Structure of the polymerase ε holoenzyme and atomic model of the leading strand replisome

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The eukaryotic leading strand DNA polymerase (Pol) ε contains 4 subunits, Pol2, Dpb2, Dpb3 and Dpb4. Pol2 is a fusion of two B-family Pols; the N-terminal Pol module is catalytic and the C-terminal Pol module is non-catalytic. Despite extensive efforts, there is no atomic structure for Pol ε holoenzyme, critical to understanding how DNA synthesis is coordinated with unwinding and the DNA path through the CMG helicase-Pol ε-PCNA clamp. We show here a 3.5-Å cryo-EM structure of yeast Pol ε revealing that the Dpb3–Dpb4 subunits bridge the two DNA Pol modules of Pol2, holding them rigid. This information enabled an atomic model of the leading strand replisome. Interestingly, the model suggests that an OB fold in Dbp2 directs leading ssDNA from CMG to the Pol ε active site. These results complete the DNA path from entry of parental DNA into CMG to exit of daughter DNA from PCNA.
Chromosome replication in eukaryotes is performed by three different B-family DNA polymerases (Pol), Pol ε, Pol δ, and Pol α-primase^{1-4}. Pol ε performs bulk leading strand synthesis while Pol δ acts on the lagging strand. Pol α-primase contains both RNA primase and DNA polymerase activity and functions to generate hybrid RNA-DNA primers to initiate DNA synthesis by Pol ε and Pol δ. Pol ε is the largest of the replicative DNA polymerases and contains four subunits (Fig. 1a)^5,6. The Pol2 subunit harbors the catalytic DNA polymerase and proofreading 3′–5′ exonuclease in the N-terminal half. The three accessory subunits include the essential Dpb2 and two small nonessential Dpb3 and Dpb4 subunits. The small Dpb3

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\text{Dpb3} \quad \text{Pol2} \quad \text{Dpb2} \quad \text{Dpb4}
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Fig. 1 Cryo-EM structure of the S. cerevisiae leading strand Pol ε. a Domain architecture of the four subunits of the holoenzyme: Pol2 contains two polymerase domains, the catalytic NTD and the non-catalytic CTD. There is a Dpb2-binding domain (Dpb2-BD) in the CTD that further contains two Cys motifs (CysA and CysB). Dpb2 has an OB domain and a calcineurin-like PDE domain. Dpb3 and Dpb4 each contain a histone fold domain (HF) and a C-terminal region (C). b 2D class averages of Pol ε showing the rigid state (I) and flexible state (II). c 3D map in front and back views, with each subunit shown in a distinct color.
and Dpb4 subunits each adopt histone folds that form a tight Dpb3–4 complex. Pol ε physically associates with the replicative CMG (Cdc45, Mcm2-7, and GINS) helicase to assemble a molecular machine termed the leading strand replisome that couples continuous DNA unwinding with high fidelity and processive DNA synthesis. Pol2 contains two DNA polymerase modules covalently linked in a 2222-residue long polypeptide chain; the catalytic polymerization and proofreading nuclease action are contained in the N-terminal (NTD) module of Pol2 while the C-terminal (CTD) module of Pol2 encodes a non-catalytic DNA polymerase that likely serves a structural role. Genetic studies in Saccharomyces cerevisiae (S.c.) show that the inactive polymerase module of Pol2 is essential, while the catalytic N-terminal module of Pol2 is not essential, although cell growth is quite compromised. The Dpb2 subunit is also essential, and studies indicate that it functions with the CTD inactive polymerase module of Pol2 in assisting initiation factors in the formation of CMG helicase at origins. Genetic studies reveal that the Dpb3 and Dpb4 histone fold subunits are not essential but are required for preservation of epigenetic information during replication.

Structures of individual subunits and domains of S.c. Pol2 have previously been determined. The active NTD of Pol2 bound to primed DNA is solved to 2.5 Å resolution and the inactive CTD of Pol2 is determined to 4.5–7 Å resolution. The structure of Dpb2 and crystal structure of the histone fold subunits Dpb3–Dpb4 are also previously reported. However, the arrangement of these subunits and domains within the Pol ε holoenzyme is unknown due to the inability to trap a rigid form of the holoenzyme. Thus, the location of Dpb3–4 complex in the Pol ε holoenzyme and the orientation of the Pol2 NTD and CTD in the holoenzyme are not known. Furthermore, the Dpb3–4 complex is demonstrated to bind double-strand DNA and enhance the processivity of Pol ε.

This report determines the structure of the S.c. Pol ε holoenzyme, revealing the juxtaposition of each of the subunits. A most interesting finding is that the active and inactive polymerase modules of Pol2 are spatially separate and are held together by the Dpb3–Dpb4 histone fold subunits. Importantly, the Pol ε structure has enabled us to build a pseudo atomic model of the leading strand replisome, revealing the orthogonal path of the parental DNA entering CMG and the nascent daughter DNA exiting from PCNA, and how the leading single-strand DNA is directed by the Dpb2 OB domain from the CMG helicase to the Pol ε active site.

**Results and discussion**

**The Pol ε holoenzyme is held by Dpb3–4 into a rigid state.** In electron micrographs, Pol ε is a flexible two-lobed structure, with the Pol2 NTD in lobe 1, the Pol2 CTD and Dpb2 in lobe 2, and the Dpb3–4 position unknown. Our 2D classification of a large cryo-EM dataset of Pol ε revealed the full Pol ε holoenzyme in a rigid form and reveals that Dpb3–4 binds between lobe 1 and lobe 2, holding them rigid. We also observed particles that displayed the previously observed flexibility in which the image classes only resolved lobe 2 (Fig. 1b, bottom row). Specifically, we observed averaged class images with all subunits, including both Pol2 NTD and CTD domains, Dpb2, and Dpb3–4 complex (lobes 1 and 2), but also class averages with Pol2 CTD and Dpb2 (lobe 1) and blurry Pol2 NTD lobe and missing or blurry Dpb3–4. Previous cryo-EM studies of Pol ε (and Pol ε–CMG complex) have only visualized the lobe 1 state of Pol ε. Thus, we were surprised to observe class averages in which both lobes 1 and 2 had well-defined structural features (Fig. 1a), showing that Pol ε holoenzyme can exist in a rigid form. We presume that the plunge-freezing process needed to make cryo-EM grids often disrupts the Pol ε holoenzyme, although it remains possible that Pol ε has two functional forms, rigid and flexible. The possible function of a flexible form of Pol ε holoenzyme, assuming it exists in the cell, will be considered below.

**The structure of the rigid state Pol ε holoenzyme.** Through large-scale data collection including recording data at a tilted angle of 30° and 3D classification selecting the rigid particles, we obtained a cryo-EM 3D map at 3.5 Å of the Pol ε holoenzyme (Fig. 1c, Supplementary Figs. 1–3, and Supplementary Table 1). At this resolution one could observe side chains at numerous positions in the 3D map (Supplementary Fig. 4). Atomic model building was facilitated by the large side chain densities, as well as the previously determined structures of each component of Pol ε. This is the first time that the structure of a eukaryotic leading strand DNA polymerase holoenzyme has been determined to atomic resolution, revealing the position and orientation of each of the four subunits.

Lobe 1 of the holoenzyme contains the Pol2 catalytic domain with an N-terminal subdomain (31–281), an exonuclease (282–527), a palm (528–950), a finger (769–833), and a thumb (951–1186) domain that are organized into a toroid (Fig. 2a). The Pol2 NTD structure is an open circle compared with the crystal structure of the DNA template/primer (T/P)-bound Pol2 NTD (Supplementary Fig. 5a, b). The difference in our apo structure and the ternary complex reveals a 27° tilt of the finger domain. These observations indicate that the native state of the Pol2 apo-enzyme forms a gapped circle, and that DNA/dNTP binding induces the finger domain to clamp down to form the conformation that completely encircles the DNA.

Lobe 2 contains Pol2 CTD and Dpb2. Dpb2 is the B subunit conserved in eukaryotic Pols α, δ, and ε and contains an N-terminal largely helical domain, an OB domain and an inactivated calcineurin-like phosphoesterase domain (PDE) (Figs. 1a and 2b). In our structure, the PDE domain (residues 168–209 and 349–689) and the embedded OB domain (residue 210–348) are well ordered, but are disordered. The Dpb2 subunit forms intimate contacts to the Pol2 CTD via the PDE but has few contacts with the Pol2 NTD or with the Dpb3–4 complex. The crystal structure of the B subunit (p59) in complex with the p261 C-terminal Zn-coordinating fragment (p261c CysA and CysB) of human Pol ε was previously solved to 2.35 Å. The human crystal structure superimposes well with the corresponding region in the yeast Pol ε (Supplementary Fig. 5c). Furthermore, the entire lobe 2 (Pol2 CTD and Dpb2) was recently determined by cryo-EM to 5 Å resolution revealing that the polymerase fold has a wide-open jaw that is partially blocked by one of the two Cys motifs (CysA). It was unknown if the jaw-blocking CysA motif observed in the Pol2 NTD-truncated structure moves out in the holoenzyme. In our 3.5 Å structure of the holoenzyme, we found the overall structure of Pol2 CTD is similar and the CysA motif remains in place to block the jaw, providing further support that the Pol2 CTD has neither polymerase activity nor DNA-binding activity.

The Dpb3–4 heterodimer is known to have a histone fold, but its position within the Pol ε has been unknown. Interestingly, we found that Dpb3–4 is wedged in the middle of the holoenzyme between lobes 1 and 2, and that there is a solvent exposed and positively charged surface in the Dpb3–4 (Supplementary Fig. 6). Dpb3–4 enhances the processivity of Pol ε holoenzyme and can directly bind DNA in vitro. It is currently unclear if the
positive patch accounts for the reported DNA-binding activity of Dpb3–4, and if so, whether it binds DNA in the context of the replisome. Importantly, we found that the overall architecture of the Pol ε observed in the rigid state is consistent with our previous cross-linking mass spectrometry of the yeast Pol ε in complex with the CMG helicase 10 (Supplementary Fig. 7), indicating the physiological relevance of the rigid state.

A mooring helix in the Pol2 NTD–CTD linker anchors Dpb3–4. The 112-residue NTD–CTD linker (Thr-1186–Ser-1308) is widely assumed to be disordered accounting for the flexible association between the two Pol ε lobes. Interestingly, the last one third (Val-1270–Ser-1308; 38 residues) of the linker forms a long L-shaped α-helix in our structure (Fig. 2c and Supplementary Movie 1). This helix appears to be critically important, as it interacts with the Pol2 NTD and recruits Dpb3–4 into the Pol ε holoenzyme. We refer to this α-helix as the mooring helix. The mooring helix underlies the observed rigid state of Pol ε as it positions the Dpb3–4 complex into the weakest middle region of Pol ε and forms intimate contacts to both Pol ε lobes, thereby fixing their orientation relative to one another. This mooring helix was not observed in the Pol2 CTD–Dpb2 subcomplex structure 15 and likely forms a structured helix only upon interaction with Dpb3–4. The recruited Dpb3–4 then acts as a bridge to buttress the catalytically active NTD and catalytically inactive CTD polymerase modules of Pol2, holding them rigid. The fact that the rigid Pol ε state conforms with our earlier reported in vitro cross-linking/mass spectrometry results further supports our conclusion that the rigid class of particles populate the solution phase state of Pol ε 10.

Figure 3a illustrates the detailed connections among: (1) Pol2 NTD and CTD (panel 1), (2) Pol2 NTD and Dpb3 (panel 2), (3) the mooring helix and Dpb3–4 (panel 3), (4) the Pol2 CTD and Dpb4 (panel 4), and (5) the mooring helix and Pol2 NTD (panel 5). The interface between the Pol2 NTD and CTD lacks hydrophobic buried residues and consists mainly of four salt bridges wherein the NTD contains four negatively charged residues and the CTD contains four positive charged residues. These electrostatic salt bridges are unlikely to have sufficient energy to hold the two Pol2 domains in a fixed position. Panels 2 and 4 show the connections between Dpb3–4 and the Pol2 NTD and CTD, respectively. Dpb3 binds the NTD of Pol2, and Dpb4 binds the CTD of Pol2. While the Pol2 NTD forms a 988 Å2
Fig. 3 Domain–domain interactions within Pol ε. a Pull-apart of the structure. Each circle represents the contact region between two domains. The oval with a number indicates the interaction that is enlarged and shown in the numbered enlarged boxes. 1: between Pol2 NTD and CTD; 2: between Pol2 NTD and Dpb3; 3: between mooring helix and Dpb3–4; 4: between Dpb3 and Dpb4; and 5: between mooring helix and Pol2 NTD. b Sequence alignment of the mooring helix in Sc (S. cerevisiae), Sp (S. pombe), Dr (D. reos), Hs (H. sapien), Mm (M. muscarus). The red asterisks indicate conserved residues involved in interactions with Dpb3–4.
buried hydrophobic interface with Dpb3 (panel 2), the Pol2 CTD–Dpb4 interface appears less robust involving mainly salt bridges. This may seem curious, considering the established ability of Dpb3–4 (and Dpb2) to form a stable isolable complex with the CTD of Pol212,13. However, this can be explained by the extensive interface of 2307 Å² between the Pol2 mooring helix and the Dpb3–4 complex (Fig. 3a, panel 3). Hence, we conclude that the Pol2 mooring helix is in line with the leading strand replisome. This information is essential for understanding how the leading strand DNA exiting the replisome is directed to the Pol2 CTD (Supplementary Movie 2). We first docked the structures of Pol2 NTD–PCNA (Fig. 4c and Supplementary Movie 2). We then superimposed the Pol2 NTD region of the published Pol2 NTD–P/T structure14 with our apo Pol2 NTD. The binding position of the Pol2 NTD with the Pol2 CTD was then derived by superimposing the catalytic domain of the Pol2–DNA–FEN1–PCNA structure37 with the Pol2 NTD of the Pol2–DNA–FEN1 complex37. This was feasible because the structural fold of the catalytic domain of the three eukaryotic replicative polymerases are highly conserved.

The Pol2 PIP motif (1193–120135) is within the first half of the NT–CTD linker (aa 1187–1270), thus is disordered in the Pol2–DNA–FEN1 complex37. Further, a Dpb2 helix and the connecting loop (aa 378–399) and a Pol2 NTD loop (aa 1125–1139) are also close to the Pol2 NTD–CTD junction; they may interact with PCNA as well (Supplementary Fig. S9b, c). In our model, the primer and template is bound by the Pol2 NTD which is stabilized by PCNA. This may raise the question of how an RFC clamp loader interacts with Pol2. The Pol2 P domain may interact with PCNA, just like FEN1 does in the Pol2–DNA–FEN1 complex37. Further, a Dpb2 helix and the connecting loop (aa 378–399) and a Pol2 NTD loop (aa 1125–1139) are also close to the Pol2 NTD–CTD junction; they may interact with PCNA as well (Supplementary Fig. S9b, c). In our model, the primer and template is bound by the Pol2 NTD which is stabilized by PCNA. This may raise the question of how an RFC clamp loader interacts with Pol2. The Pol2 P domain may interact with PCNA, just like FEN1 does in the Pol2–DNA–FEN1 complex37. Further, a Dpb2 helix and the connecting loop (aa 378–399) and a Pol2 NTD loop (aa 1125–1139) are also close to the Pol2 NTD–CTD junction; they may interact with PCNA as well (Supplementary Fig. S9b, c). In our model, the primer and template is bound by the Pol2 NTD which is stabilized by PCNA. This may raise the question of how an RFC clamp loader interacts with Pol2. The Pol2 P domain may interact with PCNA, just like FEN1 does in the Pol2–DNA–FEN1 complex37. Further, a Dpb2 helix and the connecting loop (aa 378–399) and a Pol2 NTD loop (aa 1125–1139) are also close to the Pol2 NTD–CTD junction; they may interact with PCNA as well (Supplementary Fig. S9b, c). In our model, the primer and template is bound by the Pol2 NTD which is stabilized by PCNA. This may raise the question of how an RFC clamp loader interacts with Pol2. The Pol2 P domain may interact with PCNA, just like FEN1 does in the Pol2–DNA–FEN1 complex37. Further, a Dpb2 helix and the connecting loop (aa 378–399) and a Pol2 NTD loop (aa 1125–1139) are also close to the Pol2 NTD–CTD junction; they may interact with PCNA as well (Supplementary Fig. S9b, c). In our model, the primer and template is bound by the Pol2 NTD which is stabilized by PCNA. This may raise the question of how an RFC clamp loader interacts with Pol2. The Pol2 P domain may interact with PCNA, just like FEN1 does in the Pol2–DNA–FEN1 complex37. Further, a Dpb2 helix and the connecting loop (aa 378–399) and a Pol2 NTD loop (aa 1125–1139) are also close to the Pol2 NTD–CTD junction; they may interact with PCNA as well (Supplementary Fig. S9b, c). In our model, the primer and template is bound by the Pol2 NTD which is stabilized by PCNA. This may raise the question of how an RFC clamp loader interacts with Pol2. The Pol2 P domain may interact with PCNA, just like FEN1 does in the Pol2–DNA–FEN1 complex37. Further, a Dpb2 helix and the connecting loop (aa 378–399) and a Pol2 NTD loop (aa 1125–1139) are also close to the Pol2 NTD–CTD junction; they may interact with PCNA as well (Supplementary Fig. S9b, c). In our model, the primer and template is bound by the Pol2 NTD which is stabilized by PCNA. This may raise the question of how an RFC clamp loader interacts with Pol2. The Pol2 P domain may interact with PCNA, just like FEN1 does in the Pol2–DNA–FEN1 complex37. Further, a Dpb2 helix and the connecting loop (aa 378–399) and a Pol2 NTD loop (aa 1125–1139) are also close to the Pol2 NTD–CTD junction; they may interact with PCNA as well (Supplementary Fig. S9b, c). In our model, the primer and template is bound by the Pol2 NTD which is stabilized by PCNA. This may raise the question of how an RFC clamp loader interacts with Pol2. The Pol2 P domain may interact with PCNA, just like FEN1 does in the Pol2–DNA–FEN1 complex37. Further, a Dpb2 helix and the connecting loop (aa 378–399) and a Pol2 NTD loop (aa 1125–1139) are also close to the Pol2 NTD–CTD junction; they may interact with PCNA as well (Supplementary Fig. S9b, c).
(oligosaccharyl/oligonucleotide binding domain) such as those found in SSB and RPA is known to bind ssDNA. The Dpb2 OB domain is positioned midway in the journey of the leading strand DNA from the helicase exit to the entry of the polymerase catalytic site. Therefore, we suggest that both the Mcm5 WHD and the Dpb2 OB may play roles in guiding the leading strand.

Previous studies document a flexible form of Pol ε, and a flexible form of Pol ε is also noted here, but whether this is an artifact of cryo-grid preparation or whether a flexible form of Pol ε exists in the cell is unknown. It is possible that a flexible form of Pol ε, if it exists, serves a physiological role. An obvious possibility is that a flexible catalytic domain of Pol ε may provide access to the 3′ terminus for another DNA polymerase, such as a TLS polymerase upon encounter with a DNA lesion. It is also tempting to speculate that a flexible form of Pol ε may facilitate transfer of parental nucleosomes to the leading strand for epigenetic inheritance. Cellular studies show that Dpb3 and Dpb4 are required for epigenetic inheritance, as deletion of the gene encoding them result in deficiency in transfer of parental epigenetic information to the leading strand. Interestingly, Dpb3–4 are demonstrated to bind the H3–H4 tetramer. We note that if the binding between Dpb3–4 and H3–H4 mimics the interface between H2A–H2B and H3–H4 in the histone octamer, the rigid Pol ε holoenzyme structure determined here is...
Fig. 5 Leading ssDNA path from the CMG helicase to the Pol ε. a Top and side views of the surface presentation of the atomic model of the leading strand replisome, centering around the Dpb2 OB domain. b A sketch illustrating the midway location of Dpb2 in the shortest path of the leading strand DNA from CMG to the entrance of the Pol ε catalytic site.
incompatible with Dpb3–4 binding to H3–H4 because the Pol2 mooring helix gets in the way preventing such interaction. Perhaps a flexible form of Pol ε might possibly facilitate H3–H4 binding by Dpb3–4. The role of Dpb3–Dpb4 in epigenetics is an important issue that requires further study.

In summary, we have solved the structure of the eukaryotic Pol ε holoenzyme, revealing how Dpb3–4 is anchored in the middle of the two-lobed enzyme complex by the mooring helix which is part of the long linker between the active and inactive polymerase modules of Pol2. The structure has also enabled atomistic modeling of the entire leading strand replisome, revealing a likely path for part of the long linker between the active and inactive polymerase subunits into yeast under control of the Gal1/10 promotor. Yeast cells were then grown in YP media at 30 °C, and induced upon reaching an OD600 of 0.7 by addition of 20 g/L galactose followed by continued growth for 6 h. Pol ε holoenzyme was then purified similarly to a previously method. Briefly, cells were lysed by addition null (6970 rpm) and cell debris removed by centrifugation (19,000 r.p.m. in a SS-34 rotor for 1 h at 4 °C). The supernatant was applied to a 1 ml Anti-Flag antibody resin (Sigma), followed by elution using 0.15 mg/ml 3XFLAG peptide (EZBiolab, Carmel, Indiana, USA). Peak fractions were then pooled and further purified on a MonoS column. Peak fractions containing Pol ε holoenzyme were pooled and dialyzed against 50 mM Hepes pH 7.5, 50 mM KC1u, 200 mM Kacetate, 1 mM DTT, 4 mM MgCl2, aliquoted, snap frozen in liquid nitrogen, and stored at −80 °C. CMG was purified by integrating each of the 11 genes encoding the CMG subunits into yeast, similar to a previous protocol. Briefly, yeast cells were grown in YP media at 30 °C and induced at an OD600 of 0.7 by addition of 20 g/L galactose and incubated a further 6 h at 30 °C. CMG was then purified similar to Pol ε holoenzyme through the FLAG affinity column step. Peak fractions were then pooled and further purified on a 1 ml HisTrap HP column (GE Healthcare) and eluted with a 7.5 mM linear gradient of 0.5–750 mM imidazole. Peak fractions were pooled and dialyzed against 50 mM Hepes pH 7.5, 50 mM KC1u, 200 mM Kacetate, 1 mM DTT, 4 mM MgCl2, aliquoted, snap frozen in liquid nitrogen, and stored at −80 °C.

DNA replication assay. Experiments used a 2.7 kb forked DNA substrate having a synthetic forked DNA ligated to linearized pUC19 DNA, followed by gel filtration to remove excess forked DNA as described. The DNA template was primed using a 5′ 32P-37mer oligonucleotide as described. Reactions (25 μl final) contained 1.25 nM linear forked template in 25 mM Tris-OAc pH 7.5, 5% glycerol, 40 μM dNTPs WASA, 5 mM MnCl2, 10 mM MgOAc, 50 mM KCl, 50 mM KOAc, 40 mM MgCl2, 40 mM Kacetate, 1 mM DTT, 4 mM MgCl2, aliquoted, snap frozen in liquid nitrogen, and stored at −80 °C.

Methods
Proteins and nucleic acids. Pol ε holoenzyme was purified integrating the genes for its subunits into yeast under control of the Gal1/10 promotor. Yeast cells were then grown in YP media at 30 °C, and induced upon reaching an OD600 of 0.7 by addition of 20 g/L galactose followed by continued growth for 6 h. Pol ε holoenzyme was then purified similarly to a previously method. Briefly, cells were lysed by addition null (6970 rpm) and cell debris removed by centrifugation (19,000 r.p.m. in a SS-34 rotor for 1 h at 4 °C). The supernatant was applied to a 1 ml Anti-Flag antibody resin (Sigma), followed by elution using 0.15 mg/ml 3XFLAG peptide (EZBiolab, Carmel, Indiana, USA). Peak fractions were then pooled and further purified on a MonoS column. Peak fractions containing Pol ε holoenzyme were pooled and dialyzed against 50 mM Hepes pH 7.5, 50 mM KC1u, 200 mM Kacetate, 1 mM DTT, 4 mM MgCl2, aliquoted, snap frozen in liquid nitrogen, and stored at −80 °C.

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Atomic modeling and validation. Models of all Pol ε subunits were directly extracted from the crystal and cryo-EM structure of the yeast Pol2 NTD (PDB ID 5U8S), Pol2 CTD with Dpb2 (PDB ID 6HV8), and Dpb3–Dpb4 (PDB ID 5T26). DNA sequence was randomly assigned in the model. These models were rigid-body fitted into the 3D density map with COOT and Chimera. The entire Pol2 ε models were first refined by rigid-body refinement of individual chains using the PHENIX program, and subsequently adjusted manually in COOT guided by residues with bulky side chains like Arg, Phe, Tyr, and Trp. The electron densities at the metal binding sites in the C-terminal CysA and CysB of Pol2 CTD were weak, particularly at the CysB site. We modeled a Zn2+ in the CysA site (aa 2108–2130) but did not model a ligand in the CysB site. The model was then refined in real space by phenix.real_space_refine and in reciprocal space by PHENIX with the application of secondary structure and stereochemical constraints. The structure factors (including phases) were calculated by Fourier transformation of the experimental density map with the program Phenix.map_to_structure_factors. The final models were validated using MolProbity. Structural figures were prepared in Chimera and Pymol.

Cryo-EM. To prepare EM grids of Pol ε samples, 3 μl of Pol ε sample was applied, at a final concentration of ~1.6 mg/ml, to C-1.2/1.3/1.0 holey carbon grids, treated by glow-discharge before use. Grids were then incubated for 10 s at 6 °C and 90% humidity, blotted for 3 s and plunged into liquid ethane using a Thermo Fisher Vitrobot IV. Grids were loaded into a Titan Krios electron microscope and images at 300 kV were collected automatically using low-dose mode at a magnification of ~130,000 and a pixel size of 1.028 Å per pixel. A Gatan K2 summit direct electron detector was used for image recording with a defocus range from −1.5 to −2.5 μm under super-resolution mode. The dose rate was 10 electrons per Å2 per second and total exposure time was 6 s. The total dose was divided into 30-frame movies and each frame was exposed for 0.2 s. For cryo-EM of the CMG-Pol ε complex containing native Pol ε and recombinant CMG, the sample concentration was 1.1 mg/ml. The cryo-EM data was collected in an Arctica Talos electron microscope operated at 200 kV with a Gatan K2 summit direct electron detector. The dose rate was 10 electrons per Å2 per second and total exposure time was 6 s. The total dose was divided into 30-frame movies and each frame was exposed for 0.2 s.

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Data availability. The 3D cryo-EM map of the S. cerevisiae polymerase ε holoenzyme at 3.5 Å resolution has been deposited in the Electron Microscopy Data Bank under accession code EMD-21701. The corresponding atomic model has been deposited in the Protein Data Bank under accession code PDB 6WJV.
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Author contributions
Z.Y., R.G, M.E.O., and H.L. conceived and designed experiments. R.G. purified Pol ε and CMG-Pol ε complex. Z.Y. performed the EM experiments, image processing, 3D reconstruction, and atomic modeling. G.S. performed the DNA replication assays. Z.Y., M.E.O., and H.L. analyzed the data and wrote the paper.

Competing interests
The authors declare no competing interests.

Additional information
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