Crystal Structure of the Human Pol α B Subunit in Complex with the C-terminal Domain of the Catalytic Subunit*

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Background: DNA polymerase α (Pol α) initiates DNA synthesis and is indispensable for genome replication.

Results: We present a crystal structure of human Pol α minus the catalytic core at 2.5 Å resolution.

Conclusion: The mode of interaction between the Pol α subunits is evolutionarily conserved.

Significance: The data provide structural insight into the function of the primase-Pol α complex.

In eukaryotic DNA replication, short RNA-DNA hybrid primers synthesized by primase-DNA polymerase α (Prim-Pol α) are needed to start DNA replication by the replicative DNA polymerases, Pol δ and Pol ε. The C terminus of the Pol α catalytic subunit (p180c) in complex with the B subunit (p70) regulates the RNA priming and DNA polymerizing activities of Prim-Pol α. It tethers Pol α and primase, facilitating RNA primer handover from primase to Pol α. To understand these regulatory mechanisms and to reveal the details of human Pol α organization, we determined the crystal structure of p70 in complex with p180c. The structured portion of p70 includes a phosphodies- terase (PDE) domain and an oligonucleotide/oligosaccharide binding (OB) domain. The N-terminal domain and the linker connecting it to the PDE domain are disordered in the reported crystal structure. The p180c adopts an elongated asymmetric saddle shape, with a three-helix bundle in the middle and zinc-binding modules (Zn1 and Zn2) on each side. The extensive p180c–p70 interactions involve 20 hydrogen bonds and a number of hydrophobic interactions resulting in an extended buried surface of 4080 Å². Importantly, in the structure of the p180c–p70 complex with full-length p70, the residues from the N-terminal to the OB domain contribute to interactions with p180c. The comparative structural analysis revealed both the conserved features and the differences between the human and yeast Pol α complexes.

Accurate DNA replication is crucial for the maintenance of genome stability and for the suppression of mutagenesis and carcinogenesis (1–3). In eukaryotes, the processive replication of DNA polymerases, Pol δ and Pol ε, is needed to start DNA replication by the replicative DNA polymerases, Pol δ and Pol ε. The C terminus of the Pol α catalytic subunit (p180c) in complex with the B subunit (p70) regulates the RNA priming and DNA polymerizing activities of Prim-Pol α. It tethers Pol α and primase, facilitating RNA primer handover from primase to Pol α. To understand these regulatory mechanisms and to reveal the details of human Pol α organization, we determined the crystal structure of p70 in complex with p180c. The structured portion of p70 includes a phosphodies- terase (PDE) domain and an oligonucleotide/oligosaccharide binding (OB) domain. The N-terminal domain and the linker connecting it to the PDE domain are disordered in the reported crystal structure. The p180c adopts an elongated asymmetric saddle shape, with a three-helix bundle in the middle and zinc-binding modules (Zn1 and Zn2) on each side. The extensive p180c–p70 interactions involve 20 hydrogen bonds and a number of hydrophobic interactions resulting in an extended buried surface of 4080 Å². Importantly, in the structure of the p180c–p70 complex with full-length p70, the residues from the N-terminal to the OB domain contribute to interactions with p180c. The comparative structural analysis revealed both the conserved features and the differences between the human and yeast Pol α complexes.

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Accurate DNA replication is crucial for the maintenance of genome stability and for the suppression of mutagenesis and carcinogenesis (1–3). In eukaryotes, the processive replication of DNA polymerases of the B family, which is comprised of four members: Pol α, Pol δ, Pol ε, and Pol ζ (4). During the start of DNA synthesis de novo, RNA-DNA hybrid primers are synthesized by the primase-Pol α (Prim-Pol α)2 complex. Initially, primase synthesizes a short RNA primer of ~10 nucleotides; then the RNA-primed template DNA is translocated to the active site of Pol α with its following extension by deoxyribonucleotides (5, 6). According to the current models, after synthesis of ~20 nucleotides, Pol α yields to processive polymerases, Pol δ or Pol ε, to perform the bulk of replication on the lagging and leading DNA strands (7–9). Pol ζ plays a role in completion of replication of damaged DNA and past non-conventional DNA structures (10–13). DNA fragments synthesized by Pol α contain errors because it does not have proof-reading exonuclease activity, and, if uncorrected, these errors contribute to the overall fidelity of DNA replication (14). It was estimated that patches synthesized by Pol α comprise ~1.5% of newly replicated human genome, making Pol α an important factor modulating genome stability (15).

All four eukaryotic B family DNA polymerases share several common architectural features, including the “signature” B family catalytic domain, the C-terminal domain (CTD) that is connected to the catalytic domain by a flexible linker, and the B subunit (16, 17). The B subunits do not possess any catalytic activity. Instead, they stabilize the catalytic subunit (18, 19), have a role in regulation of the DNA synthesis in a cell cycle-dependent manner (20–23), and act as scaffolds mediating interactions with other components of the replication machinery (24–26). The CTDs of Pol α and Pol ε contain two zinc-binding modules (Zn1 and Zn2), where Zn2 is involved in interactions with the B subunit (27, 28). The CTDs of Pol δ and Pol ζ also have two putative metal-binding modules, each containing four conservative cysteines (17, 29). However, the second module is significantly different from Zn2 in the CTDs of Pol α and Pol ε because, instead of zinc, it coordinates a 4Fe-4S cluster (30, 31).

The crystal structure of the human Pol θ B subunit (p50) in complex with the N-terminal domain of the C subunit (p66c) provided the first view of the three-dimensional organization of...
B subunits in B family DNA polymerases (32). The B subunit of Pol δ is comprised of N-terminal residues followed by an oligonucleotide/oligosaccharide-binding (OB) domain and a catalytically inactive phosphodiesterase (PDE) domain. Based on structural and mutational analyses of p50-p66N, we predicted the CTD-docking surface of the B subunit (33). Based on our structural and biochemical studies, we discovered that the B and C subunits of human Pol δ bind the CTD of human Pol ζ as efficiently as the CTD of Pol δ, indicating that Pol ζ functions as a four-subunit complex (30). Subsequent studies led to isolation of four-subunit active forms of Pol ζ in three different laboratories (34–36).

The crystal structure of the yeast Pol α B subunit in complex with the cognate CTD reported by the Pellegrini group (27) confirmed that the overall structure of the B subunits of replicative DNA polymerases is conserved, and the described mode of interaction between the Pol α subunits is consistent with our analysis of the CTD-docking site of the B subunit (33). However, some putatively important details are missing in the structure of the yeast B subunit, because the construct used lacked its 247 N-terminal amino acid residues. Here we report the crystallization, structure determination, and comparative structural analysis of the full-length human Pol α B-subunit in complex with p180C.

**Experimental Procedures**

Protein Expression and Purification—The cDNA encoding for p180 (1–1462) was generously provided by Dr. Motoshi Suzuki (Nagoya University), and the cDNA for p70 (1–598) was purchased from the Open Biosystems (clone ID 2822514). A pETHSUL vector expressing the first 98 residues of *Saccharomyces cerevisiae* SMT3 (SUMO) with an N-terminal His tag and a pSUPER vector expressing SUMO-specific protease dtUD1 (doubly tagged UD1) were a generous gift from Dr. Patrick Loll.

**Table 1** Data collection and refinement statistics

| Data collection | Native |
|-----------------|--------|
| Space group     | P2₁    |
| Cell dimensions |        |
| a, b, c (Å)    | 103.59, 17.14, 132.09 |
| β (°)           | 90, 100.97, 90 |
| Resolution (Å) | 50-2.5 (2.54-2.50) |
| Unique reflections | 116,900 |
| Rmerge (%)     | 8.4 (39.6) |
| I/σ(I)         | 13.3 (2.1) |
| Completeness (%) | 95.0 (88.5) |
| Redundancy     | 2.8 (2.1) |
| Temperature (K) | 100 |

| Refinement      |        |
|-----------------|--------|
| Resolution (Å) | 50-2.50 |
| No. reflections | 116,874 |
| Rmerge/I/σ(I)  | 21.78/25.77 |

| No. atoms/B-factors (Å²) |
|-----------------|--------|
| Protein         | 19,516/50.9 |
| Zinc            | 8/62.4 |
| Solvent         | 202/36.5 |

| R.m.s. deviation |
|------------------|
| Bond length (Å)  | 0.008 |
| Bond angles (°)  | 1.69 |

| Ramachandran plot |
|--------------------|
| Favored (%)        | 88.1 |
| Allowed (%)        | 11.8 |
| Disallowed (%)     | 0.1 |

**FIGURE 1. Overall structure of human p180C-p70.** A, schematic representation of the domain organization. The red lines in the schematics of p180C present the relative positions of the zinc-coordinating residues in two zinc-binding modules: Zn1 (Cys-1283, Cys-1286, Cys-1310, and Cys-1315) and Zn2 (Cys-1348, Cys-1353, Cys-1371, and Cys-1374). B, schematic representation of p180C-p70. The p180C is colored light pink; the PDE domain (excluding region 158–195), OB domain, and the linker regions between domains are colored cyan, green, and gray, respectively. The N-terminal portion of the PDE domain (residues 158–195) with surrounding linkers, which are absent in the yeast Pol α CTD-B subunit structure, are highlighted in red. Zinc atoms are depicted as orange spheres.
dtUD1 was expressed and purified as described in Ref. 37. The genes for the full-length p70 and p180C (1265–1444) with an N-terminal His8-SUMO tag were subcloned into the pETDuet-1 vector (Novagen) using NcoI/BamHI and NdeI/KpnI (TaKaRa) restriction sites, respectively. The DNA coding for the His8-SUMO tag was amplified from a pETHSUL vector (two histidines were added to original His6 tag at that time) and attached to the fragment encoding p180C by fusion PCR (38). The human His-SUMO-p180C-p70 complex was expressed for 16 h (18 °C) in *Escherichia coli* strain Rosetta-2(DE3) in LB medium after induction with 1 mM isopropyl/β-D-1-thiogalactopyranoside. After cell disruption using Emulsiflex-C5, the protein complex was purified using sequential Ni-IDA (Bio-Rad), HiTrap Q, and HiTrap SP (GE Healthcare) columns. His6-tagged dtUD1 protease was added at a 1:200 molar ratio in the process of purification to cleave the His8-SUMO tag. Finally, the protein solution was applied to a HiLoad 16/60 Superdex 200 gel filtration column (GE Healthcare) and peak fractions were combined and concentrated to 9.7 mg ml\(^{-1}\) in buffer containing 10 mM Tris-HCl, pH 7.7, 50 mM NaCl, and 2 mM DTT. The concentrated protein solution was frozen in liquid nitrogen and stored at 193 K until use.

**Crystallization**—Crystallization—The aliquots of protein solution were defrosted and centrifuged to remove the precipitate, and the sample monodispersity was verified with dynamic light scattering. The screening of crystallization conditions was performed with the sitting drop vapor diffusion method at 295 K by mixing 1 μl of protein solution with 1 μl of reservoir solution. The reservoirs contained 2-fold diluted Crystal Screen and Crystal Screen 2 (Hampton Research) solutions with the addition of 2 mM tris-(2-carboxyethyl) phosphine hydrochloride, pH 7.5. Needle-shaped crystal clusters appeared in the ninth condition of the Crystal Screen kit containing 0.1M ammonium acetate, 50 mM sodium citrate, pH 5.6, and 15% (w/v) PEG 4000. Optimization of the condition was performed with variation of the PEG 4000 concentration and an Additive Screen kit (Hampton Research). The diffraction quality elongated plate crystals that began growing in 2–3 weeks were obtained at 295 K in 100 mM ammonium acetate, 50 mM sodium citrate, pH 5.6, 10–10.4% (w/v) PEG 4000, 2 mM tris-(2-carboxyethyl) phosphine hydrochloride, and 50 mM guanidine HCl.

**Data Collection**—For diffraction data collection, the crystals were soaked in a cryoprotectant solution (50 mM sodium citrate, pH 5.6, 0.1 M ammonium acetate, 10% (w/v) PEG 4000, and 15% (v/v) PEG 200) for a few seconds, scooped in a nylon fiber loop, and flash-cooled in a dry nitrogen stream at 100 K. Preliminary characterizations of crystal were performed on a Rigaku R-AXIS IV imaging plate using Osmic VariMax™ HR.

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**FIGURE 2. Structural comparison of the human and yeast Pol α CTDs and B subunits.** A, alignment of p180C and the yeast Pol α CTD (PDB code 3FLO) using the conserved α2 and α3 helices. The color scheme for p180C is the same as in Fig. 1B; the yeast analog is colored gray, with zins depicted as light green spheres. B, close-up view of the Zn1 module in the CTD of human and yeast Pol α. C, alignment of p70 and the yeast Pol α B subunit. p70 is colored as in Fig. 1B, and the B subunit of yeast Pol α is colored light orange. The insertion regions in the yeast B subunit are indicated.
mirror-focused CuKβ radiation from a Rigaku FR-E rotating anode operated at 45 kV and 45 mA. Complete diffraction data sets were collected using synchrotron x-rays on the Argonne National Laboratory Advanced Photon source Beamline 24ID-E with the Area Detector Systems Corporation Quantum 315 detector. All intensity data were indexed, integrated, and scaled with DENZO and SCALEPACK from the HKL-2000 program package (39). The crystals belong to monoclinic space group P21 and diffract up to 2.5 Å resolution. The asymmetric unit contains four independent p180c-p70 molecules with a solvent content of 53.48%. The crystal parameters and data processing statistics of the best data set used for structure determination and refinement are summarized in Table 1.

Crystal Structure Determination—The crystal structure of the human p180c-p70 complex was determined by the molecular replacement method using the previously reported C-terminal domain of yeast Pol α in complex with its B subunit (27) (Protein Data Bank code 3FL0) as a search model. The positions of zinc ions were confirmed with an anomalous difference Fourier map. Molecular replacement and initial automated model rebuilding performed with Phenix (40) revealed over 60% of correctly built structure. The remaining crystallographic computing was performed with CNS version 1.1 (41) using phases from the automatically built partial model. Non-crystallographic symmetry restraints and density modification with averaging were applied during the initial stages of model refinement and map calculations for manual model building with Coot (42). Final inspection and adjustments in the structure were performed with Turbo-Frodo. Application of zonal scaling (43) improved the quality of the electron density maps. After the addition of solvent molecules, the model was refined at 2.5 Å resolution to an Rcryst of 22.2% and an Rfree of 25.0%. The final refinement statistics are provided in Table 1. The figures containing molecular structures were prepared with PyMOL (44).

Results

Overall Structure of the p180c-p70 Complex—The crystal contains four independent p180c-p70 molecules, 1–4, in an
Crystal Structure of the Human Pol α CTD-B Subunit Complex

FIGURE 4. Structural comparison of the p180c and p70 molecules in an asymmetric unit. The right panel shows an overall view of the aligned molecules 1 and 2 from the same asymmetric unit, and the left panel shows a close-up view of intermolecular interactions between loop β₁α₁ of molecule 1 and a symmetry related molecule, p70 and p180c from molecule 1 are colored slate and magenta, whereas p70 and p180c from molecule 2 are colored cyan and light pink, respectively. Zinc atoms are colored light green (molecule 1) and orange (molecule 2). The symmetry related molecule is colored gray; the nitrogens and oxygens are colored blue and red, respectively. The side chains or main chain of the residues making the intermolecular hydrogen bonds are shown as sticks, and the hydrogen bonds are depicted as red dashed lines.

The C-terminal Domain of p180—p180c adopts an elongated asymmetric saddle shape (Figs. 1B and 2A) with a three-helix bundle (α₂, α₃, and α₅) in the middle and zinc-binding modules (Zn1 and Zn2) on each side, where the highly conserved cysteine residues form the tetrahedral coordination geometries with zinc ions. Zn1 contains two antiparallel β strands, β₁ and β₂, that together with the β₁β₂ loop hold the first two zinc-coordinating cysteines, Cys-1283 and Cys-1286. The rest of Zn1 is a coil that continues with a short β₁₀ helix. The position of Zn1 is well anchored by a number of hydrophobic interactions with helices α₂ to α₅.

The structure of Zn1 in the human complex is different than in the yeast complex (Fig. 2B). Instead of folding as an extended coil and a β₁₀ helix, the corresponding sequences in the yeast complex are folded as an antiparallel β sheet with three strands. The majority of Zn1 hydrophobic interactions formed between the extended coil and the helices α₄ and α₅ in the human complex are substituted by interactions of Zn1 with an extended loop that is inserted between helices α₄ and α₅ in the yeast complex. The Zn2 module contains a small antiparallel β sheet with strands β₃, β₄, and β₅. The α2 and α3 helices together with the Zn2 module are well superimposed with the corresponding region of the yeast complex (rmsd is 0.82 Å for 62 superimposed α carbons) (Fig. 2A).

The relatively small difference in the overall shape of human Zn2 compared with Zn2 in yeast is caused by a four-residue insertion between β₄ and the third zinc-coordinating cysteine (Cys-1371), as well as by deletion of two residues prior to and one residue insertion after the fourth zinc-coordinating cysteine (Cys-1374) (Fig. 3A). These results coincide with previous sequence analysis of CTDs in eukaryotes showing that Zn2 modules exhibit higher amino acid sequence conservation than Zn1 (17, 27).

The B Subunit—The p70 subunit is comprised of three domains: an N-terminal domain (NTD), residues 1–78; a PDE domain, residues 158–195 and 332–598; and an OB domain, residues 212–320 (Figs. 1 and 3A). Both the NTD and an extended linker (residues 79–156) between the NTD and the PDE are not visible in crystal formation, pointing to the absence of stabilizing interactions with the PDE, the OB, and p180c. The only exception is molecule 2 residues 151–156 that are stabilized by interactions with the neighboring molecule. NMR and structural studies of NTD revealed a globular domain with four α helices (25, 46). The PDE domain is comprised of a central two-layer β sheet (layer 1 ↑ β₁₁↓ β₁₂↓ β₁₃↑ β₁₄↓ β₁₅↑ β₁₆↓ β₁₇↑ β₁₈↓ β₁₉↑ β₁ₐ↓ β₁₄↑ β₁₅↓ β₁₆↑ β₁₇↓ β₁₈↑ β₁₉↓ β₁ₐ↑ β₁₄↓ β₁₅↑ β₁₆↓ β₁₇↑ β₁₉↓ β₁ₐ↑ β₁₄↓ β₁₅↑ β₁₆↓ β₁₇↑ β₁₉↓ β₁ₐ↑ β₁₄↓ β₁₅↑ β₁₆↓ β₁₇↑ β₁₉↓ β₁ₐ↑ β₁₄↓ β₁₅↑ β₁₆↓ β₁₇↑ β₁₉↓ β₁ₐ↑ β₁₄↓ β₁₅↑ β₁₆↓ β₁₇↑ β₁₉↓ β₁ₐ↑ β₁₄↓ β₁₅↑ β₁₆↓ β₁₇↑ β₁₉↓ β₁ₐ↑ β₁₄↓ β₁₅↑ β₁₆↓ β₁₇↑ β₁₉↓ β₁ₐ↑ β₁₄↓ β₁₅↑ β₁₆↓ β₁₇↑ β₁₉↓ β₁ₐ↑ β₁₄↓ β₁₅↑ β₁₆↓ β₁₇↑ β₁₉↓ β₁ₐ↑ β₁₄↓ β₁₅↑ β₁₆↓ β₁₇↑ β₁₉↓ β₁ₐ↑ β₁₄↓ β₁₅↑ β₁₆↓ β₁₇↑ β₁₉↓ β₁ₐ↑ β₁₄↓ β₁₅↑ β₁₆↓ β₁₇↑ β₁₉↓ β₁ₐ↑ β₁₄↓ β₁₅↑ β₁₆↓ β₁₇↑ β₁₉↓ β₁ₐ↑ β₁₄↓ β₁₅↑ β₁₆↓ β₁₇↑ β₁₉↓ β₁ₐ↑ β₁₄↓ β₁₅↑ β₁₆↓ β₁₇↑ β₁₉↓ β₁ₐ↑ β₁₄↓ β₁₅↑ β₁₆↓ β₁₇↑ β₁₉↓ β₁ₐ↑ β₁₄↓ β₁₅↑ β₁₆↓ β₁₇↑ β₁₉↓ β₁ₐ↑ β₁₄↓ β₁₅↑ β₁₆↓ β₁₇↑ β₁₉↓ β₁ₐ↑ β₁₄↓ β₁₅↑ β₁₆↓ β₁₇↑ β₁₉↓ β₁ₐ↑ β₁₄↓ β₁₅↑ β₁₆↓ β₁₇↑ β₁₉↓ β₁ₐ↑ β₁₄↓ β₁₅↑ β₁₆↓ β₁₇↑ β₁₉↓ β₁ₐ↑ β₁₄↓ β₁₅↑ β₁₆↓ β₁₇↑ β₁₉↓ β₁ₐ↑ β₁₄↓ β₁₅↑ β₁₆↓ β₁₇↑ β₁₉↓ β₁ₐ↑ β₁₄↓ β₁₅↑ β₁₆↓ β₁₇↑ β₁₉↓ β₁ₐ↑ β₁₄↓ β₁₅↑ β₁₆↓ β₁₇↑ β₁₉↓ β₁ₐ↑ β₁₄↓ β₁₅↑ β₁₆↓ β₁₇↑ β₁₉↓ β₁ₐ↑ β₁₄↓ β₁₅↑ β₁₆↓ β₁₇↑ β₁₉↓ β₁ₐ↑ β₁₄↓ β₁₅↑ β₁₆↓ β₁₇↑ β₁₉↓ β₁ₐ↑ β₁₄↓ β₁₅↑ β₁₆↓ β₁₇↑ β₁₉↓ β₁ₐ↑ β₁₄↓ β₁₅↑ β₁₆↓ β₁₇↑ β₁₉↓ β₁ₐ↑ β₁₄↓ β₁₅↑ β₁₆↓ β₁₇↑ β₁₉↓ β₁ₐ↑ β₁₄↓ β₁₅↑ β₁₆↓ β₁₇↑ β₁₉↓ β₁ₐ↑ β₁₄↓ β₁₅↑ β₁₆↓ β₁₇↑ β₁₉↓ β₁ₐ↑ β₁₄↓ β₁₅↑ β₁₆↓ β₁₇↑ β₁₉↓ β₁ₐ↑ β₁₄↓ β₁₅↑ β₁₆↓ β₁₇↑ β₁₉↓ β₁ₐ↑ β₁₄↓ β₁₅↑ β₁₆↓ β₁₇↑ β₁₉↓ β₁ₐ↑ β₁₄↓ β₁₅↑ β₁₆↓ β₁₇↑ β₁₉↓ β₁ₐ↑ β₁₄↓ β₁₅↑ β₁₆↓ β₁₇↑ β₁₉↓ β₁ₐ↑ β₁₄↓ β₁₅↑ β₁₆↓ β₁₇↑ β₁₉↓ β₁ₐ↑ β₁₄↓ β₁₅↑ β₁₆↓ β₁₇↑ β₁₉↓ β₁ₐ↑ β₁₄↓ β₁₅↑ β₁₆↓ β₁₇↑ β₁₉↓ β₁ₐ↑ β₁₄↓ β₁₅↑ β₁₆↓ β₁₇↑ β₁₉↓ β₁ₐ↑ β₁₄↓ β₁₅↑ β₁₆↓ β₁₇↑ β₁₉↓ β₁ₐ↑ β₁₄↓ β₁₅↑ β₁₆↓ β₁₇↑ β₁₉↓ β₁ₐ↑ β₁₄↓ β₁₅↑ β₁₆↓ β₁₇↑ β₁₉↓ β₁ₐ↑ β₁₄↓ β₁₅↑ β₁₆↓ β₁₇↑ β₁₉↓ β₁ₐ↑ β₁₄↓ β₁₅↑ β₁₆↓ β₁₇↑ β₁₉↓ β₁ₐ↑ β₁₄↓ β₁₅↑ β₁₆↓ β₁₇↑ β₁₉↓ β₁ₐ↑ β₁₄↓ β₁₅↑ β₁₆↓ β₁₇↑ β₁₉↓ β₁ₐ↑ β₁₄↓ β₁₅↑ β₁₆↓ β₁₇↑ β₁₉↓ β₁ₐ↑ β₁₄↓ β₁₅↑ β₁₆↓ β₁₇↑ β₁₉↓ β₁ₐ↑ β₁₄↓ β₁₅↑ β₁₆↓ β₁₇↑ \beta_{15} \text{ of the PDE domain to form a small antiparallel } \beta \text{ sheet. This...
two-strand β sheet has been also observed in the B subunit of human Pol δ and is probably a common feature of B family DNA polymerases (32).

The p70 residues 178–182 from loop β₁β₂ and 196–210 from loop β₄α₂ in two of the independent molecules adopt conformations that are different than in the two other independent molecules (Fig. 4). The different conformation of loop β₄α₂ is due to the effect of crystal packing in molecules 1 and 3. In these molecules, five intermolecular hydrogen bonds shift and stabilize this loop. This, in turn, disrupts the packing of p180C residues from the C terminus to Ala-1437, affecting the p70-p180C heterodimerization interface. In molecules 2 and 4, the p70 residues 196–210 are not affected by crystal packing and hence represent the native folding of molecule. In molecules 2 and 4, the disordered residues 203–206 of p70 are looping out into the area where the C-terminal residues 1445–1462 of p180 are expected to be located. It has been shown that the corresponding C-terminal residues in yeast interact with the primase large subunit (45, 47). Because the residues 203–206 of p70 and C-terminal residues 1445–1462 of p180 are pointing into the same direction, there is a possibility that 203–206 of p70 may augment the interaction of p180 with primase.

The B subunit of yeast Pol α superimposes with p70 with an rmsd of 1.8 Å for 312 matching carbon pairs, despite the low 27% sequence identity and a chain 105 amino acids longer in the yeast subunit (Figs. 2C and 3B). Several notable differences were observed in the PDE domains: α₅ is longer in yeast; the α₅α₆ loop with a 9-residue deletion is more compact, and α₆ has no bend in the human complex; α₇ is absent and α₈ is shorter in
yeast; the α₆α₁₀ loop with a 30-residue deletion is compact and well structured in the human complex, whereas it is disordered in yeast; the β₁₉β₂₀ loop with a 13-residue deletion is compact in the human complex, whereas it has an extended conformation in yeast; and the yeast B subunit contains four additional α helices. Unlike larger discrepancies in the yeast and human PDE domain structures that mainly affect the peripheral α helices and coils, the OB domains exhibit fewer prominent differences predominantly in loops α₂β₃ and β₃α₄. Some of these differences might be due to the deletion of 247 N-terminal amino acids in the yeast B subunit containing the N-terminal portion of the PDE domain.

Interaction between p180C and p70—The p180C-p70 interface has an extended shape, with a large buried surface area of 3484 Å² for molecules 1 and 3 and 4080 Å² for molecules 2 and 4. The complex formation is achieved by 20 intersubunit hydrogen bonds and a number of hydrophobic interactions (Figs. 3 and 5). Furthermore, the positively charged surface of p180C and the complementary negatively charged surface of p70 points to an enhancement of the complex stability by electrostatic interactions (Fig. 6A). The p70-interacting residues of p180C are clustered on helix α₂ and on the β₄β₅ loop of Zn2. Additional interactions are provided by the β sheet of Zn2, helix α₃', and the coil after α₆. The p180C-interacting residues of p70 are clustered on helix α₂', on loops β₃α₂, β₁₁α₂, and on α₅α₁₀. The p70 loops β₃β₄ and α₅α₆, strand β₁₁, and the N terminus of helix α₆ also contribute to intersubunit interactions. The β₁β₂ loop of Zn2 module is inserted into the depression formed by the OB domain and loop α₅α₁₀ of the PDE domain. Unlike Zn2, the Zn1 module is distantly located from the interaction interface.

Comparison of the human p180C-p70 complex with the yeast ortholog revealed several differences in the docking of the CTD
Crystal Structure of the Human Pol α CTD-B Subunit Complex

on the surface of the B subunit. For example, Zn1 of p180_c and α₃ of the yeast Pol α CTD do not participate in interaction with the corresponding B subunit (Figs. 1B and 3A). Although the positions of Zn2 modules nearly coincide, the positions of Zn1 modules were separated by more than 7 Å (Fig. 7). These differences were caused mainly by the differences in the structures of their CTDs, as well as in the CTD-interacting surfaces of the B subunits, especially loops β₃β₄, α₃α₄, and α₅α₆ (Fig. 3B). Moreover, deletion of the sequences from the N terminus to the OB fold in the yeast complex may also affect the CTD docking. The electrostatic charge distributions at the interacting surfaces are also different. In the human complex, p70 has predominantly elongated negatively charged interacting surface, whereas in the yeast B subunit the surface corresponding to Zn2-docking area is positively charged (Fig. 6). Despite the above listed differences, there are notable similarities in some of the intersubunit hydrogen bonds (Fig. 3). In particular, the interaction of the p180_c Arg-1358 side chain with the main-chain oxygen of p70 Pro-514 in the human complex is conserved in the yeast complex, pointing to the key role of this hydrogen bond.

Discussion

The interaction between the catalytic and B subunits plays a critical role for the proper function of DNA polymerases of the B family in vivo (28–30). The amino acid changes in the cysteine-rich region of the CTDs of Pol δ and Pol ε led to lethality or temperature sensitivity (29, 48, 49). The all-helical NTD of p70 is connected with the PDE domain by an 80-residue-long linker and has the potential for interaction with other DNA replication proteins and recruitment of Prim-Pol α to the replication fork. The NTD of p70 interacts with the hexameric helicase of SV40 large T antigen and activates the viral primosome (25, 50). The NTD of the B subunit of Pol ε, which has a similar structure (51), is important for the assembly of Cdc45-MCM-GINS helicase and integration of Pol ε into the eukaryotic replisome during initiation (26, 52). In Prim-Pol α, the CTD-B subunit complex plays an additional and unique role by tethering two catalytic domains and regulating their activities (53).

In this report, we extensively characterized the CTD-B subunit interface of human Pol α and compared it with that of yeast Pol α. We found differences in the charge distribution patterns at the CTD-B subunit interface and in the secondary structure elements involved in heterodimer formation. Some differences might be due to the lack of N-terminal residues with a portion of the PDE domain in the yeast B subunit fragment used for crystallization. Despite these significant differences, the key features like interactions of the Zn2 module with the OB domain and the α₃ helix with the PDE domain are conserved. This is consistent with the fact that the substitution of two cysteines by alanines in Zn2 resulted in disruption of the mouse Pol α complex (54).

The structure of p180_c-p70, as well as structure of the yeast Pol α CTD-B subunit complex, is in accordance with the biochemical data, demonstrating that the Zn2 site of Pol α coordinates zinc (30). We propose that the presence of an iron-sulfur cluster in the Zn2 of partially purified yeast Pol α and its CTD seen in the report by Netz et al. (31) is due to the intrinsic ability of metalloproteins to misincorporate non-native metals during high levels of artificial overproduction in heterologous hosts. We have shown that the partially purified CTD of human Pol ε contained significant levels of iron, whereas iron in the pure and stoichiometric complex of the CTD with the B subunit was not detected (30). This indicates that the CTD with an inadvertently misincorporated iron-sulfur cluster cannot form a stable complex with the B subunit. In the structures of human and yeast Pol α, Zn2 snugly fits a docking site on the B subunit. The [4Fe-4S] cluster has significantly larger size compare with the zinc ion, and its binding will definitely change the Zn2 shape and disrupt its interaction with the B subunit.

We recently solved the crystal structures of human DNA primase and the Pol α catalytic core domain (55, 56). Now we have the crystal structures covering all the functional domains of human Prim-Pol α that are necessary for building a model of the entire tetrameric complex. Additional structure-guided functional experiments together with single-molecule studies will be necessary to decipher the intriguing mechanisms of primer synthesis by primase and its switch to Pol α.

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