minor but significant, as revealed by the contact area of 398 Å². Helices $\alpha 4$ and $\alpha 5$ in the HRDC domain mediate the interaction between the HRDC domain and the C25 subunit [Fig. 3(A)]. The interaction includes a salt bridge between C17 Glu100 and C25 Arg151, and hydrogen bonds by C17 Asn93 and Ser97 with C25 Val84 and Trp202, respectively. The interaction is also supported by hydrophobic contacts between a hydrophobic patch consisting of Ala85, Leu88, and Val89 of the C17 subunit and Phe80 of the C25 subunit. In addition to the interaction with the C25 subunit, the HRDC domain is also packed against the C17 tip-associated domain and the linker region [Fig. 3(B)].

Figure 2. Comparison of known structures from the Rpb4/7 family. Structures of Rpb4/7 family members are shown in a ribbon diagram. A43, Rpb7, C25, and RpoF are colored blue. The A14/Rpb4/C17/RpoE tip-associated domains are colored red. HRDC domains are highlighted in orange. Displayed structures are (from top-left, in row-major order) A14/43 from *S. cerevisiae* (chain E/F of PDBID 2RF4), Rpb4/7 from *S. pombe* (chain D/G of PDBID 3H0G), Rpb4/7 from *S. cerevisiae* (chain A/B of PDBID 1Y14), Rpb4/7 from *H. sapiens* (chain G/H of PDBID 2C35), C17/25 from *S. pombe* (chain A/B of PDBID 3AYH, this work), C17/25 from *S. cerevisiae* (chain C/D of PDBID 2CKZ), RpoE/F from *Methanocaldococcus jannaschii* (chain E/F of PDBID 1GO3), RpoE/F from *Sulfolobus solfataricus* (chain E/F of PDBID 3HKZ), and RpoE/F from *Sulfolobus shibatae* (chain E/F of PDBID 2WAQ).

A hydrophobic patch, consisting of Ala14, Phe17, and Phe18 of the tip-associated domain and Cys62 and Leu65 in the linker region, contacts Phe70 of the HRDC domain. The stacking of Asn13 and Asn92 is also a notable feature [Fig. 3(A)].

Conserved properties of the C17 surface

To probe the conservation of the C17–C25 interaction, a sequence analysis of the C17 subunit was performed using Consurf.³² To cover a wide range of eukaryotes while reducing excess redundancy, 65 sequences from Uniref90 were used for the analysis.³³ The result revealed a strong contrast, where the C25-interacting surface is highly conserved, whereas the other side of the protein is variable [Fig. 4(A)]. The same tendency was observed when the HRDC domain alone was tested. Helix α 4, which includes important interactive residues, such as Ala65, Leu88, Val89, and Asn92, is especially well conserved [Fig. 4(B)]. In addition to the conservation, the regions suitable for interactions with other proteins were estimated by using a protein–protein interaction site prediction server, meta-PPISP.³⁴ The amino acid residues in the predicted contacting area correlate well with those with high conservation scores [Fig. 4(C)]. Furthermore, this binding propensity is almost invariant when the surface residues are substituted with those from *S. cerevisiae* or *H. sapiens* [Fig. 4(D,E)]. These analyses strongly suggest that the interdomain contacts observed in the present crystal structure are conserved.

Phylogenetic classification of the C17 subunit by linker sequences

To investigate the possible species-dependent differences, multiple-sequence alignment and phylogenetic analyses were performed for the C17 subunit, with the same sequence set utilized for the Consurf

Figure 3. Molecular interactions around the HRDC domain of the C17 subunit. (A) Close-up view of the second and third helices of the HRDC domain. Some residues are omitted for clarity. Colors are as in Figure 2. (B) Close-up view of the interactions around the C17 linker region.

Figure 4. Surface properties of the C17 subunit. (A) Consurf score plotted on the C17 surface. Conserved residues are colored violet, and variable residues are colored cyan. The C25 subunit is shown as a blue cartoon. (B) Close-up view of the conserved side of the HRDC domain. Surface residues with high conservation (Consurf score of 9 or 10) are shown in sticks. (C) meta-PPISP score plotted on the C17 surface. Residues are colored from red (higher propensity for protein binding) to blue (lower propensity). (D) meta-PPISP score plotted on the model of the *S. cerevisiae* C17 subunit. (E) meta-PPISP score plotted on the model of the *H. sapiens* C17 subunit.

analysis. The resulting tree was consistent with that expected from the conventional taxonomy [Fig. 5(A)]. The analyses showed that the C17 sequences could be classified into three groups, based on the length of the linker connecting the tip-associated domain and the HRDC domain [Fig. 5(B)]. The “short-linker group” has a short and relatively conserved six or seven amino acid residue linker. This group is the largest in eukaryotes and includes animals, plants, and many yeast species, such as *S. pombe* and *P. pastoris*. In contrast, the “long-linker group” has a long and variable linker, which often exceeds 20 amino acid residues. This group is limited to *S. cerevisiae* and its close relatives. The third “intermediate group” solely consists of yeast species from the subphylum Pezizomycotina and displays group-specific sequence conservation rich in prolines.

The *S. pombe* C17 subunit cannot assume the Type II conformation, because the short interdomain linker causes a number of steric hindrances [Fig. 6(A)]. This is also the case for the other members of the short-linker group. On the other hand, the *S. cerevisiae* C17 subunit can assume the alternative Type II conformation, because the long linker is flex-

ible enough to connect the two domains and is actually disordered in the crystal.²¹ Nevertheless, the long-linker group members, including *S. cerevisiae* C17, may be able to adopt the Type I conformation, as the linker is located on the protein surface with no steric hindrance. Moreover, in the previous crystal structure of the *S. cerevisiae* C17/25 subcomplex,²¹ the Type II conformation is probably stabilized by the interaction between the HRDC domain and the C-terminal His-tag on the C25 subunit. Indeed, a meta-PPISP analysis revealed that the His-tag itself has high protein-binding propensity [Fig. 6(B)] and that the Type I conformation seems more favorable when the tag is removed [Fig. 6(C)]. In addition, multiple studies have suggested that the cryo-EM maps of *S. cerevisiae* Pol III are better explained by the Type I conformation of the C17/25 subcomplex.^{7,29} Furthermore, the fact that the human C17, with a short linker, can substitute for *S. cerevisiae* C17 both *in vivo* and *in vitro*¹⁷ indicates that the Type II conformation of the C17 subunit is not fundamentally required for the transcription activity of Pol III. These lines of evidence suggest that, even for the long-linker species such as

Figure 5. Phylogenetic analysis of the C17 subunit. (A) Phylogenetic tree of the C17 subunit. (Nodes in red, green, and black represent species with long linkers, intermediate linkers, and short linkers, respectively.) (B) Sequence alignment and classification of the C17 linker region.

Figure 6. Analysis of the possible two conformations of the C17/25 subcomplex. (A) Unrealistic model of the *S. pombe* C17/25 subcomplex in the Type II conformation. The HRDC domain was placed on the basis of the superposition with *S. cerevisiae* C17/25. The linker region was manually built to show that clashes are unavoidable. Interfering C25 surface residues are depicted by blue spheres, and C17 linker residues are shown in red sticks. (B) meta-PPSIP analysis of the *S. cerevisiae* C17/25 subcomplex (chain C/D of PDBID: 2CKZ). The C25 subunit is depicted by spheres, colored according to the meta-PPISP score. The C17 HRDC domain is shown in orange. The C17 tip-associated domain is omitted for clarity. (C) meta-PPSIP analysis of the *S. cerevisiae* C17/25 subcomplex without the His-tag. The region corresponding to the His-tag (residues 213-217) was removed before the meta-PPISP analysis. The modeled HRDC domain in the Type I conformation is shown in orange. The C17 tip-associated domain is omitted for clarity.

S. cerevisiae, Pol III transcription is mainly mediated by the Type I conformation of the C17 subunit.

Materials and Methods

Protein expression

The cDNA fragments encoding the C17 and C25 subunits of *S. pombe* Pol III were each cloned into the pET-Duet1 vector (Novagen). The 6xHis-tag with an HRV-3C cleavage site was appended to the N-terminus of the C17 subunit. Rosetta 2 (DE3) cells (Novagen) were used as the expression host.

Protein purification

Cells were resuspended in buffer A [50 mM Tris-HCl buffer (pH 8.0), containing 700 mM NaCl and 10 mM 2-mercaptoethanol] and disrupted by sonication. The lysate was cleared by centrifugation and loaded onto a Ni-Sepharose FF column (GE Healthcare). The column was washed with buffer A containing 30 mM imidazole, and then the proteins were eluted with buffer A containing 400 mM imidazole. The His-tag was cleaved by HRV-3C protease (Novagen) and removed by Ni-Sepharose FF chromatography. The sample was then dialyzed against 20 mM Tris-HCl buffer (pH 8.0), containing 100 mM NaCl and 10 mM 2-mercaptoethanol, and further purified by anion exchange chromatography using a Resource Q column (GE Healthcare). The purified sample was concentrated to 7 mg/mL in 20 mM Tris-HCl buffer (pH 8.0) containing 10 mM 2-mercaptoethanol.

X-ray structure determination

Crystals were grown by the sitting-drop vapor-diffusion method, against a reservoir solution containing 100 mM Tris-HCl buffer (pH 8.0) with 1.3M Li₂SO₄. Crystals were harvested into a cryoprotectant containing 20% ethylene glycol and flash cooled in liquid nitrogen. X-ray diffraction data were obtained at BL-5A, Photon Factory (Tsukuba, Japan). The dataset was processed using XDS³⁵ and Scala.³⁶ Phasing was performed by molecular replacement with PHASER,³⁷ using the crystal structure of *S. cerevisiae* C17/25 without the HRDC domain (chain A/B of PDBID:2CKZ) as a search model. Further model building and structure refinement were performed manually with Coot³⁸ and phenix.refine.³⁹ Model validation was aided by MolProbity.⁴⁰

Conservation and phylogenetic analysis

C17 orthologs were obtained from Uniref90,³³ using PSI-BLAST,⁴¹ and the sequences were aligned with MAFFT.⁴² The alignment was then utilized for the surface conservation analysis and the phylogenetic tree construction with Consurf.³² Figures were prepared with PyMOL and Treeview.⁴³

Protein data bank accession number

The coordinates and structure factors were deposited in the protein data bank (PDB), with the accession number 3AYH.

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