

Chapter 13

DNA Polymerase ϵ

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Abstract DNA polymerase ϵ (Pol ϵ) is one of three replicative DNA polymerases in eukaryotic cells. Pol ϵ is a multi-subunit DNA polymerase with many functions. For example, recent studies in yeast have suggested that Pol ϵ is essential during the initiation of DNA replication and also participates during leading strand synthesis. In this chapter, we will discuss the structure of Pol ϵ , the individual subunits and their function.

Keywords DNA replication • Leading strand • Pre-loading complex • Processivity

13.1 Introduction

In the decades since the first DNA polymerase was discovered, the number and types of known polymerases has expanded dramatically. In human cells there are at least 15 DNA polymerases that play a part in a wide variety of activities in the replication and maintenance of the genome (Shcherbakova et al. 2003a). DNA polymerase ϵ (Pol ϵ) is a large, multi-subunit enzyme found in all eukaryotic organisms studied to date. The enzyme possesses two catalytic activities: template directed DNA polymerization and the exonucleolytic removal of mispaired primer termini. The role of Pol ϵ , however, is not limited to DNA replication; it has been implicated in such pathways as epigenetic silencing, cell cycle regulation, sister chromatid adhesion and possibly DNA recombination during repair of DNA lesions (Pursell and Kunkel 2008). All Pol ϵ enzymes discovered to date consist of the same basic

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architecture. The core of the holoenzyme is the large, catalytic subunit that can be divided into two subdomains: the N-terminal portion of the molecule is the catalytic domain and contains the polymerase and exonuclease active sites, while the C-terminal domain is catalytically inactive and appears to play a structural role in the enzyme. All Pol ϵ holoenzymes contain a large B-subunit as well as two small subunits. The accessory subunits in Pol ϵ do not appear to contain any catalytic activities and do not appear to influence the catalytic rates of the polymerase or exonuclease active sites of the N-terminal portion of the catalytic subunit. They do, however, play important roles in the biological pathways in which Pol ϵ is found. The following discussion looks at each of these subunits individually in terms of what is known about their structures and then discusses how they function together as higher order molecular complexes in the various cellular pathways in which Pol ϵ plays a role.

13.2 Structure of Pol ϵ Subunits

13.2.1 *Pol2*

While the crystal structure of Pol ϵ has not yet been reported, many aspects of its structure can be inferred from its classification with other DNA polymerases of known structure. The N-terminal portion of Pol2 is classified as a B-family DNA polymerase based on primary sequence similarities with other B-family DNA polymerases including eukaryotic DNA polymerase α and δ , the DNA polymerases from bacteriophages T4 and RB69 and the DNA polymerases from several archaea such as *Desulfurococcus sp.* (strain Tok), *Thermococcus gorgonarius* and *Pyrococcus furiosus*. X-ray crystal structures are available for several members of the B-family of DNA polymerases and all of these reveal a highly conserved arrangement of protein domains (Fig. 13.1). All of the B-family DNA polymerases whose structures have been solved consist of five domains: a catalytic portion likened to a right hand with a thumb, palm and fingers domain, an exonuclease domain and an N-terminal domain.

The thumb domain associates with the duplex DNA upstream from the polymerase active site and has been shown to play a critical role in establishing the balance between polymerizing and editing modes of the B-family DNA polymerases. Mutations in the thumb domain have been shown to act as mutators or antimutators depending on whether they cause the enzyme to spend more time in the polymerizing mode or the editing mode respectively (Stocki et al. 1995; Wu et al. 1998). The palm domain is the most highly conserved sub-domain among all DNA polymerases, not just those from the B-family, and contains the catalytic residues involved in the nucleotidyl transferase reaction of addition of nucleoside triphosphates to the growing 3' end of the primer terminus. All DNA polymerases in this family possess three conserved sequence motifs called motif A, B and C, with motifs A and C containing

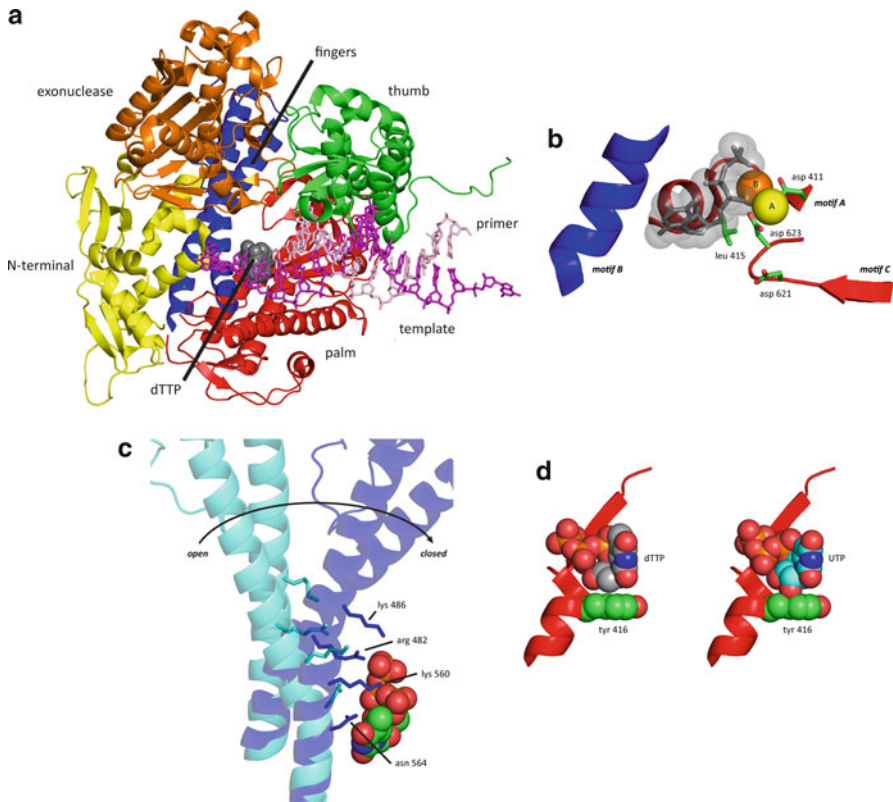


Fig. 13.1 (a) The arrangement of conserved functional domains in the B-family DNA polymerases as exemplified by the crystal structure of the ternary complex of the DNA polymerase from bacteriophage RB69 (Franklin et al. 2001). The N-terminal domain (residues 1–108 and 340–382) is shown in *yellow*, the exonuclease domain (residues 109–339) is shown in *orange*, the palm domain (residues 383–468 and 573–729) is shown in *red*, the fingers domain (residues 469–572) is shown in *blue* and the thumb domain (residues 730–903) is shown in *green*. The duplex DNA co-crystallized with the protein is represented by a *stick* model with the primer strand appearing in *pink* and the templating strand in *magenta*. The incoming dTTP molecule in the ternary complex is shown as *grey spheres*. (b) The polymerase catalytic core of the DNA polymerase from bacteriophage RB69 (Franklin et al. 2001). The three conserved motifs A, B and C (Delarue et al. 1990) are *colored* according to the scheme in part a. The incoming dTTP is shown as *grey sticks* with *transparent spheres*. The three conserved aspartates in the B-family DNA polymerases are shown as *green sticks*. The two catalytic metal ions A and B are shown as *yellow* and *orange spheres* respectively. Leu 415 corresponds to Met 644 in Pol ϵ . (c) Conformational changes in the fingers domain of the DNA polymerase from bacteriophage RB69 (Franklin et al. 2001). The fingers from the open apo complex are shown in *cyan* and those from the closed ternary complex in *blue*. The incoming dTTP is shown as *spheres*. The conserved residues that coordinate the residues of the triphosphate tail during catalysis are shown as *sticks*. (d) Sugar selectivity in the B-family DNA polymerases from bacteriophage RB69 (PDB ID: 1IG9 (Franklin et al. 2001)). The image on the *left* shows the incoming dTTP molecule in *grey spheres* and the steric gate residue tyrosine 416 in *green spheres*. The image on the *right* has a UTP ribonucleotide superimposed onto the dTTP to show the steric clash between the 2'-OH group and the tyrosine ring. Tyr 416 corresponds to Tyr 645 in Pol ϵ

the highly conserved catalytic aspartates (Fig. 13.1b) (Delarue et al. 1990). Motif C consists of a β -hairpin loop with the invariant DTD sequence at its tip. These conserved aspartate residues participate in the two-metal catalytic mechanism (Steitz 1993) that appears to be utilized by all DNA polymerases discovered to date. It has been shown in genetic experiments that substitutions of the two catalytic aspartates for alanine in the catalytic subunit of Pol ϵ (Pol2) rendered the cells inviable, suggesting that DNA synthesis by Pol ϵ is essential in yeast (Dua et al. 1999). Inserted within the palm domain sequence is the fingers domain that contains motif B. This motif consists of residues at the junction of the palm domain and the fingers domain, the movement of which is a critical component in the catalytic activity of B-family DNA polymerases. This domain also contains conserved amino acid residues that bind the triphosphate tail of the incoming nucleotide triphosphates and which undergo large conformational changes during catalysis (Fig. 13.1c) (Franklin et al. 2001; Yang et al. 1999). Another motif that may be located in the palm domain or the vicinity of the thumb domain is a 66 amino acid insertion that is only found in Pol ϵ among all B-family DNA polymerases (Shcherbakova et al. 2003b). The function of this motif is still unknown.

The exonuclease domain in B-family DNA polymerases contains four conserved residues that participate in the catalytic removal of mis-incorporated nucleotides and provide an approximately 100-fold increase in replication fidelity (McCulloch and Kunkel 2008). The exonuclease active site resides about 40 Å away from the polymerase active site in RB69 and so at least three residues must be unwound from the template in order to span this distance. Steric restraints prevent duplex DNA from entering the exonuclease active site. The Pol ϵ mutant allele *pol2-4*, carrying the double mutation D290A and E292A in the conserved motif, has no exonuclease activity in *in vitro* experiments and yeast strains with the *pol2-4* allele also have a significantly increased mutation rate (Morrison et al. 1991; Shcherbakova et al. 2003b).

The N-terminus of B-family polymerases consists of the aptly named N-terminal domain. In archaeal DNA polymerases, this domain contains conserved cysteine residues that form a binding pocket for uracil that can discriminate against the four canonical DNA bases (Fogg et al. 2002). This activity has been proposed to allow these polymerases to pause upon encountering uracil in the template to allow the cell time to repair the uracil residue before replication proceeds across this potentially mutagenic base (Connolly 2009). Interestingly, this read-ahead capability appears to be unique to the archaeal B-family DNA polymerases as no other B-family DNA polymerases, including Pol ϵ from *S. cerevisiae*, are blocked by the presence of uracil in the templating DNA (Wardle et al. 2008). A distinct function of the N-terminal domain of Pol ϵ has not yet been reported.

The catalytic core subunit of Pol ϵ is unique among all of the eukaryotic B-family DNA polymerases in that it appears to consist of two distinct polymerase domains. While all of the domains discussed above exist within the catalytic Pol2 subunit of Pol ϵ , these domains only make up about 140 kD of the estimated 259 kD enzyme in *S. cerevisiae*. Thus there is a large portion of Pol2 that appears to possess no catalytic activity. Secondary structure predictions, fold recognition and sequence similarity searches, however, result in high similarities between the 120 kD C-terminus

of human Pol ϵ and B-family DNA polymerases such as DNA polymerase II in *Escherichia coli* and the archaeal DNA polymerases from *Desulfurococcus sp.* (strain Tok) and *Thermococcus kodakaraensis* (Tahirov et al. 2009). The conserved motifs for polymerase and exonuclease domains in the C-terminus of Pol ϵ in both humans and *S. cerevisiae* align with the catalytic domains of other B-family DNA polymerases but the catalytic residues in both the polymerase and exonuclease domains are disrupted such that catalytic activity has been lost. Such a disruption in catalytic domains appears to be a common feature among a diverse group of archaeal polymerase homologs (Rogozin et al. 2008). Interestingly, the alignments of the C-terminal domain appear to be more similar to bacterial B-family DNA polymerases than the N-terminal domain of Pol ϵ itself (Tahirov et al. 2009). Thus the evolutionary pathway for the creation of Pol ϵ appears to be more complex than a simple gene duplication event of the N-terminal domain.

The very C-terminal end of the protein is a cysteine rich sequence containing two putative zinc fingers of the sequence CX₂CX₁₈CX₂CX₃₀CX₂CX₁₁CXC (Dua et al. 1998). The zinc fingers have been demonstrated to bind zinc ions, though the binding efficiency of the two fingers is not identical (Dua et al. 2002). Sequence analyses of the zinc fingers suggest that they are more similar to the single zinc finger in the archaeal PolD polymerase than to other B-family Zn fingers (Tahirov et al. 2009). The zinc-finger domain is essential and interacts with the B-subunit Dpb2 (presented below). Point mutations that support growth exhibit sensitivity to the alkylating agent methyl methane sulfonate (MMS) (Dua et al. 1999) suggesting that the mutant is deficient in DNA damage repair or avoidance. Interestingly, it is the inter-zinc finger region that is essential for cell viability, not the conserved cysteine residues that constitute the zinc fingers (Dua et al. 1998).

13.2.2 Dpb2

The B-subunit of Pol ϵ (Dpb2) consists of three domains: an N-terminal region that shows structural similarity to AAA+ proteins, a predicted OB fold in the center of the protein and a C-terminal calcineurin-like domain (Nuutinen et al. 2008). The N-terminus of human Dpb2 has been solved by NMR spectroscopy (Nuutinen et al. 2008). So far, there have not been any functions assigned to the three domains. The C-terminal calcineurin-like phosphodiesterase domain is active in archaea and appears to be involved in PCNA binding. In eukaryotic B subunits, this domain is disrupted and appears to no longer possess catalytic activity (Aravind and Koonin 1998). Dpb2 contains a putative PCNA binding domain (Dua et al. 2002). However, mutant Pol ϵ with mutations in the Dpb2 PIP (PCNA interacting protein) motif was not affected in PCNA-dependent holoenzyme assays (unpublished observations).

Dpb2 is essential for cell viability as disruption of the *DPB2* gene in *Saccharomyces cerevisiae* and in *Schizosaccharomyces pombe* is lethal to the cell. Temperature-sensitive mutants of Dpb2 showed partial defects at the restrictive temperature (Araki et al. 1991), suggesting that Dpb2 participates during the establishment

of the replication fork and is required for proper chromosomal replication in yeast. In *S. pombe*, Dpb2 binds to origins in early S-phase supporting a function during the initiation process (Feng et al. 2003). The C-terminal domain of Pol2 also associates with origin DNA at the same time as Dpb2 (Feng et al. 2003).

Dpb2 is phosphorylated by Cdc28 during late G1 phase. Inactivation of cyclin-dependent kinase (CDK) phosphorylation sites in Dpb2 leads to slow cell growth and decreased spore viability. It was suggested that phosphorylation of Dpb2 regulates its interaction with Pol2 or the activity of the Pol ϵ holoenzyme (Kesti et al. 2004), something which remains to be tested *in vitro*. Temperature sensitive *dpb2* alleles were shown to give an increased spontaneous mutation rate (Jaszczur et al. 2008). The level of spontaneous mutation rates were correlated with loss of interaction with Pol2 in two-hybrid assays (Jaszczur et al. 2009). Whether Dpb2 directly influences the fidelity of Pol2 remains to be shown.

13.2.3 *Dpb3/Dpb4 Dimer*

The primary amino acid sequence of Dpb3 and Dpb4 suggests that the N-terminal region of Dpb4 contains a histone fold motif consisting of a helix-strand-helix motif (Ohya et al. 2000). This motif was first identified as being required for dimerization of histone H2A/H2B and H3/H4 pairs (Arents and Moudrianakis 1993) and has been shown to be present in numerous other protein-protein and protein-DNA interactions (Baxevanis et al. 1995). In fact Dpb4 also has another partner, Dls1, in a chromatin remodeling complex. The structure of the Dls1/Dpb4 complex (CHRAC14-CHRAC16) from *D. melanogaster* revealed that Dpb4 indeed has a histone-fold (Hartlepp et al. 2005). The role of Dls1/Dpb4 appears to tether the chromatin remodeling complex to the double-stranded DNA, a function which also has been proposed for the Dpb3/Dpb4 complex in Pol ϵ (Dang et al. 2007; Hartlepp et al. 2005; Tsubota et al. 2003).

Pol ϵ has the ability to bind double-stranded DNA with high affinity, a property not normally associated with DNA polymerases (Tsubota et al. 2003). The double-stranded DNA binding affinity is a property of the C-terminal portion and/or the C-terminal associated subunits of Pol ϵ while the more common single-stranded DNA binding property resides in the N-terminal portion of the enzyme that contains the polymerase and exonuclease motifs. Binding of double-stranded DNA by a heterodimer of Dpb3 and Dpb4, however, was very weak. Subsequent work showed that the Dpb3/Dpb4 heterodimer acts in concert with the Pol2/Dpb2 heterodimer to bind double-stranded DNA with an affinity much higher than the individual heterodimers (Tsubota et al. 2006). When Pol2/Dpb2 and Dpb3/Dpb4 were purified separately, binding to double-stranded DNA as assayed in gel shift assays only occurred at high concentrations of protein but when the two complexes were mixed together, the affinity for double-stranded DNA was similar to that of the four subunit holoenzyme. Homology modeling of the Dpb3/Dpb4 heterodimer onto the crystal structure of the chicken H2A-H2B-dsDNA complex (Harp et al. 2000) suggested regions of the proteins that might interact with double-stranded DNA (Fig. 13.2).

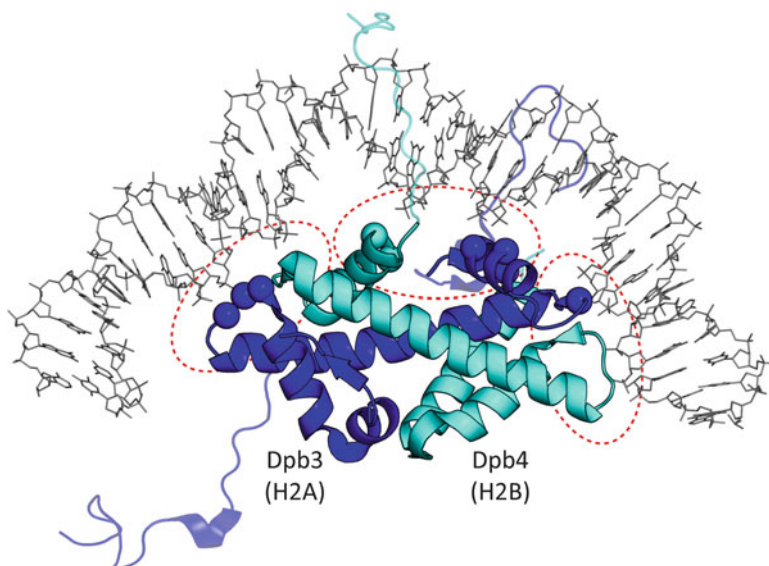


Fig. 13.2 Theoretical DNA binding motifs in Dpb3 and Dpb4 based on the crystal structure of histone fold containing proteins. The structures of H2A (blue) and H2B (cyan) are taken from PDB ID 1EQZ (Harp et al. 2000). The regions of the proteins onto which Dpb3 and Dpb4 were homology modeled are shown in darker colors with black outlines. The portion of the DNA duplex in direct contact with the H2A and H2B proteins is shown as grey sticks. The residues in H2A and H2B corresponding to those that were mutated in Dpb3 and Dpb4 are indicated by spheres and the proposed DNA binding regions in Dpb3 and Dpb4 are indicated by the red dashed lines (Tsubota et al. 2006). This figure is based on Figure 3 in Tsubota et al. (2006)

Indeed, mutations of several lysine residues in Dpb3 reduced double-stranded DNA binding and this defect could be rescued by mutations of a serine and threonine to lysine in Dpb4. Thus the conclusion was made that the heterodimer of Dpb3 and Dpb4 is involved in double-stranded DNA binding (Tsubota et al. 2006).

Dpb4 and Dpb3 are not essential for cell viability in *S. cerevisiae* and appear to offer no enhancement to the catalytic activity of Pol ϵ (Aksenova et al. 2010; Ohya et al. 2000). However, genetic interactions with Dpb11 and Rad53, as well as altered cell cycle progression during S-phase support a model where Dpb3 and Dpb4 are required for normal replication fork progression (Ohya et al. 2000).

13.3 Structure of Pol ϵ Holoenzyme

Based on a combination of sedimentation velocity experiments and gel filtration analysis, the quaternary structure of Pol ϵ appears to be a 1:1:1:1 stoichiometric ratio of all four subunits (Chilkova et al. 2003). The molecular mass of Pol ϵ was determined to be 371 kDa, demonstrating that Pol ϵ purifies as a monomer in all conditions tested (Chilkova et al. 2003).

Structural studies of Pol ϵ have been hampered by poor yields of recombinant protein and by proteolytic cleavage of the Pol2 subunit (Dua et al. 2002). Expression of the Pol ϵ holoenzyme in *S. cerevisiae*, however, yielded sufficient quantities of material to begin attempts at structural studies of the holoenzyme (Chilkova et al. 2003). The structure of Pol ϵ has been solved by cryo-electron microscopy (Asturias et al. 2006). An iterative projection mapping procedure (Penczek et al. 1994) on approximately 19,000 individual Pol ϵ holoenzyme molecules resulted in a reconstruction of the holoenzyme to about 20 Å with a volume of approximately 380 kDa that corresponded well with the previously estimated molecular weight of Pol ϵ (Fig. 13.3a) (Asturias et al. 2006). In this reconstruction, Pol ϵ appears as an elongated protein with a globular head domain and an extended tail domain. In an attempt to locate the subdomains of Pol ϵ , identical reconstructions were made using only the Pol2 subunit (Fig. 13.3b). The 20 Å reconstructions of Pol2 are strikingly similar to the globular domain of the holoenzyme suggesting that the globular head domain consists of the catalytic core. This, then, leaves the extended tail domain to contain the three subunits Dpb2, Dpb3 and Dpb4. A third construct was subjected to the same imaging protocol and consisted of the catalytic core Pol2 and the B-subunit Dpb2. This reconstruction (again to about 20 Å) resembled the structure of Pol2 alone but with extra volume (Fig. 13.3c). This extra volume, however, does not account for all of the expected volume from Dpb2 suggesting that, at least in the absence of Dpb3 and Dpb4, Dpb2 or the C-terminus of Pol2 is highly mobile in the Pol2/Dpb2 heterodimer.

While particles preserved in amorphous ice tend to adopt similar conformations, particles preserved in stain can show variability in conformational changes within a protein structure (Burgess et al. 2004). Images of single Pol ϵ molecules preserved in stain were divided into subcategories based on the relative orientations of the head and tail domains. This classification suggested that the tail domain can move up to 25° and 70° relative to the head domain depending on the direction of motion (see Figure 8 in Asturias et al. 2006). Such domain motions provide a tempting mechanism for how Pol ϵ could bind duplex DNA by way of a flexible linker between the head domain containing the catalytic subunit and the tail domain containing the three accessory subunits. Such a flexible linker domain is evidenced by the intrinsic mobility (and loss of density resolution) for Dpb2 in the Pol2/Dpb2 heterodimer. If duplex DNA were, indeed, to bind to the Pol ϵ holoenzyme in the manner suggested in Fig. 13.3d, this would imply that there would be a minimum length of DNA that would be stably bound by the holoenzyme of about 40 nucleotides. Primer extension assays support this hypothesis (Asturias et al. 2006). When duplexes of varying lengths were provided to Pol ϵ holoenzyme, processivity, as measured by the termination probability at each template position (Kokoska et al. 2003; McCulloch et al. 2004), increased once the duplex region was 40 nucleotides or greater. Such an increase in processivity did not occur with the Pol2 catalytic subunit in the absence of the three accessory subunits Dpb2, Dpb3 and Dpb4. Early experiments with Pol ϵ also showed an increase in processivity when the three accessory subunits were added to the Pol2 subunit (Hamatake et al. 1990).

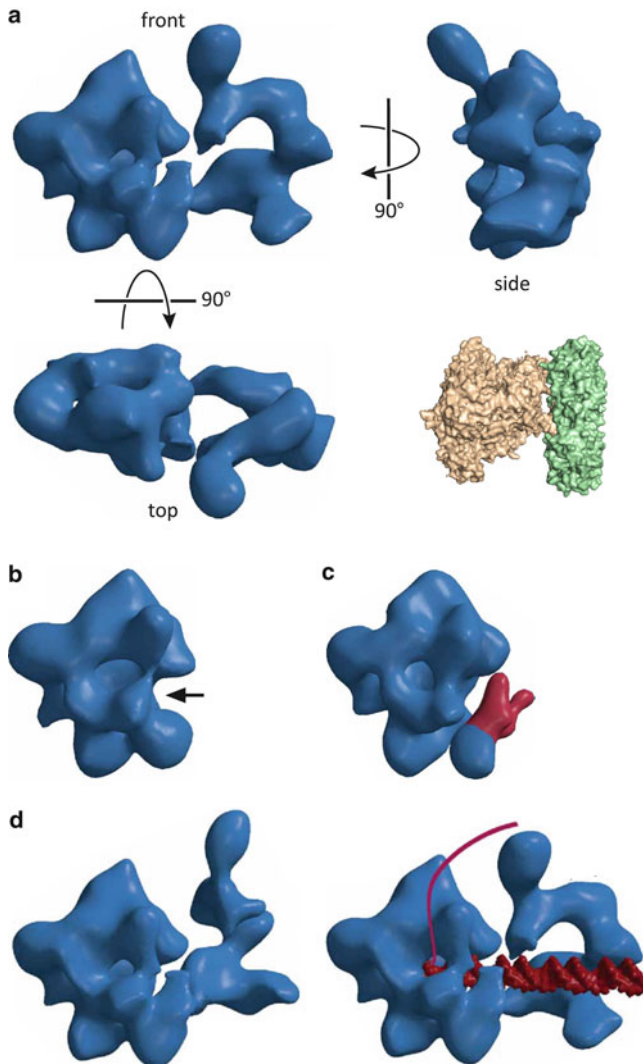


Fig. 13.3 Cryo-electron microscopy (cryo-EM) images of Pol ϵ from *S. cerevisiae*. (a) *Front*, *side* and *top* views of the four subunit holoenzyme. The crystal structure of the DNA polymerase from bacteriophage RB69 (*brown*) (Franklin et al. 2001) docked to the sliding clamp from the same organism (*green*) (Shamoo and Steitz 1999) is shown as a surface representation for scale comparison to the cryo-EM structure of Pol ϵ . (b) The cryo-EM construction of the Pol2 catalytic subunit alone. (c) The cryo-EM construction of the catalytic subunit of Pol2 in complex with Dpb2. The *red* density represents the increase in density over just the Pol2 domain alone. (d) Cryo-EM reconstructions of molecules preserved in stain show flexibility of the tail domain with respect to the globular head domain. This motion suggests a mechanism by which the active site could be made accessible to duplex DNA, which would then be held in place by the closure of the C-terminal domain of the polymerase and presumably, Dpb2, Dpb3 and Dpb4. All cryo-EM reconstructions are from (Asturias et al. 2006)

Interactions between the accessory subunits and duplex DNA as shown in Fig. 13.3d would correlate with the proposed protein folds in these subunits. Dpb3 and Dpb4 both contain histone fold motifs that are presumed to interact with duplex DNA. Indeed, deletion of the two subunits drastically reduces the processivity of the catalytic core along the DNA template (Aksenova et al. 2010). Thus the Dpb3/Dpb4 heterodimer may be functioning as a processivity factor for Pol ϵ in place of PCNA as discussed later.

One question in particular that cannot be answered by the cryo-EM structure is how the accessory subunits are associated with Pol2. It would be tempting to believe that the globular head domain contains all of Pol2 and that the tail domain contains just Dpb2, Dpb3 and Dpb4. It is known, however, that the zinc fingers in *Xenopus* Pol2 are required for binding of the B subunit p60 but not p12-p17. These interact with a motif closer to the N-terminus of Pol2 (Shikata et al. 2006). Yeast two-hybrid assays have also shown that yeast Dpb2 interacts with the zinc-finger domain (Dua et al. 1998; Jaszczur et al. 2009). It is entirely possible, therefore, that the very C-terminal domain of Pol2 extends away from the globular head domain and provides binding sites for the accessory subunits. This C-terminal tail would likely be quite flexible and thus not visible in the cryo-EM reconstructions of Pol2 alone.

13.4 Higher Order Structures

The amino terminal half of Pol ϵ that contains the polymerase and exonuclease domains is not required for cell viability in *S. cerevisiae* (Dua et al. 1999; Kesti et al. 1999) or *S. pombe* (Feng and D'Urso 2001). However, the replication process is impaired and it was suggested that Pol δ can rescue the cells from death (Dua et al. 1999; Ohya et al. 2002). In agreement with these results, the catalytic domain of Pol ϵ appears to be required for replication of the *Xenopus* genome (Shikata et al. 2006). In all cases studied, the C-terminal portion of Pol ϵ is essential for cell viability suggesting that higher order protein interactions between Pol ϵ and other cellular components are required for cellular viability.

13.4.1 Initiation of DNA Replication

The initial step in replication is the formation of a pre-replication complex at an origin of replication (autonomously replicating sequence (ARS) in yeast – see Stinchcomb et al. 1979) during the G1 phase of the cell cycle. This complex consists of the six subunit origin replication complex (ORC), which recruits Cdc6, and together these proteins load Cdt1-Mcm2-7 complexes onto DNA in an ATP hydrolysis-dependent manner (Randell et al. 2006) (see Chaps. 3, 4, 5, 6 and 7, this volume). In budding yeast, upon entry of the cell into S-phase, activated cyclin dependent kinases phosphorylate Cdc6 leading to its degradation to prevent re-initiation of

replication (Drury et al. 1997). The order by which the assembly of replication forks occurs is still not fully understood, however Pol ϵ participates in a very early step. It has recently been reported that Pol ϵ participates in a pre-loading complex in *S. cerevisiae* consisting of Pol ϵ , Dpb11, Sld2, GINS (Muramatsu et al. 2010). This complex forms in a CDK dependent manner and in the absence of association with replication origins. It was proposed that this pre-LC functions as a carrier of GINS to the replication fork, which is required to form the active helicase known as the CMG complex (Cdc45-Mcm2-7-GINS). The Dpb2 subunit has been reported to interact with the Psf1 subunit of GINS (Takayama et al. 2003) as well as with the Orc1 and Orc4 subunits of ORC (Krogan et al. 2006). Thus the C-terminal portion of Pol ϵ may be required for cell viability due to its association with Dpb2 and subsequent formation of protein complexes required for the initiation of genomic replication.

13.4.2 Role at the Replication Fork

While the N-terminal catalytic domain of Pol ϵ was not absolutely required for cell viability in *S. cerevisiae*, several lines of evidence suggested that it plays an important role during normal DNA synthesis. Thermosensitive mutants of Pol2 in *S. cerevisiae* were defective in chromosomal replication, failed to produce chromosome-sized DNA molecules and exhibited a dumbbell shape phenotype, which is indicative of DNA replication problems in budding yeast (Araki et al. 1992; Budd and Campbell 1993). Furthermore, site-directed mutagenesis of the two conserved aspartates in Pol2 that are required for polymerase activity in all other DNA polymerases demonstrated that the polymerase activity of Pol ϵ is essential in *S. cerevisiae* (Dua et al. 1999). Pol ϵ has also been demonstrated to move together with the replication fork in *S. cerevisiae* (Aparicio et al. 1997).

The current view of the role for Pol ϵ at the replication fork emerged in part from a series of studies focused on the fidelity by which Pol ϵ and Pol δ replicate DNA. Both Pol ϵ and Pol δ have proofreading activity (3'-5' exonuclease activity) that significantly increases their fidelity during DNA synthesis (Morrison et al. 1993). Early genetic experiments in *S. cerevisiae* asked if inactivation of the proofreading activity of either Pol δ or Pol ϵ would give a bias in the mutation rates of leading and lagging strand (Shcherbakova and Pavlov 1996). A nucleotide analog, 6-N-hydroxylamine (HAP), base pairs with both T and C and can lead to G-C to A-T and A-T to G-C transitions depending on whether the HAP is in the templating strand or is the incoming nucleotide. Mutagenesis by HAP is only affected by the exonuclease proofreading activity of polymerases in correcting mis-incorporations with this analog. Using the reporter gene *URA3* downstream from the origin of replication *ARS306*, it was shown that mutagenesis by proofreading deficient Pol ϵ and Pol δ changed when the orientation of the *URA3* reporter gene was changed relative to the origin of replication. Interestingly, the frequencies of reversion events were opposite when comparing exonuclease deficient Pol ϵ and Pol δ (Shcherbakova and

Pavlov 1996). Similar studies looking at mutational spectra in the *SUP4* gene with both Pol ϵ and Pol δ exonuclease deficient mutants confirmed the results (Karthikeyan et al. 2000). While these experiments do not specifically demonstrate leading or lagging strand synthesis for either enzyme, they do suggest that Pol δ and Pol ϵ proofread opposite strands.

In vitro studies of Pol ϵ revealed that Pol ϵ is a highly accurate DNA polymerase (Shcherbakova et al. 2003b). In fact, estimates suggest that Pol ϵ has the highest fidelity among all DNA polymerases in *S. cerevisiae* (Fortune et al. 2005). Still, Pol ϵ makes errors and it was found that Pol ϵ has a unique propensity for pyrimidine mismatches, in particular T-dTTP (Shcherbakova et al. 2003b). This characteristic was later enhanced in genetic experiments suggesting that Pol ϵ participates in leading strand synthesis (Pursell et al. 2007). These experiments utilized the concept of asymmetric mutators – polymerases that had a propensity for certain mismatch combinations over others. The original idea came from the E710A mutation in the large Klenow fragment of *E. coli* pol I that showed reduced fidelity (Minnick et al. 1999) but, importantly for the experiments in question, showed a strong preference for forming A-dCTP mismatches over T-dGTP mismatches (Minnick et al. 2002). These two mismatches would both lead to A-T to G-C transitions depending on whether the A or the T were in the templating strand, thus giving a mutational marker as to which strand was being copied. The glutamate at position 710 in the Klenow fragment is likely located in the same position as the invariant tyrosine at position 645 in Pol ϵ and at position 416 in RB69 (Fig. 13.1d). Replacement of the adjacent methionine in Pol ϵ with glycine (*pol2-M644G*) results in a polymerase that still retains high levels of activity but exhibits an *in vitro* error rate for forming T-dTTP mismatches that is approximately 39-fold greater than forming A-dATP mismatches (Pursell et al. 2007). When the *URA3* reporter gene was placed in both orientations both to the right and the left of the *ARS306* origin of replication, it was observed that mutational hot spots occurred in which A-T to T-A transitions were the result of T-dTTP misincorporations by *pol2-M644G*. The data lead to the conclusion that Pol ϵ participates during leading strand synthesis at the replication fork (Pursell et al. 2007). Subsequent studies, using the same approach on Pol δ with the corresponding *pol3-L612M* mutant, showed that Pol δ primarily synthesizes DNA on the lagging strand where the reporter gene was located (Nick McElhinny et al. 2008). Recently a whole genome analysis of errors made in a *pol3-L612M* strain confirmed that Pol δ is the major lagging strand polymerase (Larrea et al. 2010). Together these experiments suggests that during normal DNA replication Pol ϵ is the leading strand polymerase and Pol δ is the lagging strand polymerase.

Other observations that support this model are that Pol δ can proofread errors made by Pol α , while Pol ϵ does not (Pavlov et al. 2006). During lagging strand DNA synthesis, Pol α is continually laying down RNA primers followed by about 20 nucleotides of DNA before the primer terminus moves from Pol α to the lagging strand DNA polymerase (see Chap. 9, this volume). Because Pol α lacks 3'–5' exonuclease activity, it is more error prone than Pol ϵ and Pol δ (Kunkel et al. 1989, 1991). The mutator activity of Pol α has been shown to act synergistically with proofreading-deficient Pol δ (Pavlov et al. 2006). In these experiments, the *pol1-L868M* active site

mutant had a limited mutator effect *in vivo* and the *pol3-exo⁻* exonuclease deficient mutant of Pol δ had an approximately seven-fold increase in mutation rates. The double mutant, however, showed an approximately 70-fold increase in mutation rate. Such synergism in mutagenesis was not observed with a proofreading deficient mutant of Pol ϵ suggesting that Pol δ is able to correct errors by Pol α on the lagging strand but that Pol ϵ is not able to correct such errors and is thus not likely to be active on the lagging strand during DNA replication.

During Okazaki fragment maturation, strand displacement is carried out by the lagging strand polymerase in tight regulation with the flap endonuclease FEN1 which removes the 5' flap of RNA/DNA (see Chap. 16). Pol δ , in contrast to Pol ϵ , functionally interacts with FEN1 and DNA ligase I during the processing of primers in the Okazaki fragments (Jin et al. 2001; Garg et al. 2004). This supports the hypothesis that Pol ϵ is not a lagging strand DNA polymerase.

13.4.3 PCNA

PCNA (proliferating cell nuclear antigen, see Chap. 15, this volume) is a large, trimeric, ring-shaped molecule that wraps around duplex DNA. PCNA is loaded onto the DNA by the activity of the clamp loading protein RFC (see Chap. 14). Many molecules interact with PCNA such that it acts as a structural platform, recruiting and maintaining other molecules on the DNA. The association of DNA polymerases with PCNA at the replication fork serves to prevent dissociation of the polymerase from the DNA. Pol ϵ and Pol δ have both been shown to be stimulated by PCNA (Burgers 1991; Lee et al. 1991). Under single hit conditions in which a polymerase that dissociates from the DNA molecule will not re-associate with another previously extended DNA molecule (Bambara et al. 1995), the processivity of Pol ϵ and Pol δ were very similar, with a less than two-fold longer processivity for Pol δ compared to Pol ϵ (Chilkova et al. 2007). Interestingly, however, under these experimental conditions Pol ϵ processivity was increased only about sixfold in the presence of PCNA and clamp loader while Pol δ processivity was increased at least 100-fold. Thus, while both polymerases are stimulated by PCNA, the effect appears to be much greater for Pol δ .

Pol ϵ appears to have a canonical PCNA-interacting protein (PIP) box motif. That is a putative PCNA binding sequence of QTSLTKFF, which fits the consensus sequence Qxxhxxaa in which 'h' is a hydrophobic residue and 'a' is an aromatic residue (Warbrick et al. 1998). Unlike other proteins known to interact with PCNA, however, the PIP box is not located at the N- or C-terminal end of the protein but is instead located in the middle of the protein. The PIP box is likely where one would expect to find it at the C-terminus of the catalytic domain, but upon the gene duplication event (as discussed earlier) the PIP box became buried in front of the second set of polymerase motifs that make up the C-terminal tail of Pol ϵ . The location of the PIP box suggests that it may no longer interact directly with PCNA. Surface plasmon resonance experiments using immobilized PCNA showed no

interaction with PCNA in solution at similar concentrations at which Pol δ showed strong interactions with PCNA (Chilkova et al. 2007) suggesting that stimulation of Pol ϵ and Pol δ by PCNA occurs by two distinct mechanisms.

It is clear that PCNA stimulates Pol ϵ *in vitro* but genetic experiments demonstrated that the putative PIP-box in Pol ϵ is not necessary for cell viability. Site directed mutagenesis of the conserved residues as well as deletion of the entire PIP box had no effect on cell growth at both 23°C and 37°C (Dua et al. 2002). Instead deletion of the entire PIP box as well as mutations of the conserved hydrophobic (Leu 1196) and aromatic (Phe 1199 and Phe 1200) residues increased the sensitivity of the resulting *S. cerevisiae* cells to the DNA damaging agent methyl methanesulfate (MMS).

Pol δ has at least two different types of interactions with PCNA. It is possible that the interaction between the PIP box and PCNA is important for the loading onto the primer termini and the second interaction supports the distance by which Pol δ replicates DNA before it dissociates from the template. In contrast, Pol ϵ only interacts with PCNA when already loaded on the primer-template. Several enzymes predicted to operate on the lagging strand of the replication fork contain PIP boxes known to interact with PCNA. Pol δ interacts with PCNA through a C-terminal PIP box in its C-subunit p66, Pol32 or Cdc27 (Bermudez et al. 2002; Ducoux et al. 2001; Gerik et al. 1998; Johansson et al. 2004), the flap endonuclease FEN1 interacts with PCNA via a C-terminal PIP box (Warbrick et al. 1997) and DNA ligase I interacts with PCNA via a PIP box in its N-terminus (Levin et al. 1997). Thus there may be distinct mechanisms of PCNA utilization between the two strands at the replication fork. Leading strand replication, as carried out by Pol ϵ , was hypothesized to be PIP box-independent while lagging strand synthesis, as carried out by Pol δ , FEN1 and DNA ligase I, was proposed to be dependent on the conserved PIP box motif for interactions with PCNA (Chilkova et al. 2007). Physical interactions between Pol ϵ and the CMG complex might explain why Pol ϵ primarily replicates the leading strand: Pol ϵ is known to interact with both GINS and Cdc45 but whether this occurs when these proteins are part of the CMG complex is not yet known.

13.4.4 Checkpoint Activation in S Phase

Inhibition of replication fork progression can lead to genomic instability and subsequent chromosomal rearrangements and translocations, which play an important role in cancer development (Lengauer et al. 1998). Replication fork inhibition or blockage can be caused by natural impediments such as DNA binding proteins, collisions with the transcription machinery and aberrant DNA structures such as those caused by nucleotide repeat sequences (reviewed in Mirkin and Mirkin 2007). Replication forks are also stalled by the presence of DNA damage such as bulky adducts (Shiotani et al. 2006), abasic sites (Boiteux and Guillet 2004), DNA-protein crosslinks (Payne et al. 2006) and interstrand crosslinks (Niedernhofer et al. 2005). Sensing of replicative stress leads to a signaling cascade culminating in the phosphorylation of Rad53 in budding yeast.

Decoupling of the helicase from the replicative polymerases is suggested to generate large amounts of single-stranded DNA that recruits RPA and initiates signaling pathways (Navadgi-Patil and Burgers 2009). During chromosome replication, the lagging strand always has a certain amount of single-stranded DNA due to the synthesis of the Okazaki fragments. In contrast, it is less likely to find large amounts of single-stranded DNA on the leading strand during normal replication. Thus the leading strand polymerase is ideally positioned to participate in the sensory mechanism for the generation of checkpoint signals. Indeed, Pol ϵ has been implicated to play a role in this function. The *pol2-12* allele in *S. cerevisiae*, with Gln 2195 converted to a stop codon at the C-terminus of Pol2, leads to a loss of S phase checkpoint function (Navas et al. 1995). Pol ϵ acts as a sensor of DNA replication progression because the *pol2-12* mutants fail to activate both the Dun1 protein kinase and transcription of *RNR3* in response to DNA damaging agents, are inviable in the presence of hydroxyurea (an inhibitor of ribonucleotide reductase (Krakoff et al. 1968)), and enter into mitosis before completion of DNA replication (Navas et al. 1995). Pol ϵ was found to act as a transducer of the DNA damage checkpoint signal only during S phase and operates in a parallel pathway to *RAD9* that transduces the signal during G1 and G2 phases (Navas et al. 1996). It is still unclear how Pol ϵ participates in the checkpoint activation.

Mrc1 is a replication fork associated protein that has been implicated to be important both during DNA replication and mediating an S phase checkpoint. Interestingly, Mrc1 interacts with the helicase component Mcm6 (Komata et al. 2009) and Pol ϵ (Lou et al. 2008) and it was suggested that Mrc1 stabilizes a hypothetical interaction between the Mcm helicase and Pol ϵ (Lou et al. 2008). Pol ϵ associates with Mrc1 via both its N-terminus and C-terminus and the association with the Pol2 C-terminus appears to be modulated by Dpb2. Phosphorylation of Mrc1 during S phase checkpoint eliminates N-terminal association but not C-terminal association (Lou et al. 2008).

Another model is proposed in which stalling of leading strand synthesis by Pol ϵ signals the Dpb11/Sld2-Mec1-Rad53 signaling cascade leading finally to cell cycle arrest. This activity appears to be dependent on Dpb4 and suggests that leading and lagging strands sense DNA damage and signal this to the cell via different pathways (Puddu et al. 2011). This is an interesting model since Pol ϵ is inhibited by single-stranded DNA, while Pol δ is less sensitive to the presence of single-stranded DNA (Chilkova et al. 2007).

13.5 Ribose vs Deoxyribose Discrimination

Most DNA polymerases have mechanisms by which they can discriminate between deoxyribonucleotide and ribonucleotide triphosphates (Brown and Suo 2011; Joyce 1997). For the B-family polymerases such as Pol ϵ , this consists of a conserved tyrosine residue (position 645 in Pol ϵ) that acts as a steric gate against the 2'OH group of the sugar in ribonucleotides (Bonnin et al. 1999; Gardner and Jack 1999; Yang et al. 2002) (Fig. 13.1d). Discrimination between nucleotide sugars, however,

is not absolute and the problem is confounded by the relative abundance of the sugar moieties for which *in vivo* NTP concentrations can be orders of magnitude higher than dNTP concentrations in yeast (Nick McElhinny et al. 2010b) and mammalian cells (Traut 1994). Primer extension assays with Pol ϵ suggest that it may incorporate approximately one NMP for every 1,250 dNMPs incorporated and that DNA synthesis by Pol ϵ is inhibited by the presence of NMPs in the template (Nick McElhinny et al. 2010b; Watt et al. 2011). Pol ϵ can incorporate ribonucleotides *in vivo*, as demonstrated by the increased presence of NMP residues detected by alkali hydrolysis in the M644G mutant and the double mutant of M644G with ribonuclease H2 knockout mutation in *S. cerevisiae* (Nick McElhinny et al. 2010a). These mutations also resulted in mutator phenotypes. These results, along with the fact that ribonuclease H2 contains a PIP box and associates with PCNA (Bubeck et al. 2011), suggests that incorporation of NMP by Pol ϵ may be a significant source of genomic instability and may be repaired by ribonuclease H2 traveling with the replication fork.

13.6 Concluding Remarks

We have in this chapter only discussed some of the properties of Pol ϵ . In addition to what has been discussed, pol ϵ mutants have been reported to affect telomere length and silencing near the telomeres. There are reports suggesting that Pol ϵ interacts with proteins that participate in sister chromatid establishment and in DNA repair pathways, and which may influence chromatin structure. Its position as an important protein during the assembly of the replisome and during leading strand synthesis suggests that Pol ϵ will interact with many processes involved in the maintenance and duplication of the genome. The low-resolution cryo-EM structure has in part offered an explanation to some of the unique properties of Pol ϵ . However, it cannot give us the molecular details on how the high fidelity during DNA synthesis is achieved or why Pol ϵ incorporates ribonucleotides relatively frequently. Both these properties imply that the structure of the active site in Pol ϵ will differ from Pol δ or other B-family polymerases. A high-resolution structure would be invaluable for our understanding of how the active site functions and also for future studies of the interactions with DNA and other proteins which have been discussed in this chapter.

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References

- Aksenova A, Volkov K, Maceluch J, Pursell ZF, Rogozin IB, Kunkel TA, Pavlov YI, Johansson E (2010) Mismatch repair-independent increase in spontaneous mutagenesis in yeast lacking non-essential subunits of DNA polymerase ϵ . *PLoS Genet* 6:e1001209

- Aparicio OM, Weinstein DM, Bell SP (1997) Components and dynamics of DNA replication complexes in *S. cerevisiae*: redistribution of MCM proteins and Cdc45p during S phase. *Cell* 91:59–69
- Araki H, Hamatake RK, Johnston LH, Sugino A (1991) *DPB2*, the gene encoding DNA polymerase II subunit B, is required for chromosome replication in *Saccharomyces cerevisiae*. *Proc Natl Acad Sci U S A* 88:4601–4605
- Araki H, Ropp PA, Johnson AL, Johnston LH, Morrison A, Sugino A (1992) DNA polymerase II, the probable homolog of mammalian DNA polymerase ϵ , replicates chromosomal DNA in the yeast *Saccharomyces cerevisiae*. *EMBO J* 11:733–740
- Aravind L, Koonin EV (1998) Phosphoesterase domains associated with DNA polymerases of diverse origins. *Nucleic Acids Res* 26:3746–3752
- Arents G, Moudrianakis EN (1993) Topography of the histone octamer surface: repeating structural motifs utilized in the docking of nucleosomal DNA. *Proc Natl Acad Sci USA* 90:10489–10493
- Asturias FJ, Cheung IK, Sabouri N, Chilkova O, Wepplo D, Johansson E (2006) Structure of *Saccharomyces cerevisiae* DNA polymerase epsilon by cryo-electron microscopy. *Nat Struct Mol Biol* 13:35–43
- Bambara RA, Fay PJ, Mallaber LM (1995) Methods of analyzing processivity. *Methods Enzymol* 262:270–280
- Baxevas AD, Arents G, Moudrianakis EN, Landsman D (1995) A variety of DNA-binding and multimeric proteins contain the histone fold motif. *Nucleic Acids Res* 23:2685–2691
- Bermudez VP, MacNeill SA, Tappin I, Hurwitz J (2002) The influence of the Cdc27 subunit on the properties of the *Schizosaccharomyces pombe* DNA polymerase δ . *J Biol Chem* 277:36853–36862
- Boiteux S, Guillet M (2004) Abasic sites in DNA: repair and biological consequences in *Saccharomyces cerevisiae*. *DNA Repair (Amst)* 3:1–12
- Bonnin A, Lazaro JM, Blanco L, Salas M (1999) A single tyrosine prevents insertion of ribonucleotides in the eukaryotic-type phi29 DNA polymerase. *J Mol Biol* 290:241–251
- Brown JA, Suo Z (2011) Unlocking the sugar “steric gate” of DNA polymerases. *Biochemistry* 50:1135–1142
- Bubeck D, Reijns MA, Graham SC, Astell KR, Jones EY, Jackson AP (2011) PCNA directs type 2 RNase H activity on DNA replication and repair substrates. *Nucleic Acids Res* 39:3652–3666
- Budd ME, Campbell JL (1993) DNA polymerases delta and epsilon are required for chromosomal replication in *Saccharomyces cerevisiae*. *Mol Cell Biol* 13:496–505
- Burgers PM (1991) *Saccharomyces cerevisiae* replication factor C. II. Formation and activity of complexes with the proliferating cell nuclear antigen and with DNA polymerases δ and epsilon. *J Biol Chem* 266:22698–22706
- Burgess SA, Walker ML, Thirumurugan K, Trinick J, Knight PJ (2004) Use of negative stain and single-particle image processing to explore dynamic properties of flexible macromolecules. *J Struct Biol* 147:247–258
- Chilkova O, Jonsson BH, Johansson E (2003) The quaternary structure of DNA polymerase ϵ from *Saccharomyces cerevisiae*. *J Biol Chem* 278:14082–14086
- Chilkova O, Stenlund P, Isoz I, Stiith CM, Grabowski P, Lundstrom EB, Burgers PM, Johansson E (2007) The eukaryotic leading and lagging strand DNA polymerases are loaded onto primer-ends via separate mechanisms but have comparable processivity in the presence of PCNA. *Nucleic Acids Res* 35:6588–6597
- Connolly BA (2009) Recognition of deaminated bases by archaeal family-B DNA polymerases. *Biochem Soc Trans* 37:65–68
- Dang W, Kagalwala MN, Bartholomew B (2007) The Dpb4 subunit of ISW2 is anchored to extra-nucleosomal DNA. *J Biol Chem* 282:19418–19425
- Delarue M, Poch O, Tordo N, Moras D, Argos P (1990) An attempt to unify the structure of polymerases. *Protein Eng* 3:461–467
- Drury LS, Perkins G, Diffley JF (1997) The Cdc4/34/53 pathway targets Cdc6p for proteolysis in budding yeast. *EMBO J* 16:5966–5976

- Dua R, Levy DL, Campbell JL (1998) Role of the putative zinc finger domain of *Saccharomyces cerevisiae* DNA polymerase ϵ in DNA replication and the S/M checkpoint pathway. *J Biol Chem* 273:30046–30055
- Dua R, Levy DL, Campbell JL (1999) Analysis of the essential functions of the C-terminal protein/protein interaction domain of *Saccharomyces cerevisiae* pol ϵ and its unexpected ability to support growth in the absence of the DNA polymerase domain. *J Biol Chem* 274:22283–22288
- Dua R, Levy DL, Li CM, Snow PM, Campbell JL (2002) *In vivo* reconstitution of *Saccharomyces cerevisiae* DNA polymerase ϵ in insect cells. Purification and characterization. *J Biol Chem* 277:7889–7896
- Ducoux M, Urbach S, Baldacci G, Hubscher U, Koundrioukoff S, Christensen J, Hughes P (2001) Mediation of proliferating cell nuclear antigen (PCNA)-dependent DNA replication through a conserved p21^{Cip1}-like PCNA-binding motif present in the third subunit of human DNA polymerase δ . *J Biol Chem* 276:49258–49266
- Feng W, D'Urso G (2001) *Schizosaccharomyces pombe* cells lacking the amino-terminal catalytic domains of DNA polymerase ϵ are viable but require the DNA damage checkpoint control. *Mol Cell Biol* 21:4495–4504
- Feng W, Rodriguez-Menocal L, Tolun G, D'Urso G (2003) *Schizosaccharomyces pombe* Dpb2 binds to origin DNA early in S phase and is required for chromosomal DNA replication. *Mol Biol Cell* 14:3427–3436
- Fogg MJ, Pearl LH, Connolly BA (2002) Structural basis for uracil recognition by archaeal family B DNA polymerases. *Nat Struct Biol* 9:922–927
- Fortune JM, Pavlov YI, Welch CM, Johansson E, Burgers PM, Kunkel TA (2005) *Saccharomyces cerevisiae* DNA polymerase δ : high fidelity for base substitutions but lower fidelity for single- and multi-base deletions. *J Biol Chem* 280:29980–29987
- Franklin MC, Wang J, Steitz TA (2001) Structure of the replicating complex of a pol α family DNA polymerase. *Cell* 105:657–667
- Gardner AF, Jack WE (1999) Determinants of nucleotide sugar recognition in an archaeon DNA polymerase. *Nucleic Acids Res* 27:2545–2553
- Garg P, Stith CM, Sabouri N, Johansson E, Burgers PM (2004) Idling by DNA polymerase δ maintains a ligatable nick during lagging-strand DNA replication. *Genes Dev* 18:2764–2773
- Gerik KJ, Li X, Pautz A, Burgers PM (1998) Characterization of the two small subunits of *Saccharomyces cerevisiae* DNA polymerase δ . *J Biol Chem* 273:19747–19755
- Hamatake RK, Hasegawa H, Clark AB, Bebenek K, Kunkel TA, Sugino A (1990) Purification and characterization of DNA polymerase II from the yeast *Saccharomyces cerevisiae*. Identification of the catalytic core and a possible holoenzyme form of the enzyme. *J Biol Chem* 265:4072–4083
- Harp JM, Hanson BL, Timm DE, Bunick GJ (2000) Asymmetries in the nucleosome core particle at 2.5 Å resolution. *Acta Crystallogr D Biol Crystallogr* 56:1513–1534
- Hartlepp KF, Fernandez-Tornero C, Eberharter A, Grune T, Muller CW, Becker PB (2005) The histone fold subunits of *Drosophila* CHRAC facilitate nucleosome sliding through dynamic DNA interactions. *Mol Cell Biol* 25:9886–9896
- Jaszczur M, Flis K, Rudzka J, Kraszewska J, Budd ME, Polaczek P, Campbell JL, Jonczyk P, Fijalkowska IJ (2008) Dpb2p, a noncatalytic subunit of DNA polymerase ϵ , contributes to the fidelity of DNA replication in *Saccharomyces cerevisiae*. *Genetics* 178:633–647
- Jaszczur M, Rudzka J, Kraszewska J, Flis K, Polaczek P, Campbell JL, Fijalkowska IJ, Jonczyk P (2009) Defective interaction between Pol2p and Dpb2p, subunits of DNA polymerase ϵ , contributes to a mutator phenotype in *Saccharomyces cerevisiae*. *Mutat Res* 669:27–35
- Jin YH, Obert R, Burgers PM, Kunkel TA, Resnick MA, Gordenin DA (2001) The 3'→5' exonuclease of DNA polymerase δ can substitute for the 5' flap endonuclease Rad27/Fen1 in processing Okazaki fragments and preventing genome instability. *Proc Natl Acad Sci USA* 98:5122–5127
- Johansson E, Garg P, Burgers PM (2004) The Pol32 subunit of DNA polymerase δ contains separable domains for processive replication and proliferating cell nuclear antigen (PCNA) binding. *J Biol Chem* 279:1907–1915
- Joyce CM (1997) Choosing the right sugar: how polymerases select a nucleotide substrate. *Proc Natl Acad Sci U S A* 94:1619–1622

- Karthikeyan R, Vonarx EJ, Straffon AF, Simon M, Faye G, Kunz BA (2000) Evidence from mutational specificity studies that yeast DNA polymerases δ and ϵ replicate different DNA strands at an intracellular replication fork. *J Mol Biol* 299:405–419
- Kesti T, Flick K, Keranen S, Syvaoja JE, Wittenberg C (1999) DNA polymerase ϵ catalytic domains are dispensable for DNA replication, DNA repair, and cell viability. *Mol Cell* 3:679–685
- Kesti T, McDonald WH, Yates JR 3rd, Wittenberg C (2004) Cell cycle-dependent phosphorylation of the DNA polymerase ϵ subunit, Dpb2, by the Cdc28 cyclin-dependent protein kinase. *J Biol Chem* 279:14245–14255
- Kokoska RJ, McCulloch SD, Kunkel TA (2003) The efficiency and specificity of apurinic/apryrimidinic site bypass by human DNA polymerase η and *Sulfolobus solfataricus* Dpo4. *J Biol Chem* 278:50537–50545
- Komata M, Bando M, Araki H, Shirahige K (2009) The direct binding of Mrc1, a checkpoint mediator, to Mcm6, a replication helicase, is essential for the replication checkpoint against methyl methanesulfonate-induced stress. *Mol Cell Biol* 29:5008–5019
- Krakoff IH, Brown NC, Reichard P (1968) Inhibition of ribonucleoside diphosphate reductase by hydroxyurea. *Cancer Res* 28:1559–1565
- Krogan NJ, Cagney G, Yu H, Zhong G, Guo X, Ignatchenko A, Li J, Pu S, Datta N, Tikuisis AP, Punna T, Peregrin-Alvarez JM, Shales M, Zhang X, Davey M, Robinson MD, Paccanaro A, Bray JE, Sheung A, Beattie B, Richards DP, Canadien V, Lalev A, Mena F, Wong P, Starostine A, Canete MM, Vlasblom J, Wu S, Orsi C, Collins SR, Chandran S, Haw R, Rilstone JJ, Gandi K, Thompson NJ, Musso G, St Onge P, Ghanny S, Lam MH, Butland G, Altaf-Ul AM, Kanaya S, Shilatifard A, O'Shea E, Weissman JS, Ingles CJ, Hughes TR, Parkinson J, Gerstein M, Wodak SJ, Emili A, Greenblatt JF (2006) Global landscape of protein complexes in the yeast *Saccharomyces cerevisiae*. *Nature* 440:637–643
- Kunkel TA, Hamatake RK, Motto-Fox J, Fitzgerald MP, Sugino A (1989) Fidelity of DNA polymerase I and the DNA polymerase I-DNA primase complex from *Saccharomyces cerevisiae*. *Mol Cell Biol* 9:4447–4458
- Kunkel TA, Roberts JD, Sugino A (1991) The fidelity of DNA synthesis by the catalytic subunit of yeast DNA polymerase α alone and with accessory proteins. *Mutat Res* 250:175–182
- Larrea AA, Lujan SA, Nick McElhinny SA, Mieczkowski PA, Resnick MA, Gordenin DA, Kunkel TA (2010) Genome-wide model for the normal eukaryotic DNA replication fork. *Proc Natl Acad Sci U S A* 107:17674–17679
- Lee SH, Pan ZQ, Kwong AD, Burgers PM, Hurwitz J (1991) Synthesis of DNA by DNA polymerase ϵ *in vitro*. *J Biol Chem* 266:22707–22717
- Lengauer C, Kinzler KW, Vogelstein B (1998) Genetic instabilities in human cancers. *Nature* 396:643–649
- Levin DS, Bai W, Yao N, O'Donnell M, Tomkinson AE (1997) An interaction between DNA ligase I and proliferating cell nuclear antigen: implications for Okazaki fragment synthesis and joining. *Proc Natl Acad Sci U S A* 94:12863–12868
- Lou H, Komata M, Katou Y, Guan Z, Reis CC, Budd M, Shirahige K, Campbell JL (2008) Mrc1 and DNA polymerase ϵ function together in linking DNA replication and the S phase checkpoint. *Mol Cell* 32:106–117
- McCulloch SD, Kunkel TA (2008) The fidelity of DNA synthesis by eukaryotic replicative and translesion synthesis polymerases. *Cell Res* 18:148–161
- McCulloch SD, Kokoska RJ, Chilkova O, Welch CM, Johansson E, Burgers PM, Kunkel TA (2004) Enzymatic switching for efficient and accurate translesion DNA replication. *Nucleic Acids Res* 32:4665–4675
- Minnick DT, Bebenek K, Osheroff WP, Turner RM Jr, Astatke M, Liu L, Kunkel TA, Joyce CM (1999) Side chains that influence fidelity at the polymerase active site of *Escherichia coli* DNA polymerase I (Klenow fragment). *J Biol Chem* 274:3067–3075
- Minnick DT, Liu L, Grindley ND, Kunkel TA, Joyce CM (2002) Discrimination against purine-pyrimidine mispairs in the polymerase active site of DNA polymerase I: a structural explanation. *Proc Natl Acad Sci USA* 99:1194–1199
- Mirkin EV, Mirkin SM (2007) Replication fork stalling at natural impediments. *Microbiol Mol Biol Rev* 71:13–35

- Morrison A, Bell JB, Kunkel TA, Sugino A (1991) Eukaryotic DNA polymerase amino acid sequence required for 3'→5' exonuclease activity. *Proc Natl Acad Sci U S A* 88:9473–9477
- Morrison A, Johnson AL, Johnston LH, Sugino A (1993) Pathway correcting DNA replication errors in *Saccharomyces cerevisiae*. *EMBO J* 12:1467–1473
- Muramatsu S, Hirai K, Tak YS, Kamimura Y, Araki H (2010) CDK-dependent complex formation between replication proteins Dpb11, Sld2, Pol ϵ , and GINS in budding yeast. *Genes Dev* 24:602–612
- Navadgi-Patil VM, Burgers PM (2009) A tale of two tails: activation of DNA damage checkpoint kinase Mec1/ATR by the 9-1-1 clamp and by Dpb11/TopBP1. *DNA Repair (Amst)* 8:996–1003
- Navas TA, Zhou Z, Elledge SJ (1995) DNA polymerase ϵ links the DNA replication machinery to the S phase checkpoint. *Cell* 80:29–39
- Navas TA, Sanchez Y, Elledge SJ (1996) RAD9 and DNA polymerase ϵ form parallel sensory branches for transducing the DNA damage checkpoint signal in *Saccharomyces cerevisiae*. *Genes Dev* 10:2632–2643
- Nick McElhinny SA, Gordenin DA, Stith CM, Burgers PM, Kunkel TA (2008) Division of labor at the eukaryotic replication fork. *Mol Cell* 30:137–144
- Nick McElhinny SA, Kumar D, Clark AB, Watt DL, Watts BE, Lundstrom EB, Johansson E, Chabes A, Kunkel TA (2010a) Genome instability due to ribonucleotide incorporation into DNA. *Nat Chem Biol* 6:774–781
- Nick McElhinny SA, Watts BE, Kumar D, Watt DL, Lundstrom EB, Burgers PM, Johansson E, Chabes A, Kunkel TA (2010b) Abundant ribonucleotide incorporation into DNA by yeast replicative polymerases. *Proc Natl Acad Sci U S A* 107:4949–4954
- Niedernhofer LJ, Lalai AS, Hoeijmakers JH (2005) Fanconi anemia (cross)linked to DNA repair. *Cell* 123:1191–1198
- Nuutinen T, Tossavainen H, Fredriksson K, Pirila P, Permi P, Pospiech H, Syvaoja JE (2008) The solution structure of the amino-terminal domain of human DNA polymerase ϵ subunit B is homologous to C-domains of AAA+ proteins. *Nucleic Acids Res* 36:5102–5110
- Ohya T, Maki S, Kawasaki Y, Sugino A (2000) Structure and function of the fourth subunit (Dpb4p) of DNA polymerase ϵ in *Saccharomyces cerevisiae*. *Nucleic Acids Res* 28:3846–3852
- Ohya T, Kawasaki Y, Hiraga S, Kanbara S, Nakajo K, Nakashima N, Suzuki A, Sugino A (2002) The DNA polymerase domain of pol(ϵ) is required for rapid, efficient, and highly accurate chromosomal DNA replication, telomere length maintenance, and normal cell senescence in *Saccharomyces cerevisiae*. *J Biol Chem* 277:28099–28108
- Pavlov YI, Frahm C, Nick McElhinny SA, Niimi A, Suzuki M, Kunkel TA (2006) Evidence that errors made by DNA polymerase α are corrected by DNA polymerase δ . *Curr Biol* 16:202–207
- Payne BT, van Knippenberg IC, Bell H, Filipe SR, Sherratt DJ, McGlynn P (2006) Replication fork blockage by transcription factor-DNA complexes in *Escherichia coli*. *Nucleic Acids Res* 34:5194–5202
- Penczek PA, Grassucci RA, Frank J (1994) The ribosome at improved resolution: new techniques for merging and orientation refinement in 3D cryo-electron microscopy of biological particles. *Ultramicroscopy* 53:251–270
- Puddu F, Piergiorganni G, Plevani P, Muzi-Falconi M (2011) Sensing of replication stress and Mec1 activation act through two independent pathways involving the 9-1-1 complex and DNA polymerase ϵ . *PLoS Genet* 7:e1002022
- Pursell ZF, Kunkel TA (2008) DNA polymerase epsilon: a polymerase of unusual size (and complexity). *Prog Nucleic Acid Res Mol Biol* 82:101–145
- Pursell ZF, Isov I, Lundstrom EB, Johansson E, Kunkel TA (2007) Yeast DNA polymerase ϵ participates in leading-strand DNA replication. *Science* 317:127–130
- Randell JC, Bowers JL, Rodriguez HK, Bell SP (2006) Sequential ATP hydrolysis by Cdc6 and ORC directs loading of the Mcm2-7 helicase. *Mol Cell* 21:29–39
- Rogozin IB, Makarova KS, Pavlov YI, Koonin EV (2008) A highly conserved family of inactivated archaeal B family DNA polymerases. *Biol Direct* 3:32

- Shamoo Y, Steitz TA (1999) Building a replisome from interacting pieces: sliding clamp complexed to a peptide from DNA polymerase and a polymerase editing complex. *Cell* 99:155–166
- Shcherbakova PV, Pavlov YI (1996) 3'→5' Exonucleases of DNA polymerases ϵ and δ correct base analog induced DNA replication errors on opposite DNA strands in *Saccharomyces cerevisiae*. *Genetics* 142:717–726
- Shcherbakova PV, Bebenek K, Kunkel TA (2003a) Functions of eukaryotic DNA polymerases. *Sci Aging Knowledge Environ* 2003:RE3
- Shcherbakova PV, Pavlov YI, Chilkova O, Rogozin IB, Johansson E, Kunkel TA (2003b) Unique error signature of the four-subunit yeast DNA polymerase ϵ . *J Biol Chem* 278:43770–43780
- Shikata K, Sasa-Masuda T, Okuno Y, Waga S, Sugino A (2006) The DNA polymerase activity of Pol ϵ holoenzyme is required for rapid and efficient chromosomal DNA replication in *Xenopus* egg extracts. *BMC Biochem* 7:21
- Shiotani B, Kobayashi M, Watanabe M, Yamamoto K, Sugimura T, Wakabayashi K (2006) Involvement of the ATR- and ATM-dependent checkpoint responses in cell cycle arrest evoked by p18INK4. *Mol Cancer Res* 4:125–133
- Steitz TA (1993) DNA- and RNA-dependent DNA polymerases. *Curr Opin Struct Biol* 3:31–38
- Stinchcomb DT, Struhl K, Davis RW (1979) Isolation and characterisation of a yeast chromosomal replicator. *Nature* 282:39–43
- Stocki SA, Nonay RL, Reha-Krantz LJ (1995) Dynamics of bacteriophage T4 DNA polymerase function: identification of amino acid residues that affect switching between polymerase and 3'→5' exonuclease activities. *J Mol Biol* 254:15–28
- Tahirov TH, Makarova KS, Rogozin IB, Pavlov YI, Koonin EV (2009) Evolution of DNA polymerases: an inactivated polymerase-exonuclease module in Pol ϵ and a chimeric origin of eukaryotic polymerases from two classes of archaeal ancestors. *Biol Direct* 4:11
- Takayama Y, Kamimura Y, Okawa M, Muramatsu S, Sugino A, Araki H (2003) GINS, a novel multiprotein complex required for chromosomal DNA replication in budding yeast. *Genes Dev* 17:1153–1165
- Traut TW (1994) Physiological concentrations of purines and pyrimidines. *Mol Cell Biochem* 140:1–22
- Tsubota T, Maki S, Kubota H, Sugino A, Maki H (2003) Double-stranded DNA binding properties of *Saccharomyces cerevisiae* DNA polymerase ϵ and of the Dpb3p-Dpb4p subassembly. *Genes Cells* 8:873–888
- Tsubota T, Tajima R, Ode K, Kubota H, Fukuhara N, Kawabata T, Maki S, Maki H (2006) Double-stranded DNA binding, an unusual property of DNA polymerase ϵ , promotes epigenetic silencing in *Saccharomyces cerevisiae*. *J Biol Chem* 281:32898–32908
- Warbrick E, Lane DP, Glover DM, Cox LS (1997) Homologous regions of Fen1 and p21Cip1 compete for binding to the same site on PCNA: a potential mechanism to co-ordinate DNA replication and repair. *Oncogene* 14:2313–2321
- Warbrick E, Heatherington W, Lane DP, Glover DM (1998) PCNA binding proteins in *Drosophila melanogaster*: the analysis of a conserved PCNA binding domain. *Nucleic Acids Res* 26:3925–3932
- Wardle J, Burgers PM, Cann IK, Darley K, Heslop P, Johansson E, Lin LJ, McGlynn P, Sanvoisin J, Stith CM, Connolly BA (2008) Uracil recognition by replicative DNA polymerases is limited to the archaea, not occurring with bacteria and eukarya. *Nucleic Acids Res* 36:705–711
- Watt DL, Johansson E, Burgers PM, Kunkel TA (2011) Replication of ribonucleotide-containing DNA templates by yeast replicative polymerases. *DNA Repair (Amst)* 10:897–902
- Wu P, Nossal N, Benkovic SJ (1998) Kinetic characterization of a bacteriophage T4 antimutator DNA polymerase. *Biochemistry* 37:14748–14755
- Yang G, Lin T, Karam J, Konigsberg WH (1999) Steady-state kinetic characterization of RB69 DNA polymerase mutants that affect dNTP incorporation. *Biochemistry* 38:8094–8101
- Yang G, Franklin M, Li J, Lin TC, Konigsberg W (2002) A conserved Tyr residue is required for sugar selectivity in a Pol α DNA polymerase. *Biochemistry* 41:10256–10261