Published in final edited form as:
Prog Nucleic Acid Res Mol Biol. 2008 ; 82: 101–145. doi:10.1016/S0079-6603(08)00004-4.

DNA Polymerase ε: A Polymerase Of Unusual Size (and Complexity)

Zachary F. Pursell and **Thomas A. Kunkel***
Laboratory of Molecular Genetics and Laboratory of Structural Biology National Institute of Environmental Health Sciences, National Institutes of Health, Department of Health and Human Services, Research Triangle Park, NC 27709

A. Introduction

Shortly after Arthur Kornberg and colleagues identified the first DNA polymerase in the mid-twentieth century (1), the first eukaryotic DNA polymerase was discovered (2), and was eventually named DNA Pol α. Pol α was initially believed to be the sole polymerase responsible for eukaryotic DNA replication, but that view changed two decades later with the discovery of a second replicative polymerase, Pol δ (3). Although the catalytic subunit of Pol δ is 125 kDa, soon after its discovery even larger polymerases were purified that had somewhat similar properties and were therefore variously called Pol δII, Pol δ*, and big Pol δ (4–6). However, biochemical studies eventually led to the realization that these larger enzyme forms were actually a distinct polymerase, Pol ε (7). Although much of the early work on Pol ε centered on its initially defined role in mammalian DNA repair, by the early 1990's the gene encoding Pol ε was cloned (8,9), making possible the many genetic and biochemical studies that are the subject of this review. From these studies, we now know that Pol ε is involved in several processes that are central to maintaining the stability of the eukaryotic nuclear genome (Fig. 1). These include DNA replication, repair of DNA damage, control of cell cycle progression, chromatin remodeling and epigenetic regulation of the stable transfer of information from mother to daughter cells. Here we consider the evidence supporting these many functions for Pol ε, and in so doing point out that a great deal remains to be learned about this large and complex polymerase.

B. Pol ε Structure

1. The Catalytic Subunit

DNA polymerase ε is a member of the B family of DNA polymerases that share sequence homology with the catalytic subunit of bacterial Pol II, the product of the *E. coli polB* gene. The open reading frame for the *POLE1* gene encoding the Pol ε catalytic subunit (Fig. 2A) is among the longest of the many known eukaryotic polymerases (10), rivaled only by those of its B family sibling Pol ζ and the A family enzyme, Pol θ. The catalytic subunits of human (Fig. 2A) and yeast Pol ε contain 2286 and 2222 amino acids, respectively. The 140 kDa N-terminal half of the protein is fairly well conserved across different species, with 63% sequence identity shared between the yeast and human enzymes (9). This conservation reflects the fact that the amino terminal residues of Pol ε harbor the polymerase and exonuclease activities.

The X ray crystal structure of Pol ε has not yet been reported. However, based on homology to two B family siblings for which X ray crystal structures are available (RB69 Pol (11) and

*To whom correspondence should be addressed. Tel: +1 919-541-2644; Fax: +1 919-541-7613; kunkel@niehs.nih.gov.

ϕ29 Pol (12)), the polymerase active site is within the palm of a polymerase domain comprised of the palm, fingers and thumb sub-domains (colored purple, blue and green, respectively, for RB69 Pol in Fig. 2B) that are characteristic of all polymerases regardless of family (13). The structural framework for catalysis of the polymerization reaction is comprised of highly conserved Motifs A, B and C (Fig. 2C/D) that are characteristic of the "right-handed" polymerases, e.g., those in families A, B and Y. Within this framework are three carboxylates thought to coordinate two metal ions (blue spheres in Fig. 2B) required for catalysis, two of which are in the invariant DTD sequence in motif C and a third in motif A (black boxes in the alignments in Fig. 2C).

The amino terminus of Pol ε also includes residues critical for its intrinsic 3´ exonuclease activity (Fig. 2A). This activity is contained in a domain (colored red in the structure of RB69 Pol in Fig. 2B) that is physically separated from the polymerase domain by many angstroms (Fig. 2B). The exonuclease active site is comprised of amino acids in conserved motifs designated Exo I, II and III (Fig. 2A/E), again including three catalytic carboxylates. While a similar domain is present within all B family polymerases, the catalytic carboxylates are not present in some other B family members, including eukaryotic Pols α and ζ. These latter two polymerases naturally lack intrinsic exonuclease activity, such that they cannot proofread replication errors and synthesize DNA less accurately than does Pol ε, or Pol δ, which also has an intrinsic 3´ exonuclease activity.

The 120 kDa C-terminal half of the catalytic subunit of Pol ε does not harbor a known catalytic activity but nonetheless shares 25% sequence homology between yeast and humans. The last one hundred residues at the C-terminus of yeast Pol ε contain two C4 Zinc-fingers and a spacer region that contains an essential function of Pol ε (see below), and this C-terminal half also mediates interactions with the smaller subunits of the holoenzyme (14,15). Also present is a putative PCNA interaction motif that is not necessary for normal DNA replication, but which when mutated leads to increased sensitivity to MMS (16).

2. Pol ε Holoenzyme

In all species studied to date, the Pol ε holoenzyme is comprised of four subunits (Fig. 3A/B and Table 1). The second subunit of Pol ε is Dpb2 in budding yeast and p59 in humans, the latter based on its predicted molecular weight. This subunit has no known catalytic activity, but it interacts with the catalytic subunit so tightly that it dissociates only under denaturing conditions (5). The absence of Dpb2 does not reduce Pol ε catalytic activity but does reduce Pol ε stability during purification (9), leading to the idea that it has an important role in stabilizing the catalytic subunit. Dpb2 is essential in yeast. Moreover, it is phosphorylated in S phase in a Cdc28-dependent manner, suggesting a potential regulatory role (17). Based on extensive yeast two-hybrid analysis, Dpb2 interaction with the C-terminus of Pol2 was disrupted with Pol2 mutants that disrupt C-terminus self-interaction in the yeast two-hybrid assay and disrupt normal replication *in vivo*, indicating that the stabilizing effects of Dpb2 on the holoenzyme may allow for an active, possibly dimeric form of Pol ε (14). This possibility is somewhat tempered by the extensive biochemical characterization, including analytical ultracentrifugation and sedimentation analysis of the native holoenzyme purified from yeast, showing a 1:1:1:1 stoichiometry for each of the four subunits (18). Additionally, it is likely the Pol2-Dpb2 interaction itself, and therefore likely the presence of all four subunits, that enables normal replication since mutant alleles of Dpb2 that disrupt this interaction show reduced survival (19). These Dpb2 mutant alleles are also mutators, making errors during replication, though it is unclear if this is due to affecting holoenzyme stability, processivity, or some other process (19). Both Dpb2 and its human homolog, p59, contain consensus PCNA interaction motifs, though only the human protein has been shown to physically interact with PCNA (20).

Like Dpb2, the two smallest subunits of human Pol ε, p12 and p17, also lack catalytic activity and also physically interact with the C-terminal half of the catalytic subunit. They also interact with each other through histone-fold motifs similar to those found in histone H2A and H2B (15). Yeast strains with deletions in Dpb3 or Dpb4, the yeast homologs of human p12 and p17, are viable (21,22). Thus neither subunit is essential for growth or chromosomal replication. However, *dpb3Δ* strains have slightly elevated spontaneous mutation rates (21), indicating that Dpb3 is important for genome stability and may possibly modulate the fidelity of DNA synthesis conducted by Pol ε *in vivo*. While this phenotype could result from reduced fidelity during replication, Dpb3 and Dpb4 both have roles outside of normal DNA replication, including chromatin remodeling and DNA transactions at the ribosomal DNA repeats (see below).

In an exciting recent development (23), the structures of the 4-subunit yeast Pol ε holoenzyme (Fig. 3C) and of the 2-subunit Pol2-Dpb2 enzyme have been solved by cryo-EM. These structures suggest that the three accessory subunits are connected to the catalytic subunit in such a manner that they contact the double-stranded DNA duplex upstream of the polymerase active site (Fig. 3C), possibly reducing polymerase dissociation and increasing processivity. However this linkage is flexible, indicating that the multi-subunit tail may adopt different conformations, perhaps making additional contacts with factors involved in replication or checkpoint activation. A groove is present that is large enough to accommodate approximately 40 nucleotides of double-stranded DNA upstream of the primer terminus. This is the same length of dsDNA that maximizes the intrinsic processivity of Pol ε when copying long primer-templates *in vitro* (23). The position of Dpb2 in the structure suggests the potential to interact with PCNA upstream of the active site, which is likely where RB69 Pol interacts with its sliding clamp (24).

C. Physical and Functional Interactions of Pol ε

In addition to interactions among the four subunits of the holoenzyme, Pol ε has also been shown to interact with other proteins (Table 2). One interacting partner is yeast Dpb11 (25), known as TopBP1 in humans and variously known as Rad4, Cut5, and Mus101 in other species. Dpb11 is a BRCT repeat-containing protein initially identified as a multicopy suppressor of temperature sensitive mutants in the C-terminus of Pol2 and of mutants in Dpb2 (26), that is loaded onto origins after pre-replication complex formation, and is required for loading Pol α and Pol ε at origins (25). Dpb11 interacts with phosphorylated Sld2 and Sld3, an interaction that is necessary to initiate replication (27,28). Dpb11 interacts genetically with components of the four-subunit GINS complex that is important in replication initiation (29,30) and fork progression (31). In addition to Dpb11, the GCM complex contains Cdc45 and a heterohexamer of MCM, contains ATP-dependent helicase activity and may be the replicative helicase (32).

TopBP1, the human homolog of Dpb11, interacts with the full-length Pol ε catalytic subunit and a 180 kDa variant of pol ε in human cells that over-express TopBP1. The functional significance of this interaction has not been explored (33). TopBP1 deficiency is not lethal in human cells but does perturb cell cycle progression and leads to genome instability due to DNA strand breaks accumulated in S phase (34). Human Pol ε is part of a complex with RNA Pol II that increases transcription activation (35). This complex contains a number of repair factors. The Pol ε interaction was later shown to be with the hyperphosphorylated elongation form of RNA polII, and occurred throughout the cell cycle (36), possibly pointing to a link between transcription and DNA repair synthesis.

Mdm2 is an oncoprotein that is up regulated in many human tumors (37) and is a major negative regulator of p53, acting as an E3 ubiquitin ligase and targeting p53 for degradation

(38). An N-terminal 166 amino acid region of Mdm2 physically interacts with the C-terminal, non-catalytic half of Pol ε (39) and to also stimulate Pol ε activity (40). It was suggested that Mdm2, and perhaps other factors, may modulate Pol ε functions in response to changing conditions. For example, binding of Mdm2 may displace Pol ε-bound factors in response to stress, thus reconfiguring Pol ε to perform a role in DNA repair and/or checkpoint control. Pol ε interactions with other proteins (Table 2) are discussed below in relationship to the many functions proposed for Pol ε (Fig. 1). Given the large size of the essential non-catalytic portion of the Pol ε catalytic subunit (Fig. 2A), the fact that the holoenzyme contains four subunits, and has been understudied due to past challenges in obtaining and working with Pol ε holoenzyme, the number of interacting partners can be expected to increase in the future, especially since some interactions may be condition-specific and/or transient.

D. Biochemical Properties of Pol ε

1. Polymerization

Pol ε catalyzes DNA template-dependent DNA synthesis by a phosphoryl transfer reaction involving nucleophilic attack by the 3′ hydroxyl of the primer terminus on the α-phosphate of the incoming deoxynucleoside triphosphate (dNTP). The products of this reaction are pyrophosphate and a DNA chain increased in length by one nucleotide. The catalytic mechanism is conserved among DNA polymerases (41). It begins with binding of a primer-template to the polymerase. The primer terminus is bound at the polymerase active site, which is largely comprised of the A, B and C sequence motifs mentioned above (Fig. 2C), which harbor the three carboxylate residues that coordinate two divalent metal ions, usually Mg^{2+}. Binding of a correct dNTP results in conformational changes in both the polymerase and the DNA. While the specific nature of these changes depends on the polymerase (reviewed in (42)), the dNTP-induced conformational changes result in assembly of an active site (Fig. 4) with geometry appropriate for the phosphoryl transfer reaction, which proceeds via in-line displacement and results in inversion of the stereochemical configuration of the α-phosphorous atom, as structurally observed for the family X member Pol λ (43). Pyrophosphate is released and translocation of the polymerase allows the newly incorporated base pair to serve as the primer terminus for the next cycle of catalysis.

Pol ε usually translocates following nucleotide incorporation without dissociation from DNA, i.e., it synthesizes DNA processively. Indeed, Pol ε was initially described as a larger variant of Pol δ (4,5) that was highly processive (>5 kb) in the absence of PCNA (6). Later work demonstrated that Pol ε processivity can in fact be stimulated by PCNA (44), but not to the extent that PCNA stimulates Pol δ (7). Another early biochemical distinction between these two polymerases is that poly(dA)•oligo(dT) is a preferred substrate for Pol ε, while Pol δ prefers an alternating poly(dA-dT) substrate (7,45). Interestingly, >100 mM KCl and NaCl strongly inhibits Pol ε synthesis (44,46), while potassium glutamate actually slightly stimulates Pol ε activity (44). Glutamate is the physiologically relevant anion in bacteria (47) and is less disruptive to *E. coli* DNA polymerase III complex stability than is chloride (48). A recent study provides evidence that in the presence of PCNA the processivities of Pol ε and Pol δ are actually quite similar (49). Under reaction conditions where DNA synthesis results from a single polymerase-DNA binding event, the processivities of both pol ε and pol δ are low (< 600 nt). This is likely due to more efficient loading of Pol δ on the RFC-PCNA-containing primer terminus via its much stronger interactions with PCNA. In the absence of PCNA, Pol ε is remarkably only able to synthesize up to ~50nt in one binding event. These assays were performed in sodium acetate, and while yeast Pol ε holoenzyme stability was enhanced in sodium acetate (18), its optimal concentration for Pol ε activity has not been extensively investigated.

2. Exonuclease

Pol ε and Pol δ are the only two eukaryotic nuclear DNA polymerases with an intrinsic 3'–5' exonucleolytic activity with which to excise primer terminal nucleotides (10). This activity is especially useful for proofreading of errors made by these polymerases. Proofreading of polymerization errors occurs when the presence of a mismatch at or within a few nucleotides upstream of the primer terminus in the polymerase active site slows further polymerization. This increases the time available for the primer terminus to fray, generating a single stranded primer that can move to the exonuclease active site for excision of the nascent error. The exonuclease active site is thought to contain two divalent metal ions coordinated by the carboxylate residues in Exo motifs I and III. These metals catalyze an in-line displacement reaction similar to that described above for polymerization, thereby liberating a dNMP from the primer terminus. The exonuclease activity of Pol ε is robust, such that it substantially increases replication fidelity (see below), and can also excise correctly paired bases. The latter may occur during bypass of lesions in DNA (50) and possibly during DNA mismatch repair, where genetic studies have implicated the exonuclease activity of Pol ε in excision of the nascent strand containing the mismatch (51).

3. DNA Binding

An additional biochemical property of Pol ε that likely has a direct bearing on in vivo function is its ability to bind dsDNA through its small subunits (52) (Fig. 3 and see below). Pol ε can also bind ssDNA, the presence of which actually inhibits Pol ε activity in the absence of RPA (52). While ssDNA binding is mediated through the N-terminal catalytic half, the four-subunit holoenzyme dissociates from primer-template DNA almost two orders of magnitude faster in the presence of ssDNA than does the catalytic half (53). This ssDNA binding is proposed to play an integral role in the Pol ε checkpoint function.

4. Fidelity

Initial measurements of the fidelity of DNA synthesis by Pol ε were made with enzyme purified from calf thymus tissue at a time when Pol ε was still referred to as Pol δII (54,55). More recent measurements have been performed with recombinant yeast Pol ε holoenzyme, as well as with a mutant holoenzyme that lacks 3´ exonuclease activity due to the alanine replacements for the catalytic carboxylates in the exonuclease active site (56,57). These studies all reveal that, as expected for a polymerase that has a major role in DNA replication, mammalian and yeast Pol ε synthesize DNA with high fidelity. This high fidelity sharply contrasts with the much lower fidelity of other polymerases, e.g., the translesion synthesis enzyme Pol η and the DNA repair polymerase yeast Pol IV (Table 3). The major contribution to the fidelity of DNA synthesis by Pol ε is the high nucleotide selectivity of the polymerase itself, as illustrated by the low rates at which exonuclease-deficient Pol ε generates the two most common polymerization errors, single base substitution and single base deletion errors (Table 3). Such high fidelity is thought to partly depend on rigorous selection for correct Watson-Crick base pairing geometry in the binding pocket for the nascent base pair (see (58–63) for recent reviews on fidelity mechanisms).

An additional contribution to Pol ε fidelity comes from proofreading by the 3´ exonuclease. This is revealed by the lower error rates for the wild type holoenzyme as compared to its exonuclease-deficient derivative (Table 3). The contribution of proofreading to fidelity varies depending on the composition of the mismatch and the local sequence environment. Such variations are expected because the degree to which different Pol ε errors slow polymerization and/or sensitize the helix to fraying can vary. On average, proofreading improves Pol ε fidelity about 10- to 100-fold, consistent with the mutator effects seen in yeast when the exonuclease activity of Pol ε is inactivated (57,64,65).

Like all polymerases, Pol ε ultimately does not generate all types of errors at equal rates, but rather has a distinctive error specificity (57). Two features of Pol ε error specificity are particularly interesting in light of its proposed biological roles in DNA replication. One is that Pol ε is among the most accurate of DNA polymerases for single base deletion/insertion errors. Because indels are typically generated more frequently within repetitive sequences (66), this property may be relevant to the proposal that Pol ε has a particularly important role in replicating heterochromatic DNA, which is enriched in repetitive sequences (67). Another is that a mutant derivative of Pol ε has a unique base substitution error specificity that has been useful for inferring its role in replication of the leading strand template (see below).

E. Pol ε in DNA Replication

In order for eukaryotic cells to divide and pass along their genetic complement to each daughter cell, the entire genome must be replicated accurately once, and only once, per division. This is an ordered process whereby origins of replication, ranging from well-defined 125 bp ARS sequences in *S. cerevisiae* to relatively poorly understood zones of replication of many kb in mammalian cells, are licensed by the binding of pre-RC (replication complex) components, including the ORC complex, during the G1 phase of the cell cycle (68,69). These origins are then activated for replication during S phase by the binding and phosphorylation of a number of factors including Cdc45, Sld2, Sld3, and Dpb11 (27) that enable the replicative helicase, likely the CMG complex consisting of Cdc45/Mcm2–7/GINS, to unwind the DNA in a bidirectional manner in order to establish replication forks (32,70). Once the origins have been properly licensed and activated, replication can proceed. Since the two DNA strands are antiparallel and DNA polymerases synthesize DNA in only one direction (5’ to 3’ on each nascent strand), replication is inherently asymmetric, with synthesis thought to be highly processive on the leading strand and discontinuous on the lagging strand (71). Since no DNA polymerase can conduct *de novo* DNA synthesis, a 2-subunit primase that is tightly associated with Pol α initiates replication by synthesizing short RNA chains. The primase initiates synthesis both at origins of replication and at the beginning of every Okazaki fragment during lagging strand replication. These primers are then extended by Pol α, which synthesizes DNA chains of up to 20 nucleotides. This synthesis is followed by a switch to Pol δ and/or Pol ε, which then perform the bulk of chain elongation.

1. Evidence that Pol ε is a major replicative polymerase

That yeast Pol ε is involved in replication is indicated by a number of studies. Early evidence came from studies revealing phenotypic similarities between the initial pol2 yeast mutants, primarily disruptions of the newly cloned ORF (72), and those from mutants of pol1 and pol3, as well as other mutants known to be essential for replication (73,74). Later screens for single amino acid residue changes leading to temperature-sensitive alleles identified mutations in two different regions of the catalytic subunit, the polymerase active site and the non-catalytic C-terminus (75,76). Both types of alleles had similar phenotypes but later studies also indicated a role for Pol ε in checkpoint activation ((77) and see below). At the restrictive temperature, these temperature-sensitive mutants ceased DNA synthesis, accumulated sub-chromosomal size DNA fragments and arrested with unreplicated chromosomes, all similar to Pol α and Pol δ mutants, indicating that, in addition to an essential role for Pol δ in replication, faithful chromosomal replication was also Pol ε-dependent (75,76). Peak transcript expression during the cell cycle was also coincident with Pol α (75) and subsequent microarray analysis found that mRNA for catalytic and non-catalytic subunits of the replicative DNA polymerases peak just prior to S phase (78). That Pol ε participates in replication is also indicated by the fact that it is loaded onto DNA at

origins of replication. Pol ε in yeast is found associated with replication origins prior to Pol α and along with Dpb11 (25), which is essential for chromosomal replication (79,80).

Observations derived from inactivation of its intrinsic 3'–5' exonuclease proofreading activity provide further support for a role of Pol ε in DNA replication. When the exonuclease activity of Pol ε was first identified and inactivated (64), the resulting yeast *pol2–4* mutant had an elevated mutation rate that was further increased by inactivating DNA mismatch repair, indicating that the mutations observed in the *pol2–4* strain are indeed replication errors (51,57,64). Additionally, depletion of Pol ε from a Xenopus extract results in a chromosomal replication defect, and this defect is restored upon addition of purified Xenopus Pol ε (81). Further evidence that Pol ε is involved in replication came from immunohistochemical studies showing Pol ε colocalization with actively replicating foci of DNA in normal human fibroblasts (67). This colocalization was observed late in S phase, leading to the suggestion that Pol ε is particularly important for replicating heterochromatin.

2. Division of Labor at the Replication Fork

A two polymerase model of replication fork progression derives from extensive studies of the *E. coli* replication fork which consists of two molecules of DNA polymerase III coordinately synthesizing DNA, with one operating on the continuous leading strand and the other operating on the discontinuous lagging strand (reviewed in (82)). Coordination of these two polymerases at the fork is mediated by a multisubunit complex with the τ subunit at the functional center. Following the identification of Pol ε and Pol δ as eukaryotic replicative polymerases, three different models have been put forth regarding the division of labor between Pol ε and Pol δ at the fork. One suggests that Pol δ is primarily responsible for replicating the leading strand and that Pol ε synthesizes the lagging strand. This model arose from early biochemical studies of the two polymerases ((83,84) and reviewed in (82)). Based on substantial evidence now suggesting an important role for Pol δ in lagging-strand synthesis (85–87), this model is currently disfavored.

A second model posits that Pol δ is responsible for the majority of synthesis on both the leading and lagging strands. This model is supported by the fact that SV40 origin dependent replication of double stranded DNA by primate cells requires Pols α and δ, but not Pol ε (88,89). A caveat here is that, unlike chromosomal replication, replication from the SV40 origin relies on SV40 T antigen (Tag) for initiation and helicase activities (90). Moreover, SV40 is a polyomavirus and its replication is not subject to the same cell cycle controls as is normal, chromosomal DNA (91). The latter may be particularly relevant, given the proposed role of Pol ε in checkpoint functions (see below). That Pol ε is dispensable for replication is belied by evidence that Pol ε can be cross-linked to nascent chromosomal DNA in mammalian cells (89), suggesting that, as in yeast cells (75), mammalian Pol ε is involved in chromosomal replication.

Additional support for the model suggesting that Pol δ is responsible for the majority of synthesis on both the leading and lagging strands comes from experiments in budding and fission yeast using Pol2 mutants lacking the open reading frame for the polymerase catalytic activity (92–94). While deleting the entire Pol2 gene is lethal, deleting only the N-terminus encoding the polymerase activity is not. This indicates that Pol ε activity *per se* is not absolutely required for chromosomal replication, and that the essential function of the Pol2 gene is contained within its C-terminal residues. Nonetheless, cells lacking the N-terminus of *Pol2* encoding Pol ε activity are not completely healthy and they have a prolonged S phase. In contrast to the viability of an in frame deletion of the N-terminus, mutation of two of the catalytic aspartic acid residues of yeast Pol ε to alanines is lethal (92). This is likely to encode a dominant negative polymerase that binds to the replication fork and inhibits replication.

A third model (72) posits that Pol ε is the primary leading strand DNA polymerase while Pol δ is primarily responsible for lagging-strand synthesis. This model is supported by the fact that Pol ε is intrinsically more processive than Pol δ and therefore is better suited for continuous leading strand replication (16). In addition, Pol δ is clearly implicated in lagging strand replication because its 3´ exonuclease cooperates with FEN1 to generate ligatable nicks at the 5´ ends of Okazaki fragments (86) and because it participates in completing the replication of telomeres (95), a lagging strand replication process. Genetic evidence from yeast indicating that the exonuclease activities of Pol ε and Pol δ proofread replication errors on opposite strands during replication (65,96), leads to the further inference that Pol ε participates in leading strand replication.

Strong additional evidence for Pol ε replicating the leading strand came from a combination of biochemical and genetic evidence in yeast (97). A M664G mutant allele of yeast Pol ε with reduced fidelity was identified (Fig 2C/D) that possessed a unique in vitro mutational signature, while otherwise retaining normal biochemical properties. When this allele was placed in a haploid yeast strain, an *in vivo* mutational signature was observed which was similar to that seen *in vitro* and was dependent upon both the position and orientation of a reporter gene relative to efficient replication origins. The mutational signature was consistent with participation of Pol ε in leading strand replication. This mutational signature pattern consistent with leading strand replication occurred on two different chromosomes and at both early and late-firing origins.

Recent evidence using a similar approach has now firmly implicated Pol δ in replicating the lagging strand (98). A mutant allele of Pol δ made by changing a leucine that is homologous to the methionine changed in Pol ε also has reduced fidelity but otherwise retains wild type catalytic properties (98). Yeast strains with this allele retain relatively normal replicative capacity and growth rate (98,99), but have an elevated mutation rate and a unique mutational signature (98). As in the Pol ε study (Pursell), this Pol δ mutational signature is both position- and orientation-dependent and is consistent with a primary role for Pol δ in replicating the lagging strand. Taken together, the results of both studies imply that under normal circumstances, Pol ε is primarily responsible for replicating the leading strand and Pol δ is primarily responsible for replicating the lagging strand (Fig. 5).

It remains to be seen if this pattern is observed throughout the yeast genome, or changes with chromatin status, genomic location, or other parameters. It also remains to be seen if the roles of Pol ε in yeast differ in any way from its roles in higher organisms.

F. The Role of Pol ε in Checkpoint Control

1. Cell Cycle Progression and Replication

The cell cycle is an orderly progression of events that allows the genome to be completely duplicated prior to the onset of mitosis. Unrepaired DNA damage sustained at any point in the cell cycle can disrupt this progression. DNA repair and checkpoint activation are the two means by which cells can avoid the potentially deleterious uncoupling of normal cell cycle progression. Checkpoints are cellular pathways that involve slowing or blocking cell cycle progression to allow time for repair of the lesion. There are four main DNA damage checkpoints: G1/S, intra-S, S/M, and G2/M (reviewed in (100)). DNA damage incurred prior to the cell fully committing to entering S phase and subsequent DNA replication activates the G1/S arrest primarily by inhibiting origin activation. DNA damage incurred once the cell has fully committed to replication activates the intra-S checkpoint, which acts by inhibiting late-firing origins, thus prolonging S phase, and stabilizing stalled replication forks to allow for productive restart once the replication block is removed. The S/M checkpoint is one of the least well understood, preventing catastrophic mitosis from

occurring in the absence of complete replication. The G2/M checkpoint arrests the cell cycle prior to cell division in the presence of DNA damage. Replication in general, and Pol ε in particular, is implicated primarily in the intra-S and S/M checkpoints.

Replication forks arising at replication origins proceed until termination upon meeting a fork traveling in the opposite direction from an adjacent origin. There are a number of reasons why a fork may stop prior to termination, and so mechanisms exist to ensure fork progression, to stabilize stalled forks and prevent collapse and strand breaks, and to signal for a delay in cell division. The two most well studied causes of fork stalling are low dNTP pools resulting from treatment with hydroxyurea (HU) and blocking lesions in DNA. In both cases, stalled forks are sensed by a PI(3)K-like kinase, ATR in humans and Mec1 in yeast, which phosphorylates downstream components of the checkpoint pathway. The ultimate responses to both types of damage are to inhibit the initiation of late-firing origins, to stabilize the stalled forks, and to allow the cell sufficient time to restart the stalled fork prior to proceeding with cell division. Failure to achieve any of these outcomes can result in fork collapse followed by double strand breaks, hyper-recombination, genome rearrangement, and catastrophic attempts at cell division prior to replicating the complete genome.

2. Pol ε Linked to Cell Cycle Progression and Replication

Pol ε was initially implicated as a component of the S/M replication checkpoint because mutants in the extreme C-terminus of the yeast catalytic subunit are sensitive to HU and are unable to activate the RNR3 transcriptional response and prevent catastrophic mitosis as a consequence of HU treatment (77). This defect in RNR3 transcriptional activation and in Rad53 phosphorylation were subsequently shown to be specific to S phase (101), similar to what is seen in Mec1 mutants. Rad53 is a protein kinase and checkpoint transducer that, when phosphorylated, in turn phosphorylates targets directly involved in initiating checkpoint responses. Additionally, the human Pol ε C-terminal half interacts with Mdm2 (39) and Mdm2 in turn stimulates Pol ε activity *in vitro* (40). As mentioned above, Mdm2 is an E3 ubiquitin ligase for p53 (38) and is involved in a complex regulatory loop resulting in activation or inhibition of p53 activity depending on Mdm2 interaction with a network of proteins (37). Thus Pol ε is linked to the regulation of cell cycle progression in human cells, though its involvement is less well understood than in the model yeast system.

Yeast Pol ε involvement in the intra-S checkpoint was first proposed based on its genetic association with Sgs1. Sgs1 is the lone budding yeast RecQ family helicase and is non-essential. Deletion mutants are sensitive to MMS-induced replication blocks and allow replication fork progression in the presence of HU. Intriguingly, sgs1 mutants also show elevated levels of rDNA recombination (102). Humans have five RecQ family members, including BLM and WRN, and mutants in these helicases can cause genomic instability and cancer. C-terminal Pol ε mutants are epistatic with the HU-induced Δsgs1 defects, and mutants in both the helicase and polymerase lose viability when removed from HU, pointing to an intra-S checkpoint defect (103). Pol ε associates with stalled forks at early and late-firing origins in the presence of HU and this stable association is dependent on both helicase-active Sgs1 (104) and on Rad51 (105) and is also a component of a paused replisome, along with Pol α and the CMG replicative helicase (106). However, Pol ε no longer associates with early-firing origins in the absence of a functional Mec1, which likely acts directly at the fork to aid in stabilizing stalled forks (107). That Pol ε associates with late origins inappropriately firing in mec1 mutants in the presence of HU indicates that the assembly of Pol ε at replication forks remains intact, but it is the stabilization defect that causes loss of Pol ε (104).

3. Dpb2 and the Cell Cycle

Dpb2 phosphorylation is another means of regulating Pol ε function at the replication fork. It is phosphorylated in S phase by Cdc28 (17), the yeast CDK1 homolog which phosphorylates a number of replication proteins in order to help control replication initiation and prevent re-replication (108,109). Dpb2 dephosphorylation is linked to proper S phase progression and exit from mitosis. Cdc14 is a phosphatase whose targets generally include Cdc28 phosphorylation substrates, of which Dpb2 is an S phase-specific substrate (17). A cdc14 mutant that mislocalizes away from its normal, nucleolar site deregulates Cdc14 activity, allowing Dpb2 and Sld2 to be prematurely dephosphorylated and causing defective S phase progression (110). While Dpb2 phosphorylation is not essential for yeast growth, it was hypothesized that Dpb2 phosphorylation can regulate holoenzyme formation and DNA-binding, much like other B family accessory subunit phosphorylation events (111).

G. Pol ε Involvement in Regulating Chromatin States

1. Pol ε Influence on Gene Silencing

Heterochromatic DNA is transcriptionally silent, and silenced regions are able to epigenetically influence neighboring regions through the use of Sir proteins, including the NAD+-deacetylase Sir2. Silencing involves establishment, maintenance, and inheritance of the silent state (112). Budding yeast establish, maintain, and propagate three different silent regions through differential involvement of the Sir proteins: rDNA, silent mating-type loci, and telomeres. The rDNA exists in an array of up to 200 repeats, each of which contains an origin of replication along with the heavily transcribed rRNA genes. More than half of these repeats may be silenced in a SIR2-dependent manner. The silent mating-type loci are located 16 and 23 kb from the left and right telomere, respectively, of yeast chromosome III. Silencing at these loci requires the full complement of Sir proteins, Sir1–4. Silencing at telomeric regions of the yeast genome requires only Sir2–4. Pol ε is implicated in silencing at all three types of silenced loci in yeast, indicating a fundamental role in the propagation of transcriptional states during replication. The role of Pol ε in determining chromatin status may involve the stability of the Pol ε complex, rather than a biochemical activity *per se*, because this function is affected by truncations at the C-terminus of Pol2, which lacks catalytic activity and is involved in forming a complex with the smaller subunits (14,113), and by mutations in Dpb3, which contains no identified catalytic activity and is not essential for viability (21). DPB3 was identified in a screen for deletion mutants that lost the ability to silence a marker located in the rDNA (114), indicating a role for Pol ε in positively regulating gene silencing. Mutants in Pol α and RFC were also identified, however these mutants resulted in shortened telomeres, while telomere length was unaffected in the Δdpb3 mutant (114). Ehrenhofer-Murray and coworkers (115) established an allele of the HMRa locus which was deficient in silencing, and the strain could thus not mate with MATα strains. They then screened a large set of replication mutants for those able to restore silencing and allow mating. A Pol ε C-terminal truncation mutant, *pol2–12*, restored silencing along with mutants of RFC, PCNA, and CDC45. This allele of Pol ε is temperature-sensitive and disrupts the four subunit complex. Alleles of Pol α and δ were both tested and observed to have no effect on silencing. It should be noted that in one case silencing is lost by disrupting the pol ε holoenzyme, while in the other case silencing is restored by disrupting the pol ε holoenzyme. This may reflect different roles for pol ε involved in DNA synthesis at different chromosomal locations with different silencing mechanisms.

2. Pol ε and Telomeres

Telomeres are protein-bound repetitive sequence elements that cap the ends of eukaryotic linear chromosomes (116). The normal replication machinery has the capacity to gradually

shorten chromosomes due to the "end-replication" problem (reviewed in (117)), ultimately resulting in senescence or apoptosis (118). This shortening is prevented by telomerase, a reverse-transcriptase-like enzyme that adds repetitive sequence elements to chromosomal ends to maintain telomere length (119). Subtelomeric regions in both yeast and mammals are subject to epigenetic silencing (reviewed in (120)), specifically through a heterochromatic spreading mechanism in yeast (121). These subtelomeric regions in budding yeast contain dormant or very late-firing origins of replication that are suppressed, in part, by this silencing. When telomere length is artificially reduced, either through inactivation of Terc, the RNA component of telomerase, in mouse (122) or by recombination in yeast (123), the heterochromatic state is lost and yeast ARS sequences promote origin firing early in S phase. In telomerase-proficient yeast, this early origin firing in turn promotes telomere lengthening (123). This coupling of replication timing and telomere length was demonstrated through the use of wild type Pol ε in ChIP experiments. The telomeres in pol2–16 mutants after passaging are shorter than in wild type yeast, and these cells senesce much earlier than wild type cells (22). The role of Pol ε in establishing and promoting the epigenetic silencing of regions of DNA during replication might possibly be linked to maintaining telomere length.

3. Telomere-proximal Effect

Pol ε also has a role in telomere-proximal silencing, as shown in colorimetric and viability assays using reporter genes immediately adjacent to the telomere in which both pol2 C-terminal truncation and Δdpb3 mutants are defective for silencing telomere-proximal reporter genes. Iida et al. (124) used a clever single-cell assay to further determine that Pol ε normally functions to silence this region in opposition to the yeast CHRAC remodeling complex, which operates by activating this region. This is striking given that CHRAC and Pol ε share a subunit in all organisms studied (124,125) (see below). Disruption of the shared subunit shows equal amounts of silencing and activation in this assay, clearly demonstrating an independent role in two complexes for the shared subunit.

H. Pol ε Relationship with Chromatin Remodeling Complexes

1. Chromatin Remodeling

In order to package the entire genome into the nucleus and to control gene expression, DNA in eukaryotic cells is organized into chromatin (126). At the nucleic acid level, 146 bp of DNA is wrapped in two turns around a histone octamer comprised of a core of two H3/H4 histone dimers flanked by two pairs of H2A/H2B histone dimers forming the nucleosome. These nucleosomes are separated by histone-free linker DNA and are arrayed along the DNA, which coils into 30 nm helices known as chromatin fibers, which in turn are packed upon each other to form the higher order structure known as chromatin. The net result of this packaging is that the DNA is rendered less accessible to DNA binding proteins, including DNA polymerases involved in replication and DNA repair. Cells have a large complement of multi-subunit protein complexes called chromatin remodeling complexes that are able to reorganize the histone octamers to grant enzymes like transcription factors and components of the replication machinery access to the DNA (127). These are distinct from histone-modifying enzymes, as they do not covalently alter the histone proteins. Nucleosomes are stable structures due in large part to a high number of DNA-protein contacts either directly via the histone proteins or mediated through water. Each of these chromatin remodeling complexes couples the energy released through ATP hydrolysis with a rearrangement of the nucleosomal array.

All chromatin remodeling complexes contain a catalytic subunit that is a member of the DEAD/H ATPase Swi2/Snf2 family (see (128) and references contained therein). These

members are organized into four groups based on their homology to canonical family members containing a unique complement of protein domains: the SWI/SNF, CHD, INO80, and ISWI groups. Along with the ATPase domain, the ISWI class contains a SANT domain, which has homology to a class of transcription factor DNA binding domains and, importantly, may be involved in coupling interaction with histone tails to histone modification.

Drosophila has one member of the ISWI family that forms three different complexes in vitro, called NURF, ACF, and CHRAC, based on their associated subunits and biochemical activities. CHRAC (CHRomatin Accessibility Complex) from Drosophila is comprised of four subunits: ISWI, the ATPase-containing subunit; Acf1, which contains, among other characterized and uncharacterized domains, a bromodomain involved in binding histone acetyl-lysine residues and a WAC domain that targets proteins to heterochromatin in mouse (129); and two small histone fold motif (HFM)-containing proteins, p14 and p16 (130). CHRAC was initially purified from Drosophila extracts as a complex that allowed restriction enzymes to digest their target sequence in chromatin (131). An additional activity of CHRAC is its ability to organize ordered arrays of nucleosomes in the presence of ATP from disorganized nucleosomes formed in the absence of ATP (131).

2. Dpb4/p17: The Shared Subunit

The first evidence that DNA polymerase ε played some role in these chromatin remodeling complexes came with the purification of HuCHRAC, the human homolog of the Drosophila CHRAC (125). Two homologues of the DmCHRAC HFM-containing subunits, called p15 and p17 in humans, were identified and cloned. At approximately the same time, the two HFM-containing subunits of human Pol ε, p12 and p17, were cloned (15) and it was independently determined that p17 from HuCHRAC was identical to p17 from human Pol ε.

Two yeast homologs of ISWI, ISW1 and ISW2 were identified in a chromatin remodeling complex (132) before the genes were cloned for any of the HFM subunits from either complex. It was later determined that ISW2 exists in an ATPase chromatin remodeling complex containing two HFM subunits: DPB4, a small subunit of yeast Pol ε and DLS1 (Dpb3-like subunit), a novel yeast protein with homology to the unique HFM subunits in CHRAC (124,133). The shared subunit is conserved throughout evolution. Why two entirely separate enzymatic activities should have such a shared subunit remains an open question. The shared subunit could bridge an interaction between the two complexes, but this appears unlikely given that p12, the Pol ε-specific HFM protein, is not found in purified CHRAC in multiple species (134,135), and given that hSNF2H is not found in purified human Pol ε (Pursell & Linn, unpublished observations). Based on sequence homology to the CCAAT-binding factor subunits, CBF-A and -C, it was originally proposed that the human p12/p17 heterodimer might form an interaction surface, much like CBF-A/-C enables binding of CBF-B and subsequent transactivation, thus allowing Pol ε to interact with other factors, possibly involved in influencing chromatin structure (15).

One possible explanation for the shared subunit is to coordinately regulate Pol ε and CHRAC. Both the CHRAC and Pol ε HFM subunits from humans (125) and from yeast (52) are able to bind dsDNA. This enables the four-subunit Pol ε holoenzyme to tightly bind dsDNA (136), unique among replicative DNA polymerases. The shared subunit in yeast, Dpb4, can be cross-linked to extranucleosomal DNA, but not nucleosomal (137). The HFM subunits may bind dsDNA, either in complex with the catalytic subunits or in their absence in a manner similar to the histone H2A/H2B heterodimer (see (138) and Fig. 6), to mark regions of DNA to be operated on by either Pol ε, likely to propagate a silenced state, or by CHRAC, likely to propagate a derepressed nucleosomal state (115). The decision to target Pol ε or CHRAC may be made at the epigenetic level through histone modifications.

Histones were found to co-immunoprecipitate with both yCHRAC and Pol ε through Dpb4, with a Dpb4-associated histone H4 hypoacetylation pattern different from bulk genomic H4 (135). A genome-wide microarray of DNA bound to Dpb4-histone complexes showed enrichment near telomeres and boundary regions separating the silenced HM loci from the surrounding euchromatin, consistent with the silencing data observed by both Iida et al. and Ehrenhofer-Murray et al. (115) It may be that Pol ε and CHRAC are directed to silenced and non-silenced regions of DNA, respectively, in order to ensure that the chromatin status is properly maintained during replication and then propagated to the progeny. Switching states, if necessary, would be carried out by other factors, possibly HATs or HDACs, whose actions would dictate the switching of the targeting signals for Pol ε and CHRAC.

3. Centromeres and Sister Chromatid Cohesio

Evidence from metazoan cells indicates that Pol ε and CHRAC may be targeted to regions of silenced DNA as well. Two components of CHRAC, Acf1 and Snf2h, colocalize with HP1β, which aids in the formation of pericentromeric heterochromatin by binding K9-methylated histone H3, and BrdU, during S phase (139). Additionally, reduction of both Acf1 and Snf2h causes a delay in S phase specific to elongation (139). Pericentromeric heterochromatin is a region of specialized heterochromatin surrounding the centromeres that is important for spindle attachment during mitosis. While budding yeast lack true pericentromeric heterochromatin, Pol ε physically interacts with and is stimulated by Trf4 (140), which is required for sister chromatid cohesion and completion of S phase (141). Trf4, and the redundant Trf5, was initially believed to be a novel DNA polymerase involved in this process, but was subsequently shown to have poly(A) polymerase activity (142). Snf2h has been found in a complex separate from CHRAC that contains hRad21 and is involved in loading cohesin onto DNA (143). Pol ε from non-transformed human fibroblasts was also found to colocalize with both PCNA and BrdU at sites of active DNA replication late in S phase, during which time heterochromatin is replicated in mammalian cells (67). Taken together, these data suggest that the shared p17 subunit may target both CHRAC and Pol ε to repetitive DNA, to facilitate replication of heterochromatin to ensure that the epigenetic state of heterochromatic DNA is maintained and propagated. The differences between yeast and human CHRAC-pol ε relationships may also suggest a difference in the balance of CHRAC and Pol ε activities between budding yeast, with its SIR2-dependent silencing and absence of true heterochromatin, and metazoan and fission yeast, with HP1-dependent heterochromatic DNA.

CHRAC may also contribute, directly or indirectly, to Pol ε during replication at the origin-unwinding stage. The initiation of SV40 origin-containing DNA into a nucleosomal template using cell-free extracts normally inhibits in vitro replication (144). Purified CHRAC was able to rearrange the nucleosomes on an SV40 template and relieve this inhibition of replication (145). While this system relies on Tag both for origin unwinding and for helicase activity, and while Pol ε is dispensible for this reaction (88), nevertheless it is intriguing that a nucleosome remodeling complex is able to facilitate replication origin firing and also shares a subunit with a DNA polymerase known to bind replication origins prior to origin firing (25).

I. The Roles of Pol ε in Excision Repair of DNA Damage

Much of the early work implicating Pol ε in DNA repair synthesis was based in large part on studies making use of the DNA polymerase inhibitor, aphidicolin (5,146). This was done primarily to distinguish aphidicolin-sensitive DNA synthesis from aphidicolin-insensitive DNA polymerases like Pol β. Unfortunately, aphidicolin inhibits both Pol δ and Pol ε, making distinctions between the two using this strategy difficult. Even today it remains difficult to distinguish the involvement of the two polymerases in DNA repair synthesis,

though there is much evidence to suggest that Pol ε does play a role in filling gaps generated during several types of excision repair.

1. Base Excision Repair

The cell is constantly exposed to a wide variety of agents that can chemically modify the DNA, posing a challenge to the normal progression of replication forks and transcription complexes. These insults include reactive oxygen species created within the cell, and external sources like ultraviolet irradiation and chemicals that introduce DNA base adducts. In order to deal with these lesions and prevent mutagenesis and genome instability, multiple pathways exist to identify, excise, and correct these lesions (147). DNA base modifications that do not generally distort DNA helix geometry are primarily repaired by base excision repair (148). Multiple BER subpathways exist to repair the various non-distorting lesions (reviewed in (149)).

In mammals, Pol β has a major role in BER, being essential for the repair synthesis of a single nucleotide to replace the damaged nucleotide (150). However, Pols δ and ε have both been implicated in the PCNA-dependent BER, which involves synthesis patch sizes greater than one nucleotide and likely serves as a backup to Pol β-dependent BER (151,152). Pol ε was implicated in BER in yeast when extracts made from temperature-sensitive yeast Pol ε mutants were deficient in repairing three different lesions, a plasmid containing dUMP, an OsO_4-treated plasmid, and a UV-irradiated plasmid (153). Purified yeast Pol ε was able to restore DNA repair, thus implicating Pol ε in BER.

2. Nucleotide Excision Repair

Many lesions that strongly distort the DNA helix are repaired by nucleotide excision repair (NER). NER requires synthesis of about 30 nucleotides to fill the gap generated by excision of the lesion from the DNA (147). Early evidence that Pol ε plays a role in NER-dependent DNA synthesis came from studies in human cells that preceded knowledge that pols δ and ε were distinct enzymes. Postconfluent, normal diploid human fibroblasts, which do not exhibit semiconservative (replicative) DNA synthesis, undergo conservative (repair) DNA synthesis after UV irradiation (5). When cells were permeabilized after UV treatment, a repair factor was lost along with the repair synthesis, which was complemented by addition of HeLa extracts. Extensive fractionation and biochemical characterization revealed this soluble repair factor to be DNA polymerase ε (6,7) (described above and below).

Complete reconstitution of mammalian nucleotide excision repair was initially performed using calf thymus Pol ε as the gap-filling DNA polymerase (154). A subsequent study determined that Pol ε was better able than Pol δ to perform gap-filling synthesis that ultimately generated DNA ligase I-ligatable products (155). Unlike with pols α and δ, DNA synthesis efficiency by Pol ε increased as gap size decreased (156), another desirable characteristic for a gap-filling polymerase. Another study pointing to Pol ε and Pol δ redundancy in excision repair showed that only mutants in both polymerases showed an accumulation of single strand DNA breaks in response to UV irradiation (157). Whereas extracts from temperature-sensitive Pol δ or Pol ε mutants show reduced NER activity, Pol α is not involved in yeast NER (158). At least the DNA polymerase component of Pol δ is able to completely compensate for the absence of Pol ε during budding yeast NER *in vivo*, as pol2–16 mutants lacking the catalytic domain are not sensitive to UV (94). However, extracts prepared from pol2–16 cells do show reduced NER synthesis *in vitro*, indicating that Pol ε does play a substantial role in NER (158).

A recent study combined whole-cell repair assays with ChIP and immunofluorescence microscopy in both proliferating and quiescent cells to identify XRCC1 and DNA ligase

IIIα as novel core components of NER in humans (159). DNA ligase I is essential for chromosomal replication, plays an important role in NER (160), and has also been implicated in long-patch BER (113) along with the scaffold protein XRCC1 (161). For these reasons, DNA ligase I was used in the excision repair reconstitution assays that implicated both Pol δ and Pol ε, without providing a clear distinction between the two polymerases (154,155). Interestingly, Pol δ was found to be present at sites of NER involving XRCC1-DNA ligase IIIα in both actively cycling and quiescent cells, while Pol ε was only found with DNA ligase I at sites of NER and only in cycling cells (159). This is curious given results with the original repair assays that identified Pol ε in confluent cells (5), which may indicate that the repair role of Pol ε in post-mitotic cells relies on an as yet uncharacterized DNA ligase, since ligase I is not present in non-replicating cells (159).

Interstrand cross-links (ICLs) are extremely toxic to cells and particularly relevant to human health as many anti-cancer drugs induce ICLs. In order to deal with this high toxicity, a number of repair pathways exist to accurately repair these lesions (reviewed in (162)). The predominant ICL repair pathways involve the NER, recombinational repair, and translesion DNA synthesis systems, which pathway is used appears to vary considerably with both the cell cycle and type of lesion (163). While overall ICL repair is only poorly understood in eukaryotes, the use of NER and recombinational repair, both of which involve Pol ε to some degree, raises the possibility that Pol ε is involved in ICL repair.

3. Does Pol ε function in Mismatch Repair?

Biochemical evidence has implicated Pol δ in MMR (164–166). Nonetheless, those studies noted that a possible role for Pol ε should not be excluded. In support of this possibility is one study providing genetic data consistent with a role for the 3´ exonuclease activity of Pol ε in the excision step of MMR. In this study, exonuclease-deficient Pol ε mutants, which alone show very little effect on frameshift fidelity in long (≥8 nt) homonucleotide runs, showed a synergistic mutator effect in long homonucleotide runs with inactivation of exo1, which is known to be involved in mismatch repair (51). Based on the even more severe defect seen with exonuclease-deficient Pol δ mutants in combination with exo1 inactivation, the authors proposed that Exo1 competes with the 3’ exonuclease activities of both pols ε and δ in the excision step of mismatch repair. Biochemical studies with human cell extracts showed that the excision step of MMR is aphidicolin-insensitive, arguing against a direct role for either Pol ε or Pol δ. However, the authors note that the resistance varies somewhat with extract preparation and that their assay does not require the presence of a replication fork. If the 3’ excision step is dependent upon a replication fork, it would not be observed in their assay.

J. Pol ε in Recombination

1. Double Strand Break Repair

Double-strand breaks (DSB) in DNA are among the most toxic of lesions; even one DSB can be lethal (167). DSBs arise through a number of different endogenous and exogenous sources including γ-irradiation, mating type switching, immunoglobulin rearrangement, crossing over during meiosis, and stalled replication forks (reviewed in (147)). Two major types of repair abrogate the deleterious effects of DSBs, non-homologous end-joining, and homologous recombination (168,169). The latter can be subdivided into gene conversion and break-induced replication (BIR), both of which involve a number of replication fork proteins, likely including Pol ε.

2. Gene Conversion

Double-strand breaks repaired by gene conversion likely do so via a modified replication fork consisting of leading and lagging strand polymerases. Temperature-sensitive mutants of pols α, δ, and ε are each defective in gene conversion at the MAT locus in budding yeast upon generation of an HO-induced DSB (170). While an early study in asynchronous yeast cells reported that Pol α-primase mutants had the most serious defect, later examination of this mutant in synchronized cells revealed that Pol α is unnecessary, questioning the importance of Okazaki fragment processing in recombinational repair through gene conversion (171). A Pol ε mutant gave a more severe defect in gene conversion than did a Pol δ mutant, consistent with the idea that leading strand DNA synthesis might play a more prominent role in gene conversion, or that Pol ε may be able to more effectively substitute for Pol δ lagging strand synthesis than can Pol δ substitute for Pol ε. The gene conversion process occurs with fast kinetics and involves little, if any, checkpoint activation.

3. Break-induced Replication

The break-induced replication (BIR) pathway may operate at replication forks that stall at a DNA lesion. Strand invasion mediated by Rad51 allows the reestablishment of a unidirectional replication fork that can proceed to the end of the chromosome (172). A Rad51-independent mode of BIR exists (173) which is dependent upon Rad50 and Rad59, although no direct involvement of a replicative DNA polymerase has been reported for this pathway (174). However, in an assay designed to investigate the role of the replicative polymerases in Rad51-dependent BIR, yeast pols α and δ were found to be required for the initiation step (175). Pol ε was found to be dispensable for the initiation step but required for the elongation step and formation of long products of up to 30 kb. The Pol ε mutant used to probe this BIR was a C-terminal mutant that disrupts the holoenzyme. This region contains the portion of Pol ε implicated in damage sensing and checkpoint activation, indicating that perhaps these activities are the required activities of Pol ε in BIR.

4. Mammalian Recombination Complex

That Pol ε may participate in recombinational repair in metazoan cells is suggested by its association with recombination-like activities. Pol ε from bovine tissue co-purifies with the multiprotein RC-1 complex that can transfer a homologous sequence from one closed, circular DNA template to another *in trans* (176–179). Also copurifying with Pol ε were DNA ligase III and a structure-specific endonuclease that may help to resolve Holliday junctions arising during recombination. During mouse testis development, Pol ε mRNA and protein levels persist through the late pachytene stage of meiosis, when Rad51 levels peak and the meiotic chromosomes are fully synapsed and recombination is completed (180). Human Pol ε may thus function as a DNA polymerase in a recombinational repair complex in a similar fashion to DNA synthesis during BIR in yeast, with a primary role in the elongation/completion phase of recombination repair.

5. rDNA Recombination

Pol ε is involved in silencing at the yeast rDNA loci (114), likely acting during replication to propagate the silenced state like it does at the boundary regions near the HM loci (115). The rDNA loci in yeast are recombinigenic and defects that reduce the replicative lifespan of yeast cause a hyperrecombination phenotype resulting in the accumulation of extrachromosomal rDNA minicircles, eventually causing the mother cell to die (181). This process of creating rDNA minicircles is promoted by Fob1, which stalls replication forks at Fob1-binding sites located within each rDNA repeat, and is repressed by Sir2 (and the Sir2-like Hst2), which silences rDNA through the formation of heterochromatin (182). rDNA is in metazoans is heterochromatic and primarily replicates late in S phase (183), the same time

that the replication-linked function of Pol ε is occurring (67). It is tempting to speculate that when replication forks are stalled to the point where a recombinational repair mechanism is invoked (e.g. a specific protein block, heavy transcriptional activity, a single-strand break at a replication fork), Pol ε is the primary elongation polymerase. In order to prevent premature mitosis Pol ε is then able to signal through its C-terminal half the presence of the crossed-over replication intermediate, which is lethal if left unresolved. What makes this even more intriguing are observations with mutants lacking the Pol ε catalytic domain, which lose replicative capacity much earlier than do wild type cells (22). A defect in Pol ε that causes reduced lifespan might thus be due to a defect in rDNA recombination. In addition, Pol ε physically interacts with Trf4, which is involved in sister chromatid cohesion (141). Trf4 is a component of a complex that binds the rDNA intergenic spacer region and helps regulate rDNA copy number (184), and Δtrf4 mutants are sensitive to MMS (185). This role is consistent with the major observations of Pol ε being essential for chromosomal replication, and that the essential portion in yeast is its C-terminal, subunit-interacting half. It is also consistent with Pol ε being important for Rad51-dependent BIR and the observation that Pol ε foci colocalize with replication foci only late in S phase. This may be a general mechanism that is observed primarily at the rDNA loci due to the naturally high degree of recombination occurring there and its regular assembly into nucleolar foci (186).

K. Schizosaccharomyces pombe Pol ε

1. Catalytic Subunit

S. pombe Pol ε, or cdc20, was initially described as a mutant allele defective in the initiation of DNA replication (187) and with reduced sporulation efficiency (188). Thermosensitive mutants in cdc20, along with mutants of DNA polymerase δ and MCM4, undergo mitosis prior to completing replication (189), much like the *S. cerevisiae* C-terminal truncation mutants, linking Pol ε to the DNA damage checkpoint in fission yeast. When the cdc20 gene was cloned and found to encode Pol ε by virtue of sequence homology, disruption of the coding sequence was lethal, consistent with the idea that S. pombe Pol ε is essential for DNA replication (190). Haploids generated from two different mutants had reduced sporulation but showed no replication or other mitotic defects. Additionally, C-terminal mutants mimicking those C-terminal truncation mutants that are defective for the S-phase checkpoint arrest with the checkpoint intact, indicating that S. pombe Pol ε may differ from *S. cerevisiae* Pol ε in having little or no role in S-phase checkpoint integrity. However, when replication checkpoint-proficient cdc20 mutants are combined with a similarly replication checkpoint-proficient deletion of cid1, one of six *S.pombe* homologs of the budding yeast Trf4/Trf5 proteins involved in sister chromatid cohesion, the resulting cid1Δ cdc20 double mutant is defective for the replication checkpoint (191).

As seen in budding yeast, deletion of the catalytic portion of pombe Pol ε is not lethal but does prolong S phase (93). This supports the idea that Pol δ catalytic activity is able to partially compensate for Pol ε catalytic deficiency, and that the C-terminal half of the catalytic subunit contains the essential activity. This function likely involves the ability of the C-terminal half to interact with its own subunits and possibly other factors on DNA (192). The viability of this mutant depends on components of the DNA damage checkpoint, i.e., the catalytic mutant is synthetically lethal when combined with deletion mutants of chk1, rad3 (ATR), and hus1 (9-1-1 clamp), as well as with cdc6 (pol δ). In contrast to observations in budding yeast (94), S. pombe catalytic deletion mutants are insensitive to HU and highly sensitive to MMS (93).

2. Accessory Subunits

As in budding yeast, the dpb2 subunit of S. pombe Pol ε is essential (192). In a system designed to downregulate dpb2 in the presence of thiamine, cells with reduced Dpb2 have delayed replication initiation and increased nuclear defects, including missegregated chromosomes and anucleate cells. Also as in budding yeast, dpb2 binds to ARS elements early in S phase and this binding depends on pre-RC formation, indicating a role in initiation or early elongation of replication. However, unlike budding yeast, S. pombe dpb3 is essential (193). Moreover, down regulation of dpb3 results in a cell cycle delay and an increase in multinucleate cells. This contrasts with *S. cerevisiae*, where Δdpb3 mutants are viable and the only observed phenotype is a moderate increase in mutation rate (21), a phenotype that has yet to be examined with *S. pombe* Pol ε mutants. Dpb4 is not essential for viability, but unlike in *S. cerevisiae*, a dpb4 deletion mutant is synthetic lethal in combination with mutants in DNA replication initiation and DNA damage checkpoint signaling (193).

Rad4/Cut5 is a BRCT-repeat containing protein that is essential for replication initiation (194), likely through linking pre-RC formation to CDK signaling. It shares homology to the human TopBP1 and budding yeast DPB11, which has a role in checkpoint activation (80) as well as replication initiation (79) and physically associates with Pol ε (25). Although Rad4 binds chromatin in the absence of DNA damage, this association, as well as the stability of the Rad4 protein, depends on the presence of Pol ε (194), indicating that Rad4 and Pol ε may function together in replication initiation.

L. Xenopus Pol ε

Understanding Pol ε function has been facilitated using the powerful cell free replication system of Xenopus (81). Immunodepleting Pol ε from Xenopus extracts leads to a defect in bulk DNA synthesis (81) that is complemented by recombinant Xpol ε (195). This defect is more pronounced in extracts depleted of Pol δ, which also accumulated large amounts of ssDNA (85), consistent with a role for Pol δ in lagging strand synthesis. As in *S. cerevisiae* (196), Xenopus Pol ε binds to chromatin in a Cdc45-dependent manner (197). One major difference between the frog and yeast systems is in the division of labor involved in activating replication origins and stabilizing stalled replication forks. Whereas yeast separates these two processes, in part by maintaining the Sld2-Dpb11 origin activation pathway separate from the Sgs1 helicase, the Xenopus RecQ homolog, RecQ4, or xRTS, contains helicase and Sld2-like domains in a single polypeptide (198). This Sld2-like domain interacts with xCut5, the Xenopus Dpb11 homolog, though not with the replicative DNA polymerases. xWRN is another RecQ family member, in which mutations cause the premature aging disease Werner's syndrome (199). The WRN helicase associates with replication forks (200) but when mutated lacks the severe DNA synthesis defects observed in Bloom's syndrome-derived cells (201). A role was proposed for WRN in unwinding dsDNA byproducts generated during lagging strand synthesis (202). Consistent both with Pol ε carrying out leading strand synthesis and with Pol ε being involved in stalled replication fork signaling, xWRN/FFA-1 and Pol δ accumulate on chromatin after aphidicolin treatment, while the chromatin-bound level of Pol ε does not increase (203). Claspin, the Xenopus homolog of the mediator protein Mrc1, associates with replication forks during S phase (204) in a xCdc45-dependent manner. Claspin is also required for replication fork-stalled xChk1 activation by xAtr phosphorylation (205). Underscoring the specificity of Pol ε involvement in this checkpoint pathway, xClaspin physically interacts with xPol ε, but not with either xPol α or xPol δ (206).

M. Concluding Remarks

Pol ε plays a central role in replication fork establishment, progression, and maintenance of fork stability (Fig 1). In addition, Pol ε plays important roles in the establishment and maintenance of a silenced chromatin state, the repair of DNA base damage, and the restart of stalled replication forks. Each of these processes must occur faithfully and in a regulated manner in order to allow duplication of the eukaryotic genome and stable transmission of this genetic information to the daughter cells. Disruptions to these processes can lead to mutagenesis, genome instability, aneuploidy and cell death. Pol ε also plays a role in DNA transactions at the rDNA locus and at telomeres, both of which are important in regulating the aging process. It will therefore be important to continue to characterize in detail the precise functions of Pol ε in order to determine how Pol ε contributes directly to genome stability and ultimately human health.

Acknowledgments

The authors would like to thank Drs. Kasia Bebenek and Stephanie Nick McElhinny for thoughtful discussion and comments on the manuscript. This work was supported by the Intramural Research Program of the National Institutes of Health, National Institute of Environmental Health Sciences.

References

1. Bessman MJ, Kornberg A, Lehman IR, Simms ES. Enzymic synthesis of deoxyribonucleic acid. Biochimica et biophysica acta. 1956; 21:197–198. [PubMed: 13363894]
2. Bollum FJ, Potter VR. Incorporation of thymidine into deoxyribonucleic acid by enzymes from rat tissues. J Biol Chem. 1958; 233:478–482. [PubMed: 13563524]
3. Byrnes JJ, Downey KM, Black VL, So AG. A new mammalian DNA polymerase with 3' to 5' exonuclease activity: DNA polymerase delta. Biochemistry. 1976; 15:2817–2823. [PubMed: 949478]
4. Crute JJ, Wahl AF, Bambara RA. Purification and characterization of two new high molecular weight forms of DNA polymerase delta. Biochemistry. 1986; 25:26–36. [PubMed: 3954990]
5. Nishida C, Reinhard P, Linn S. DNA repair synthesis in human fibroblasts requires DNA polymerase delta. J Biol Chem. 1988; 263:501–510. [PubMed: 3335506]
6. Syvaoja J, Linn S. Characterization of a large form of DNA polymerase delta from HeLa cells that is insensitive to proliferating cell nuclear antigen. J Biol Chem. 1989; 264:2489–2497. [PubMed: 2563372]
7. Syvaoja J, Suomensaari S, Nishida C, Goldsmith JS, Chui GS, Jain S, Linn S. DNA polymerases alpha, delta, and epsilon: three distinct enzymes from HeLa cells. Proc Natl Acad Sci U S A. 1990; 87:6664–6668. [PubMed: 1975694]
8. Kesti T, Frantti H, Syvaoja JE. Molecular cloning of the cDNA for the catalytic subunit of human DNA polymerase epsilon. J Biol Chem. 1993; 268:10238–10245. [PubMed: 8486689]
9. Li Y, Asahara H, Patel VS, Zhou S, Linn S. Purification, cDNA cloning, and gene mapping of the small subunit of human DNA polymerase epsilon. J Biol Chem. 1997; 272:32337–32344. [PubMed: 9405441]
10. Bebenek K, Kunkel TA. Functions of DNA polymerases. Advances in protein chemistry. 2004; 69:137–165. [PubMed: 15588842]
11. Franklin MC, Wang J, Steitz TA. Structure of the replicating complex of a pol alpha family DNA polymerase. Cell. 2001; 105:657–667. [PubMed: 11389835]
12. Kamtekar S, Berman AJ, Wang J, Lazaro JM, de Vega M, Blanco L, Salas M, Steitz TA. Insights into strand displacement and processivity from the crystal structure of the protein-primed DNA polymerase of bacteriophage phi29. Mol Cell. 2004; 16:609–618. [PubMed: 15546620]
13. Doublie S, Sawaya MR, Ellenberger T. An open and closed case for all polymerases. Structure. 1999; 7:R31–R35. [PubMed: 10368292]

14. Dua R, Levy DL, Campbell JL. Role of the putative zinc finger domain of Saccharomyces cerevisiae DNA polymerase epsilon in DNA replication and the S/M checkpoint pathway. J Biol Chem. 1998; 273:30046–30055. [PubMed: 9792727]
15. Li Y, Pursell ZF, Linn S. Identification and cloning of two histone fold motif-containing subunits of HeLa DNA polymerase epsilon. J Biol Chem. 2000; 275:23247–23252. [PubMed: 10801849]
16. Dua R, Levy DL, Li CM, Snow PM, Campbell JL. In vivo reconstitution of Saccharomyces cerevisiae DNA polymerase epsilon in insect cells. Purification and characterization. J Biol Chem. 2002; 277:7889–7896. [PubMed: 11756442]
17. Kesti T, McDonald WH, Yates JR 3rd, Wittenberg C. Cell cycle-dependent phosphorylation of the DNA polymerase epsilon subunit, Dpb2, by the Cdc28 cyclin-dependent protein kinase. J Biol Chem. 2004; 279:14245–14255. [PubMed: 14747467]
18. Chilkova O, Jonsson BH, Johansson E. The quaternary structure of DNA polymerase epsilon from Saccharomyces cerevisiae. J Biol Chem. 2003; 278:14082–14086. [PubMed: 12571237]
19. Jaszczur M, Flis K, Rudzka J, Kraszewska J, Budd ME, Polaczek P, Campbell JL, Jonczyk P, Fijalkowska IJ. Dpb2p, a Noncatalytic Subunit of DNA Polymerase {varepsilon}, Contributes to the Fidelity of DNA Replication in Saccharomyces cerevisiae. Genetics. 2008; 178:633–647. [PubMed: 18245343]
20. Li, Y. Ph.D. Berkeley, CA: University of California, Berkeley; 1998.
21. Araki H, Hamatake RK, Morrison A, Johnson AL, Johnston LH, Sugino A. Cloning DPB3, the gene encoding the third subunit of DNA polymerase II of Saccharomyces cerevisiae. Nucleic Acids Res. 1991; 19:4867–4872. [PubMed: 1923754]
22. Ohya T, Kawasaki Y, Hiraga S, Kanbara S, Nakajo K, Nakashima N, Suzuki A, Sugino A. The DNA polymerase domain of pol(epsilon) is required for rapid, efficient, and highly accurate chromosomal DNA replication, telomere length maintenance, and normal cell senescence in Saccharomyces cerevisiae. J Biol Chem. 2002; 277:28099–28108. [PubMed: 12015307]
23. Asturias FJ, Cheung IK, Sabouri N, Chilkova O, Wepplo D, Johansson E. Structure of Saccharomyces cerevisiae DNA polymerase epsilon by cryo-electron microscopy. Nature structural & molecular biology. 2006; 13:35–43.
24. Shamoo Y, Steitz TA. Building a replisome from interacting pieces: sliding clamp complexed to a peptide from DNA polymerase and a polymerase editing complex. Cell. 1999; 99:155–166. [PubMed: 10535734]
25. Masumoto H, Sugino A, Araki H. Dpb11 controls the association between DNA polymerases alpha and epsilon and the autonomously replicating sequence region of budding yeast. Mol Cell Biol. 2000; 20:2809–2817. [PubMed: 10733584]
26. Araki H, Leem SH, Phongdara A, Sugino A. Dpb11, which interacts with DNA polymerase II(epsilon) in Saccharomyces cerevisiae, has a dual role in S-phase progression and at a cell cycle checkpoint. Proc Natl Acad Sci U S A. 1995; 92:11791–11795. [PubMed: 8524850]
27. Tanaka S, Umemori T, Hirai K, Muramatsu S, Kamimura Y, Araki H. CDK-dependent phosphorylation of Sld2 and Sld3 initiates DNA replication in budding yeast. Nature. 2007; 445:328–332. [PubMed: 17167415]
28. Zegerman P, Diffley JF. Phosphorylation of Sld2 and Sld3 by cyclin-dependent kinases promotes DNA replication in budding yeast. Nature. 2007; 445:281–285. [PubMed: 17167417]
29. Kubota Y, Takase Y, Komori Y, Hashimoto Y, Arata T, Kamimura Y, Araki H, Takisawa H. A novel ring-like complex of Xenopus proteins essential for the initiation of DNA replication. Genes Dev. 2003; 17:1141–1152. [PubMed: 12730133]
30. Takayama Y, Kamimura Y, Okawa M, Muramatsu S, Sugino A, Araki H. GINS, a novel multiprotein complex required for chromosomal DNA replication in budding yeast. Genes Dev. 2003; 17:1153–1165. [PubMed: 12730134]
31. Kanemaki M, Labib K. Distinct roles for Sld3 and GINS during establishment and progression of eukaryotic DNA replication forks. Embo J. 2006; 25:1753–1763. [PubMed: 16601689]
32. Moyer SE, Lewis PW, Botchan MR. Isolation of the Cdc45/Mcm2-7/GINS (CMG) complex, a candidate for the eukaryotic DNA replication fork helicase. Proc Natl Acad Sci U S A. 2006; 103:10236–10241. [PubMed: 16798881]

33. Makiniemi M, Hillukkala T, Tuusa J, Reini K, Vaara M, Huang D, Pospiech H, Majuri I, Westerling T, Makela TP, et al. BRCT domain-containing protein TopBP1 functions in DNA replication and damage response. J Biol Chem. 2001; 276:30399–30406. [PubMed: 11395493]
34. Kim JE, McAvoy SA, Smith DI, Chen J. Human TopBP1 ensures genome integrity during normal S phase. Mol Cell Biol. 2005; 25:10907–10915. [PubMed: 16314514]
35. Maldonado E, Shiekhattar R, Sheldon M, Cho H, Drapkin R, Rickert P, Lees E, Anderson CW, Linn S, Reinberg D. A human RNA polymerase II complex associated with SRB and DNA-repair proteins. Nature. 1996; 381:86–89. [PubMed: 8609996]
36. Rytkonen AK, Vaara M, Nethanel T, Kaufmann G, Sormunen R, Laara E, Nasheuer HP, Rahmeh A, Lee MY, Syvaoja JE, et al. Distinctive activities of DNA polymerases during human DNA replication. Febs J. 2006; 273:2984–3001. [PubMed: 16762037]
37. Iwakuma T, Lozano G. MDM2, an introduction. Mol Cancer Res. 2003; 1:993–1000. [PubMed: 14707282]
38. Haupt Y, Maya R, Kazaz A, Oren M. Mdm2 promotes the rapid degradation of p53. Nature. 1997; 387:296–299. [PubMed: 9153395]
39. Vlatkovic N, Guerrera S, Li Y, Linn S, Haines DS, Boyd MT. MDM2 interacts with the C-terminus of the catalytic subunit of DNA polymerase epsilon. Nucleic Acids Res. 2000; 28:3581–3586. [PubMed: 10982879]
40. Asahara H, Li Y, Fuss J, Haines DS, Vlatkovic N, Boyd MT, Linn S. Stimulation of human DNA polymerase epsilon by MDM2. Nucleic Acids Res. 2003; 31:2451–2459. [PubMed: 12711691]
41. Steitz TA, Smerdon SJ, Jager J, Joyce CM. A unified polymerase mechanism for nonhomologous DNA and RNA polymerases. Science. 1994; 266:2022–2025. [PubMed: 7528445]
42. Kunkel TA, Bebenek K. DNA replication fidelity. Annu Rev Biochem. 2000; 69:497–529. [PubMed: 10966467]
43. Moon AF, Garcia-Diaz M, Batra VK, Beard WA, Bebenek K, Kunkel TA, Wilson SH, Pedersen LC. The X family portrait: structural insights into biological functions of X family polymerases. DNA repair. 2007; 6:1709–1725. [PubMed: 17631059]
44. Chui G, Linn S. Further characterization of HeLa DNA polymerase epsilon. J Biol Chem. 1995; 270:7799–7808. [PubMed: 7536191]
45. Burgers PM, Bauer GA. DNA polymerase III from Saccharomyces cerevisiae. II. Inhibitor studies and comparison with DNA polymerases I and II. J Biol Chem. 1988; 263:925–930. [PubMed: 3121622]
46. Hamatake RK, Hasegawa H, Clark AB, Bebenek K, Kunkel TA, Sugino A. 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. 1990; 265:4072–4083. [PubMed: 2406268]
47. Richey B, Cayley DS, Mossing MC, Kolka C, Anderson CF, Farrar TC, Record MT Jr. Variability of the intracellular ionic environment of Escherichia coli. Differences between in vitro and in vivo effects of ion concentrations on protein-DNA interactions and gene expression. J Biol Chem. 1987; 262:7157–7164. [PubMed: 3108249]
48. Griep MA, McHenry CS. Glutamate overcomes the salt inhibition of DNA polymerase III holoenzyme. J Biol Chem. 1989; 264:11294–11301. [PubMed: 2567734]
49. Chilkova O, Stenlund P, Isoz I, Stith CM, Grabowski P, Lundstrom EB, Burgers PM, Johansson E. 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. 2007; 35:6588–6597. [PubMed: 17905813]
50. McCulloch SD, Kokoska RJ, Chilkova O, Welch CM, Johansson E, Burgers PM, Kunkel TA. Enzymatic switching for efficient and accurate translesion DNA replication. Nucleic Acids Res. 2004; 32:4665–4675. [PubMed: 15333698]
51. Tran HT, Gordenin DA, Resnick MA. The 3'-->5' exonucleases of DNA polymerases delta and epsilon and the 5'-->3' exonuclease Exo1 have major roles in postreplication mutation avoidance in Saccharomyces cerevisiae. Mol Cell Biol. 1999; 19:2000–2007. [PubMed: 10022887]
52. Tsubota T, Tajima R, Ode K, Kubota H, Fukuhara N, Kawabata T, Maki S, Maki H. Double-stranded DNA binding, an unusual property of DNA polymerase epsilon, promotes epigenetic

silencing in Saccharomyces cerevisiae. J Biol Chem. 2006; 281:32898–32908. [PubMed: 16916794]

53. Maki S, Hashimoto K, Ohara T, Sugino A. DNA polymerase II (epsilon) of Saccharomyces cerevisiae dissociates from the DNA template by sensing singlestranded DNA. J Biol Chem. 1998; 273:21332–21341. [PubMed: 9694894]
54. Kunkel TA, Sabatino RD, Bambara RA. Exonucleolytic proofreading by calf thymus DNA polymerase delta. Proc Natl Acad Sci U S A. 1987; 84:4865–4869. [PubMed: 3474631]
55. Thomas DC, Roberts JD, Sabatino RD, Myers TW, Tan CK, Downey KM, So AG, Bambara RA, Kunkel TA. Fidelity of mammalian DNA replication and replicative DNA polymerases. Biochemistry. 1991; 30:11751–11759. [PubMed: 1751492]
56. Pursell ZF, Isoz I, Lundstrom EB, Johansson E, Kunkel TA. Regulation of B family DNA polymerase fidelity by a conserved active site residue: characterization of M644W, M644L and M644F mutants of yeast DNA polymerase epsilon. Nucleic Acids Res. 2007; 35:3076–3086. [PubMed: 17452367]
57. Shcherbakova PV, Pavlov YI, Chilkova O, Rogozin IB, Johansson E, Kunkel TA. Unique error signature of the four-subunit yeast DNA polymerase epsilon. J Biol Chem. 2003; 278:43770–43780. [PubMed: 12882968]
58. Beard WA, Wilson SH. Structural insights into the origins of DNA polymerase fidelity. Structure. 2003; 11:489–496. [PubMed: 12737815]
59. Goodman MF, Fygenson KD. DNA polymerase fidelity: from genetics toward a biochemical understanding. Genetics. 1998; 148:1475–1482. [PubMed: 9560367]
60. Johnson KA. Conformational coupling in DNA polymerase fidelity. Annu Rev Biochem. 1993; 62:685–713. [PubMed: 7688945]
61. Joyce CM, Benkovic SJ. DNA polymerase fidelity: kinetics, structure, and checkpoints. Biochemistry. 2004; 43:14317–14324. [PubMed: 15533035]
62. Kool ET. Active site tightness and substrate fit in DNA replication. Annu Rev Biochem. 2002; 71:191–219. [PubMed: 12045095]
63. Kunkel TA. DNA replication fidelity. J Biol Chem. 2004; 279:16895–16898. [PubMed: 14988392]
64. Morrison A, Bell JB, Kunkel TA, Sugino A. Eukaryotic DNA polymerase amino acid sequence required for 3'---5' exonuclease activity. Proc Natl Acad Sci U S A. 1991; 88:9473–9477. [PubMed: 1658784]
65. Shcherbakova PV, Pavlov YI. 3'-->5' exonucleases of DNA polymerases epsilon and delta correct base analog induced DNA replication errors on opposite DNA strands in Saccharomyces cerevisiae. Genetics. 1996; 142:717–726. [PubMed: 8849882]
66. Garcia-Diaz M, Kunkel TA. Mechanism of a genetic glissando: structural biology of indel mutations. Trends Biochem Sci. 2006; 31:206–214. [PubMed: 16545956]
67. Fuss J, Linn S. Human DNA polymerase epsilon colocalizes with proliferating cell nuclear antigen and DNA replication late, but not early, in S phase. J Biol Chem. 2002; 277:8658–8666. [PubMed: 11741962]
68. DePamphilis ML. The 'ORC cycle': a novel pathway for regulating eukaryotic DNA replication. Gene. 2003; 310:1–15. [PubMed: 12801628]
69. Diffley JF. Regulation of early events in chromosome replication. Curr Biol. 2004; 14:R778–R786. [PubMed: 15380092]
70. Boskovic J, Coloma J, Aparicio T, Zhou M, Robinson CV, Mendez J, Montoya G. Molecular architecture of the human GINS complex. EMBO reports. 2007; 8:678–684. [PubMed: 17557111]
71. Kornberg, A.; Baker, T. DNA Replication. 2d ed.. New York: W.H. Freeman and Company; 1992.
72. Morrison A, Araki H, Clark AB, Hamatake RK, Sugino A. A third essential DNA polymerase in S. cerevisiae. Cell. 1990; 62:1143–1151. [PubMed: 2169349]
73. Johnson LM, Snyder M, Chang LM, Davis RW, Campbell JL. Isolation of the gene encoding yeast DNA polymerase I. Cell. 1985; 43:369–377. [PubMed: 3907855]
74. Newlon CS. Yeast chromosome replication and segregation. Microbiological reviews. 1988; 52:568–601. [PubMed: 3070325]

75. Araki H, Ropp PA, Johnson AL, Johnston LH, Morrison A, Sugino A. DNA polymerase II, the probable homolog of mammalian DNA polymerase epsilon, replicates chromosomal DNA in the yeast Saccharomyces cerevisiae. Embo J. 1992; 11:733–740. [PubMed: 1537345]
76. Budd ME, Campbell JL. DNA polymerases delta and epsilon are required for chromosomal replication in Saccharomyces cerevisiae. Mol Cell Biol. 1993; 13:496–505. [PubMed: 8417347]
77. Navas TA, Zhou Z, Elledge SJ. DNA polymerase epsilon links the DNA replication machinery to the S phase checkpoint. Cell. 1995; 80:29–39. [PubMed: 7813016]
78. Spellman PT, Sherlock G, Zhang MQ, Iyer VR, Anders K, Eisen MB, Brown PO, Botstein D, Futcher B. Comprehensive identification of cell cycle-regulated genes of the yeast Saccharomyces cerevisiae by microarray hybridization. Mol Biol Cell. 1998; 9:3273–3297. [PubMed: 9843569]
79. Kamimura Y, Masumoto H, Sugino A, Araki H. Sld2, which interacts with Dpb11 in Saccharomyces cerevisiae, is required for chromosomal DNA replication. Mol Cell Biol. 1998; 18:6102–6109. [PubMed: 9742127]
80. Wang H, Elledge SJ. DRC1, DNA replication and checkpoint protein 1, functions with DPB11 to control DNA replication and the S-phase checkpoint in Saccharomyces cerevisiae. Proc Natl Acad Sci U S A. 1999; 96:3824–3829. [PubMed: 10097122]
81. Waga S, Masuda T, Takisawa H, Sugino A. DNA polymerase epsilon is required for coordinated and efficient chromosomal DNA replication in Xenopus egg extracts. Proc Natl Acad Sci U S A. 2001; 98:4978–4983. [PubMed: 11296256]
82. Johnson A, O'Donnell M. Cellular DNA replicases: components and dynamics at the replication fork. Annu Rev Biochem. 2005; 74:283–315. [PubMed: 15952889]
83. McHenry CS. DNA polymerase III holoenzyme. Components, structure, and mechanism of a true replicative complex. J Biol Chem. 1991; 266:19127–19130. [PubMed: 1918028]
84. Podust VN, Hubscher U. Lagging strand DNA synthesis by calf thymus DNA polymerases alpha, beta, delta and epsilon in the presence of auxiliary proteins. Nucleic Acids Res. 1993; 21:841–846. [PubMed: 8451186]
85. Fukui T, Yamauchi K, Muroya T, Akiyama M, Maki H, Sugino A, Waga S. Distinct roles of DNA polymerases delta and epsilon at the replication fork in Xenopus egg extracts. Genes Cells. 2004; 9:179–191. [PubMed: 15005706]
86. Garg P, Stith CM, Sabouri N, Johansson E, Burgers PM. Idling by DNA polymerase delta maintains a ligatable nick during lagging-strand DNA replication. Genes Dev. 2004; 18:2764–2773. [PubMed: 15520275]
87. Jin YH, Obert R, Burgers PM, Kunkel TA, Resnick MA, Gordenin DA. The 3'-->' exonuclease of DNA polymerase delta can substitute for the 5' flap endonuclease Rad27/Fen1 in processing Okazaki fragments and preventing genome instability. Proc Natl Acad Sci U S A. 2001; 98:5122–5127. [PubMed: 11309502]
88. Waga S, Stillman B. Anatomy of a DNA replication fork revealed by reconstitution of SV40 DNA replication in vitro. Nature. 1994; 369:207–212. [PubMed: 7910375]
89. Zlotkin T, Kaufmann G, Jiang Y, Lee MY, Uitto L, Syvaoja J, Dornreiter I, Fanning E, Nethanel T. DNA polymerase epsilon may be dispensable for SV40- but not cellular-DNA replication. Embo J. 1996; 15:2298–2305. [PubMed: 8641295]
90. Dodson M, Dean FB, Bullock P, Echols H, Hurwitz J. Unwinding of duplex DNA from the SV40 origin of replication by T antigen. Science. 1987; 238:964–967. [PubMed: 2823389]
91. Huberman JA. Cell cycle control of initiation of eukaryotic DNA replication. Chromosoma. 1991; 100:419–423. [PubMed: 1914662]
92. Dua R, Levy DL, Campbell JL. Analysis of the essential functions of the C-terminal protein/protein interaction domain of Saccharomyces cerevisiae pol epsilon and its unexpected ability to support growth in the absence of the DNA polymerase domain. J Biol Chem. 1999; 274:22283–22288. [PubMed: 10428796]
93. Feng W, D'Urso G. Schizosaccharomyces pombe cells lacking the amino-terminal catalytic domains of DNA polymerase epsilon are viable but require the DNA damage checkpoint control. Mol Cell Biol. 2001; 21:4495–4504. [PubMed: 11416129]

94. Kesti T, Flick K, Keranen S, Syvaoja JE, Wittenberg C. DNA polymerase epsilon catalytic domains are dispensable for DNA replication, DNA repair, and cell viability. Mol Cell. 1999; 3:679–685. [PubMed: 10360184]
95. Diede SJ, Gottschling DE. Telomerase-mediated telomere addition in vivo requires DNA primase and DNA polymerases alpha and delta. Cell. 1999; 99:723–733. [PubMed: 10619426]
96. Karthikeyan R, Vonarx EJ, Straffon AF, Simon M, Faye G, Kunz BA. Evidence from mutational specificity studies that yeast DNA polymerases delta and epsilon replicate different DNA strands at an intracellular replication fork. J Mol Biol. 2000; 299:405–419. [PubMed: 10860748]
97. Pursell ZF, Isoz I, Lundstrom EB, Johansson E, Kunkel TA. Yeast DNA polymerase epsilon participates in leading-strand DNA replication. Science. 2007; 317:127–130. [PubMed: 17615360]
98. Nick McElhinny SA, Gordenin DA, Stith CM, Burgers PM, Kunkel TA. Division of Labor at the Eukaryotic Replication Fork. Molecular Cell. 2008 **In press**.
99. Venkatesan RN, Hsu JJ, Lawrence NA, Preston BD, Loeb LA. Mutator phenotypes caused by substitution at a conserved motif A residue in eukaryotic DNA polymerase delta. J Biol Chem. 2006; 281:4486–4494. [PubMed: 16344551]
100. Sancar A, Lindsey-Boltz LA, Unsal-Kacmaz K, Linn S. Molecular mechanisms of mammalian DNA repair and the DNA damage checkpoints. Annu Rev Biochem. 2004; 73:39–85. [PubMed: 15189136]
101. Navas TA, Sanchez Y, Elledge SJ. RAD9 and DNA polymerase epsilon form parallel sensory branches for transducing the DNA damage checkpoint signal in Saccharomyces cerevisiae. Genes Dev. 1996; 10:2632–2643. [PubMed: 8895664]
102. Gangloff S, McDonald JP, Bendixen C, Arthur L, Rothstein R. The yeast type I topoisomerase Top3 interacts with Sgs1, a DNA helicase homolog: a potential eukaryotic reverse gyrase. Mol Cell Biol. 1994; 14:8391–8398. [PubMed: 7969174]
103. Frei C, Gasser SM. The yeast Sgs1p helicase acts upstream of Rad53p in the DNA replication checkpoint and colocalizes with Rad53p in S-phase-specific foci. Genes Dev. 2000; 14:81–96. [PubMed: 10640278]
104. Cobb JA, Bjergbaek L, Shimada K, Frei C, Gasser SM. DNA polymerase stabilization at stalled replication forks requires Mec1 and the RecQ helicase Sgs1. Embo J. 2003; 22:4325–4336. [PubMed: 12912929]
105. Bjergbaek L, Cobb JA, Tsai-Pflugfelder M, Gasser SM. Mechanistically distinct roles for Sgs1p in checkpoint activation and replication fork maintenance. Embo J. 2005; 24:405–417. [PubMed: 15616582]
106. Calzada A, Hodgson B, Kanemaki M, Bueno A, Labib K. Molecular anatomy and regulation of a stable replisome at a paused eukaryotic DNA replication fork. Genes Dev. 2005; 19:1905–1919. [PubMed: 16103218]
107. Lopes M, Cotta-Ramusino C, Pellicioli A, Liberi G, Plevani P, Muzi-Falconi M, Newlon CS, Foiani M. The DNA replication checkpoint response stabilizes stalled replication forks. Nature. 2001; 412:557–561. [PubMed: 11484058]
108. Henneke G, Koundrioukoff S, Hubscher U. Phosphorylation of human Fen1 by cyclin-dependent kinase modulates its role in replication fork regulation. Oncogene. 2003; 22:4301–4313. [PubMed: 12853968]
109. Ubersax JA, Woodbury EL, Quang PN, Paraz M, Blethrow JD, Shah K, Shokat KM, Morgan DO. Targets of the cyclin-dependent kinase Cdk1. Nature. 2003; 425:859–864. [PubMed: 14574415]
110. Bloom J, Cross FR. Novel role for Cdc14 sequestration: Cdc14 dephosphorylates factors that promote DNA replication. Mol Cell Biol. 2007; 27:842–853. [PubMed: 17116692]
111. Voitenleitner C, Fanning E, Nasheuer HP. Phosphorylation of DNA polymerase alpha-primase by cyclin A-dependent kinases regulates initiation of DNA replication in vitro. Oncogene. 1997; 14:1611–1615. [PubMed: 9129153]
112. Lau A, Blitzblau H, Bell SP. Cell-cycle control of the establishment of mating-type silencing in S. cerevisiae. Genes Dev. 2002; 16:2935–2945. [PubMed: 12435634]
113. Levin DS, McKenna AE, Motycka TA, Matsumoto Y, Tomkinson AE. Interaction between PCNA and DNA ligase I is critical for joining of Okazaki fragments and long-patch base-excision repair. Curr Biol. 2000; 10:919–922. [PubMed: 10959839]

114. Smith JS, Caputo E, Boeke JD. A genetic screen for ribosomal DNA silencing defects identifies multiple DNA replication and chromatin-modulating factors. Mol Cell Biol. 1999; 19:3184–3197. [PubMed: 10082585]

115. Ehrenhofer-Murray AE, Kamakaka RT, Rine J. A role for the replication proteins PCNA, RF-C, polymerase epsilon and Cdc45 in transcriptional silencing in Saccharomyces cerevisiae. Genetics. 1999; 153:1171–1182. [PubMed: 10545450]

116. Chan SR, Blackburn EH. Telomeres and telomerase. Philosophical transactions of the Royal Society of London. 2004; 359:109–121. [PubMed: 15065663]

117. Gilson E, Geli V. How telomeres are replicated. Nature reviews. 2007; 8:825–838.

118. Zhang X, Mar V, Zhou W, Harrington L, Robinson MO. Telomere shortening and apoptosis in telomerase-inhibited human tumor cells. Genes Dev. 1999; 13:2388–2399. [PubMed: 10500096]

119. Greider CW, Blackburn EH. The telomere terminal transferase of Tetrahymena is a ribonucleoprotein enzyme with two kinds of primer specificity. Cell. 1987; 51:887–898. [PubMed: 3319189]

120. Blasco MA. The epigenetic regulation of mammalian telomeres. Nat Rev Genet. 2007; 8:299–309. [PubMed: 17363977]

121. Hecht A, Strahl-Bolsinger S, Grunstein M. Spreading of transcriptional repressor SIR3 from telomeric heterochromatin. Nature. 1996; 383:92–96. [PubMed: 8779721]

122. Benetti R, Garcia-Cao M, Blasco MA. Telomere length regulates the epigenetic status of mammalian telomeres and subtelomeres. Nature genetics. 2007; 39:243–250. [PubMed: 17237781]

123. Bianchi A, Shore D. Early replication of short telomeres in budding yeast. Cell. 2007; 128:1051–1062. [PubMed: 17382879]

124. Iida T, Araki H. Noncompetitive counteractions of DNA polymerase epsilon and ISW2/yCHRAC for epigenetic inheritance of telomere position effect in Saccharomyces cerevisiae. Mol Cell Biol. 2004; 24:217–227. [PubMed: 14673157]

125. Poot RA, Dellaire G, Hulsmann BB, Grimaldi MA, Corona DF, Becker PB, Bickmore WA, Varga-Weisz PD. HuCHRAC, a human ISWI chromatin remodelling complex contains hACF1 and two novel histone-fold proteins. Embo J. 2000; 19:3377–3387. [PubMed: 10880450]

126. Henikoff S. Nucleosome destabilization in the epigenetic regulation of gene expression. Nat Rev Genet. 2008; 9:15–26. [PubMed: 18059368]

127. Groth A, Rocha W, Verreault A, Almouzni G. Chromatin challenges during DNA replication and repair. Cell. 2007; 128:721–733. [PubMed: 17320509]

128. Wang GG, Allis CD, Chi P. Chromatin remodeling and cancer, Part II: ATP-dependent chromatin remodeling. Trends in molecular medicine. 2007; 13:373–380. [PubMed: 17822959]

129. Tate P, Lee M, Tweedie S, Skarnes WC, Bickmore WA. Capturing novel mouse genes encoding chromosomal and other nuclear proteins. J Cell Sci. 1998; 111(Pt 17):2575–2585. [PubMed: 9701556]

130. Corona DF, Eberharter A, Budde A, Deuring R, Ferrari S, Varga-Weisz P, Wilm M, Tamkun J, Becker PB. Two histone fold proteins, CHRAC-14 and CHRAC-16, are developmentally regulated subunits of chromatin accessibility complex (CHRAC). Embo J. 2000; 19:3049–3059. [PubMed: 10856248]

131. Varga-Weisz PD, Wilm M, Bonte E, Dumas K, Mann M, Becker PB. Chromatin-remodelling factor CHRAC contains the ATPases ISWI and topoisomerase II. Nature. 1997; 388:598–602. [PubMed: 9252192]

132. Tsukiyama T, Palmer J, Landel CC, Shiloach J, Wu C. Characterization of the imitation switch subfamily of ATP-dependent chromatin-remodeling factors in Saccharomyces cerevisiae. Genes Dev. 1999; 13:686–697. [PubMed: 10090725]

133. McConnell AD, Gelbart ME, Tsukiyama T. Histone fold protein Dls1p is required for Isw2-dependent chromatin remodeling in vivo. Mol Cell Biol. 2004; 24:2605–2613. [PubMed: 15024052]

134. Kukimoto I, Elderkin S, Grimaldi M, Oelgeschlager T, Varga-Weisz PD. The histone-fold protein complex CHRAC-15/17 enhances nucleosome sliding and assembly mediated by ACF. Mol Cell. 2004; 13:265–277. [PubMed: 14759371]

135. Tackett AJ, Dilworth DJ, Davey MJ, O'Donnell M, Aitchison JD, Rout MP, Chait BT. Proteomic and genomic characterization of chromatin complexes at a boundary. J Cell Biol. 2005; 169:35–47. [PubMed: 15824130]

136. Tsubota T, Maki S, Kubota H, Sugino A, Maki H. Double-stranded DNA binding properties of Saccharomyces cerevisiae DNA polymerase epsilon and of the Dpb3p-Dpb4p subassembly. Genes Cells. 2003; 8:873–888. [PubMed: 14622139]

137. Dang W, Kagalwala MN, Bartholomew B. The Dpb4 subunit of ISW2 is anchored to extranucleosomal DNA. J Biol Chem. 2007; 282:19418–19425. [PubMed: 17491017]

138. Hartlepp KF, Fernandez-Tornero C, Eberharter A, Grune T, Muller CW, Becker PB. The histone fold subunits of Drosophila CHRAC facilitate nucleosome sliding through dynamic DNA interactions. Mol Cell Biol. 2005; 25:9886–9896. [PubMed: 16260604]

139. Collins N, Poot RA, Kukimoto I, Garcia-Jimenez C, Dellaire G, Varga-Weisz PD. An ACF1-ISWI chromatin-remodeling complex is required for DNA replication through heterochromatin. Nature genetics. 2002; 32:627–632. [PubMed: 12434153]

140. Carson DR, Christman MF. Evidence that replication fork components catalyze establishment of cohesion between sister chromatids. Proc Natl Acad Sci U S A. 2001; 98:8270–8275. [PubMed: 11459963]

141. Edwards S, Li CM, Levy DL, Brown J, Snow PM, Campbell JL. Saccharomyces cerevisiae DNA polymerase epsilon and polymerase sigma interact physically and functionally, suggesting a role for polymerase epsilon in sister chromatid cohesion. Mol Cell Biol. 2003; 23:2733–2748. [PubMed: 12665575]

142. Haracska L, Johnson RE, Prakash L, Prakash S. Trf4 and Trf5 proteins of Saccharomyces cerevisiae exhibit poly(A) RNA polymerase activity but no DNA polymerase activity. Mol Cell Biol. 2005; 25:10183–10189. [PubMed: 16260630]

143. Hakimi MA, Bochar DA, Schmiesing JA, Dong Y, Barak OG, Speicher DW, Yokomori K, Shiekhattar R. A chromatin remodelling complex that loads cohesin onto human chromosomes. Nature. 2002; 418:994–998. [PubMed: 12198550]

144. Cheng L, Kelly TJ. Transcriptional activator nuclear factor I stimulates the replication of SV40 minichromosomes in vivo and in vitro. Cell. 1989; 59:541–551. [PubMed: 2553273]

145. Alexiadis V, Varga-Weisz PD, Bonte E, Becker PB, Gruss C. In vitro chromatin remodelling by chromatin accessibility complex (CHRAC) at the SV40 origin of DNA replication. Embo J. 1998; 17:3428–3438. [PubMed: 9628878]

146. Coverley D, Kenny MK, Lane DP, Wood RD. A role for the human single-stranded DNA binding protein HSSB/RPA in an early stage of nucleotide excision repair. Nucleic Acids Res. 1992; 20:3873–3880. [PubMed: 1508673]

147. Friedberg, EC.; Walker, GC.; Siede, W.; Wood, RD.; Schultz, RA.; Ellenberger, T. DNA Repair and Mutagenesis. 2d ed.. Washington, D.C.: ASM Press; 2006.

148. Beard WA, Wilson SH. Structure and mechanism of DNA polymerase Beta. Chemical reviews. 2006; 106:361–382. [PubMed: 16464010]

149. Horton JK, Watson M, Stefanick DF, Shaughnessy DT, Taylor JA, Wilson SH. XRCC1 and DNA polymerase beta in cellular protection against cytotoxic DNA single-strand breaks. Cell research. 2008; 18:48–63. [PubMed: 18166976]

150. Sobol RW, Horton JK, Kuhn R, Gu H, Singhal RK, Prasad R, Rajewsky K, Wilson SH. Requirement of mammalian DNA polymerase-beta in base-excision repair. Nature. 1996; 379:183–186. [PubMed: 8538772]

151. Parlanti E, Locatelli G, Maga G, Dogliotti E. Human base excision repair complex is physically associated to DNA replication and cell cycle regulatory proteins. Nucleic Acids Res. 2007; 35:1569–1577. [PubMed: 17289756]

152. Stucki M, Pascucci B, Parlanti E, Fortini P, Wilson SH, Hubscher U, Dogliotti E. Mammalian base excision repair by DNA polymerases delta and epsilon. Oncogene. 1998; 17:835–843. [PubMed: 9780000]

153. Wang Z, Wu X, Friedberg EC. DNA repair synthesis during base excision repair in vitro is catalyzed by DNA polymerase epsilon and is influenced by DNA polymerases alpha and delta in Saccharomyces cerevisiae. Mol Cell Biol. 1993; 13:1051–1058. [PubMed: 8423775]

154. Aboussekhra A, Biggerstaff M, Shivji MK, Vilpo JA, Moncollin V, Podust VN, Protic M, Hubscher U, Egly JM, Wood RD. Mammalian DNA nucleotide excision repair reconstituted with purified protein components. Cell. 1995; 80:859–868. [PubMed: 7697716]

155. Shivji MK, Podust VN, Hubscher U, Wood RD. Nucleotide excision repair DNA synthesis by DNA polymerase epsilon in the presence of PCNA, RFC, and RPA. Biochemistry. 1995; 34:5011–5017. [PubMed: 7711023]

156. Mozzherin DJ, Fisher PA. Human DNA polymerase epsilon: enzymologic mechanism and gap-filling synthesis. Biochemistry. 1996; 35:3572–3577. [PubMed: 8639508]

157. Budd ME, Campbell JL. DNA polymerases required for repair of UV-induced damage in Saccharomyces cerevisiae. Mol Cell Biol. 1995; 15:2173–2179. [PubMed: 7891712]

158. Wu X, Guo D, Yuan F, Wang Z. Accessibility of DNA polymerases to repair synthesis during nucleotide excision repair in yeast cell-free extracts. Nucleic Acids Res. 2001; 29:3123–3130. [PubMed: 11452038]

159. Moser J, Kool H, Giakzidis I, Caldecott K, Mullenders LH, Fousteri MI. Sealing of chromosomal DNA nicks during nucleotide excision repair requires XRCC1 and DNA ligase III alpha in a cell-cycle-specific manner. Mol Cell. 2007; 27:311–323. [PubMed: 17643379]

160. Wu X, Braithwaite E, Wang Z. DNA ligation during excision repair in yeast cell-free extracts is specifically catalyzed by the CDC9 gene product. Biochemistry. 1999; 38:2628–2635. [PubMed: 10052932]

161. Cappelli E, Taylor R, Cevasco M, Abbondandolo A, Caldecott K, Frosina G. Involvement of XRCC1 and DNA ligase III gene products in DNA base excision repair. J Biol Chem. 1997; 272:23970–23975. [PubMed: 9295348]

162. Dronkert ML, Kanaar R. Repair of DNA interstrand cross-links. Mutat Res. 2001; 486:217–247. [PubMed: 11516927]

163. McHugh PJ, Sarkar S. DNA interstrand cross-link repair in the cell cycle: a critical role for polymerase zeta in G1 phase. Cell cycle (Georgetown, Tex. 2006; 5:1044–1047.

164. Constantin N, Dzantiev L, Kadyrov FA, Modrich P. Human mismatch repair: reconstitution of a nick-directed bidirectional reaction. J Biol Chem. 2005; 280:39752–39761. [PubMed: 16188885]

165. Dzantiev L, Constantin N, Genschel J, Iyer RR, Burgers PM, Modrich P. A defined human system that supports bidirectional mismatch-provoked excision. Mol Cell. 2004; 15:31–41. [PubMed: 15225546]

166. Longley MJ, Pierce AJ, Modrich P. DNA polymerase delta is required for human mismatch repair in vitro. J Biol Chem. 1997; 272:10917–10921. [PubMed: 9099749]

167. Valerie K, Povirk LF. Regulation and mechanisms of mammalian double-strand break repair. Oncogene. 2003; 22:5792–5812. [PubMed: 12947387]

168. Haber JE. Transpositions and translocations induced by site-specific double-strand breaks in budding yeast. DNA repair. 2006; 5:998–1009. [PubMed: 16807137]

169. Weterings E, Chen DJ. The endless tale of non-homologous end-joining. Cell research. 2008; 18:114–124. [PubMed: 18166980]

170. Holmes AM, Haber JE. Double-strand break repair in yeast requires both leading and lagging strand DNA polymerases. Cell. 1999; 96:415–424. [PubMed: 10025407]

171. Wang X, Ira G, Tercero JA, Holmes AM, Diffley JF, Haber JE. Role of DNA replication proteins in double-strand break-induced recombination in Saccharomyces cerevisiae. Mol Cell Biol. 2004; 24:6891–6899. [PubMed: 15282291]

172. Bianco PR, Tracy RB, Kowalczykowski SC. DNA strand exchange proteins: a biochemical and physical comparison. Front Biosci. 1998; 3:D570–D603. [PubMed: 9632377]

173. Malkova A, Ivanov EL, Haber JE. Double-strand break repair in the absence of RAD51 in yeast: a possible role for break-induced DNA replication. Proc Natl Acad Sci U S A. 1996; 93:7131–7136. [PubMed: 8692957]

174. Chen Q, Ijpma A, Greider CW. Two survivor pathways that allow growth in the absence of telomerase are generated by distinct telomere recombination events. Mol Cell Biol. 2001; 21:1819–1827. [PubMed: 11238918]

175. Lydeard JR, Jain S, Yamaguchi M, Haber JE. Break-induced replication and telomerase-independent telomere maintenance require Pol32. Nature. 2007; 448:820–823. [PubMed: 17671506]

176. Jessberger R, Berg P. Repair of deletions and double-strand gaps by homologous recombination in a mammalian in vitro system. Mol Cell Biol. 1991; 11:445–457. [PubMed: 1986239]

177. Jessberger R, Chui G, Linn S, Kemper B. Analysis of the mammalian recombination protein complex RC-1. Mutat Res. 1996; 350:217–227. [PubMed: 8657184]

178. Jessberger R, Podust V, Hubscher U, Berg P. A mammalian protein complex that repairs double-strand breaks and deletions by recombination. J Biol Chem. 1993; 268:15070–15079. [PubMed: 8392064]

179. Jessberger R, Riwar B, Rolink A, Rodewald HR. Stimulation of defective DNA transfer activity in recombination deficient SCID cell extracts by a 72-kDa protein from wild-type thymocytes. J Biol Chem. 1995; 270:6788–6797. [PubMed: 7896825]

180. Kamel D, Mackey ZB, Sjoblom T, Walter CA, McCarrey JR, Uitto L, Palosaari H, Lahdetie J, Tomkinson AE, Syvaoja JE. Role of deoxyribonucleic acid polymerase epsilon in spermatogenesis in mice. Biology of reproduction. 1997; 57:1367–1374. [PubMed: 9408242]

181. Kaeberlein M, McVey M, Guarente L. The SIR2/3/4 complex and SIR2 alone promote longevity in Saccharomyces cerevisiae by two different mechanisms. Genes Dev. 1999; 13:2570–2580. [PubMed: 10521401]

182. Rusche LN, Kirchmaier AL, Rine J. The establishment, inheritance, and function of silenced chromatin in Saccharomyces cerevisiae. Annu Rev Biochem. 2003; 72:481–516. [PubMed: 12676793]

183. Coffman FD, He M, Diaz ML, Cohen S. DNA replication initiates at different sites in early and late S phase within human ribosomal RNA genes. Cell cycle (Georgetown, Tex. 2005; 4:1223–1226.

184. Houseley J, Kotovic K, El Hage A, Tollervey D. Trf4 targets ncRNAs from telomeric and rDNA spacer regions and functions in rDNA copy number control. Embo J. 2007; 26:4996–5006. [PubMed: 18007593]

185. Gellon L, Carson DR, Carson JP, Demple B. Intrinsic 5'-deoxyribose-5-phosphate lyase activity in Saccharomyces cerevisiae Trf4 protein with a possible role in base excision DNA repair. DNA repair. 2008; 7:187–198. [PubMed: 17983848]

186. Guarente L. Diverse and dynamic functions of the Sir silencing complex. Nature genetics. 1999; 23:281–285. [PubMed: 10545947]

187. Nasmyth K, Nurse P. Cell division cycle mutants altered in DNA replication and mitosis in the fission yeast Schizosaccharomyces pombe. Mol Gen Genet. 1981; 182:119–124. [PubMed: 6943408]

188. Grallert B, Sipiczki M. Common genes and pathways in the regulation of the mitotic and meiotic cell cycles of Schizosaccharomyces pombe. Current genetics. 1991; 20:199–204. [PubMed: 1934126]

189. Francesconi S, De Recondo AM, Baldacci G. DNA polymerase delta is required for the replication feedback control of cell cycle progression in Schizosaccharomyces pombe. Mol Gen Genet. 1995; 246:561–569. [PubMed: 7700230]

190. D'Urso G, Nurse P. Schizosaccharomyces pombe cdc20+ encodes DNA polymerase epsilon and is required for chromosomal replication but not for the S phase checkpoint. Proc Natl Acad Sci U S A. 1997; 94:12491–12496. [PubMed: 9356477]

191. Wang SW, Toda T, MacCallum R, Harris AL, Norbury C. Cid1, a fission yeast protein required for S-M checkpoint control when DNA polymerase delta or epsilon is inactivated. Mol Cell Biol. 2000; 20:3234–3244. [PubMed: 10757807]

192. Feng W, Rodriguez-Menocal L, Tolun G, D'Urso G. Schizosacchromyces pombe Dpb2 binds to origin DNA early in S phase and is required for chromosomal DNA replication. Mol Biol Cell. 2003; 14:3427–3436. [PubMed: 12925774]

193. Spiga MG, D'Urso G. Identification and cloning of two putative subunits of DNA polymerase epsilon in fission yeast. Nucleic Acids Res. 2004; 32:4945–4953. [PubMed: 15388803]

194. Siam R, Gomez EB, Forsburg SL. Schizosaccharomyces pombe Rad4/Cut5 protein modification and chromatin binding changes in DNA damage. DNA and cell biology. 2007; 26:565–575. [PubMed: 17688408]

195. Shikata K, Sasa-Masuda T, Okuno Y, Waga S, Sugino A. The DNA polymerase activity of Pol epsilon holoenzyme is required for rapid and efficient chromosomal DNA replication in Xenopus egg extracts. BMC biochemistry. 2006; 7:21. [PubMed: 16925818]

196. Aparicio OM, Stout AM, Bell SP. Differential assembly of Cdc45p and DNA polymerases at early and late origins of DNA replication. Proc Natl Acad Sci U S A. 1999; 96:9130–9135. [PubMed: 10430907]

197. Mimura S, Masuda T, Matsui T, Takisawa H. Central role for cdc45 in establishing an initiation complex of DNA replication in Xenopus egg extracts. Genes Cells. 2000; 5:439–452. [PubMed: 10886370]

198. Matsuno K, Kumano M, Kubota Y, Hashimoto Y, Takisawa H. The N-terminal noncatalytic region of Xenopus RecQ4 is required for chromatin binding of DNA polymerase alpha in the initiation of DNA replication. Mol Cell Biol. 2006; 26:4843–4852. [PubMed: 16782873]

199. Bohr VA. Deficient DNA repair in the human progeroid disorder, Werner syndrome. Mutat Res. 2005; 577:252–259. [PubMed: 15916783]

200. Lebel M, Spillare EA, Harris CC, Leder P. The Werner syndrome gene product co-purifies with the DNA replication complex and interacts with PCNA and topoisomerase I. J Biol Chem. 1999; 274:37795–37799. [PubMed: 10608841]

201. Spanos A, Holliday R, German J. Bloom's syndrome. XIII. DNA-polymerase activity of cultured lymphoblastoid cells. Human genetics. 1986; 73:119–122. [PubMed: 3013755]

202. Brosh RM Jr, Waheed J, Sommers JA. Biochemical characterization of the DNA substrate specificity of Werner syndrome helicase. J Biol Chem. 2002; 277:23236–23245. [PubMed: 11956187]

203. Sasakawa N, Fukui T, Waga S. Accumulation of FFA-1, the Xenopus homolog of Werner helicase, and DNA polymerase delta on chromatin in response to replication fork arrest. Journal of biochemistry. 2006; 140:95–103. [PubMed: 16798775]

204. Lee J, Kumagai A, Dunphy WG. Claspin, a Chk1-regulatory protein, monitors DNA replication on chromatin independently of RPA, ATR, and Rad17. Mol Cell. 2003; 11:329–340. [PubMed: 12620222]

205. Kumagai A, Dunphy WG. Claspin, a novel protein required for the activation of Chk1 during a DNA replication checkpoint response in Xenopus egg extracts. Mol Cell. 2000; 6:839–849. [PubMed: 11090622]

206. Lee J, Gold DA, Shevchenko A, Shevchenko A, Dunphy WG. Roles of replication fork-interacting and Chk1-activating domains from Claspin in a DNA replication checkpoint response. Mol Biol Cell. 2005; 16:5269–5282. [PubMed: 16148040]

207. Fortune JM, Pavlov YI, Welch CM, Johansson E, Burgers PM, Kunkel TA. Saccharomyces cerevisiae DNA polymerase delta: high fidelity for base substitutions but lower fidelity for single- and multi-base deletions. J Biol Chem. 2005; 280:29980–29987. [PubMed: 15964835]

208. Fortune JM, Stith CM, Kissling GE, Burgers PM, Kunkel TA. RPA and PCNA suppress formation of large deletion errors by yeast DNA polymerase delta. Nucleic Acids Res. 2006; 34:4335–4341. [PubMed: 16936322]

209. Kunkel TA, Hamatake RK, Motto-Fox J, Fitzgerald MP, Sugino A. Fidelity of DNA polymerase I and the DNA polymerase I-DNA primase complex from Saccharomyces cerevisiae. Mol Cell Biol. 1989; 9:4447–4458. [PubMed: 2555694]

210. Bebenek K, Garcia-Diaz M, Patishall SR, Kunkel TA. Biochemical properties of Saccharomyces cerevisiae DNA polymerase IV. J Biol Chem. 2005; 280:20051–20058. [PubMed: 15778218]

211. McCulloch SD, Wood A, Garg P, Burgers PM, Kunkel TA. Effects of accessory proteins on the bypass of a cis-syn thymine-thymine dimer by Saccharomyces cerevisiae DNA polymerase eta. Biochemistry. 2007; 46:8888–8896. [PubMed: 17608453]

212. Nick McElhinny SA, Stith CM, Burgers PM, Kunkel TA. Inefficient proofreading and biased error rates during inaccurate DNA synthesis by a mutant derivative of Saccharomyces cerevisiae DNA polymerase delta. J Biol Chem. 2007; 282:2324–2332. [PubMed: 17121822]

213. Harp JM, Hanson BL, Timm DE, Bunick GJ. Asymmetries in the nucleosome core particle at 2.5 A resolution. Acta crystallographica. 2000; 56:1513–1534.

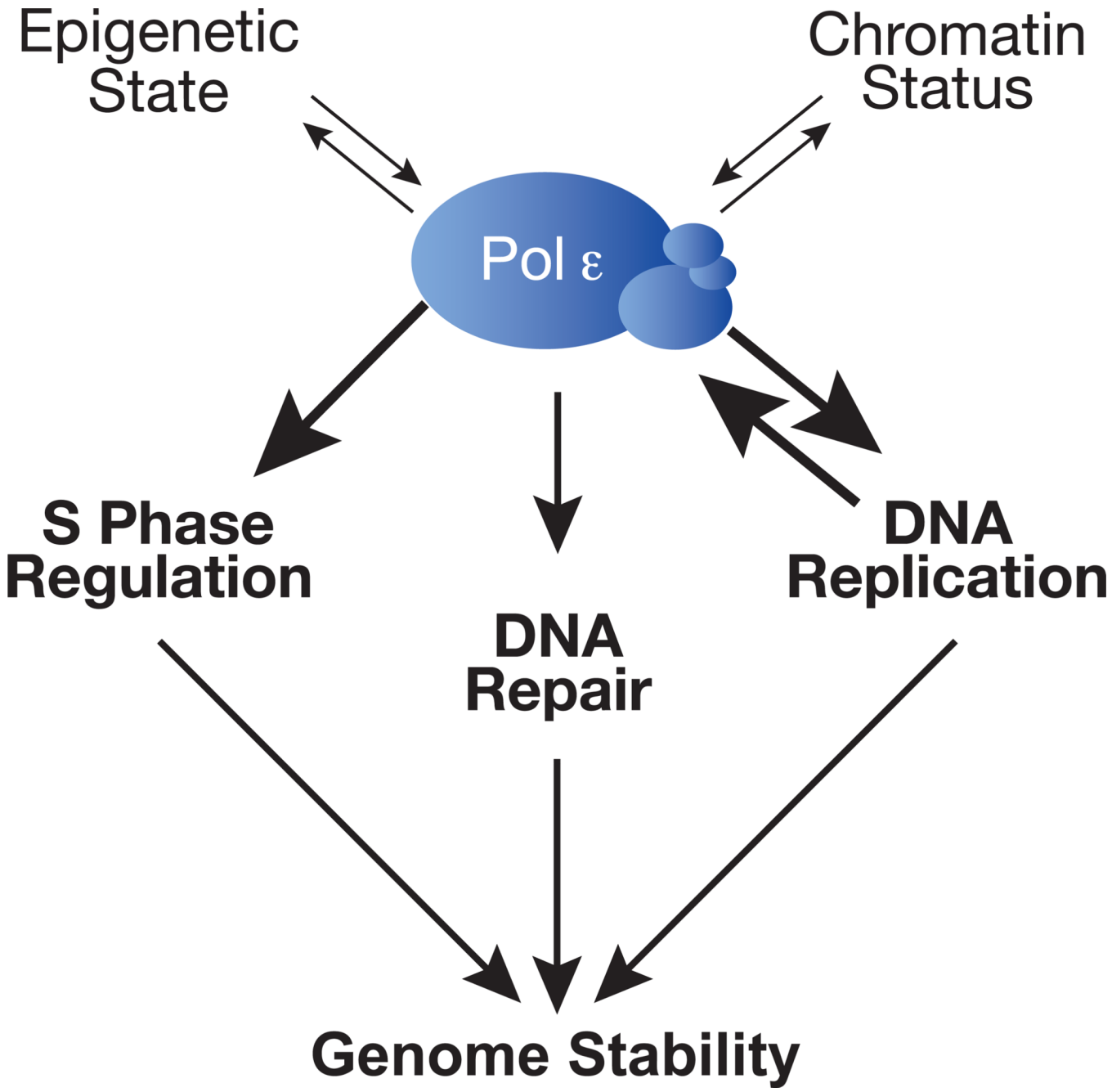
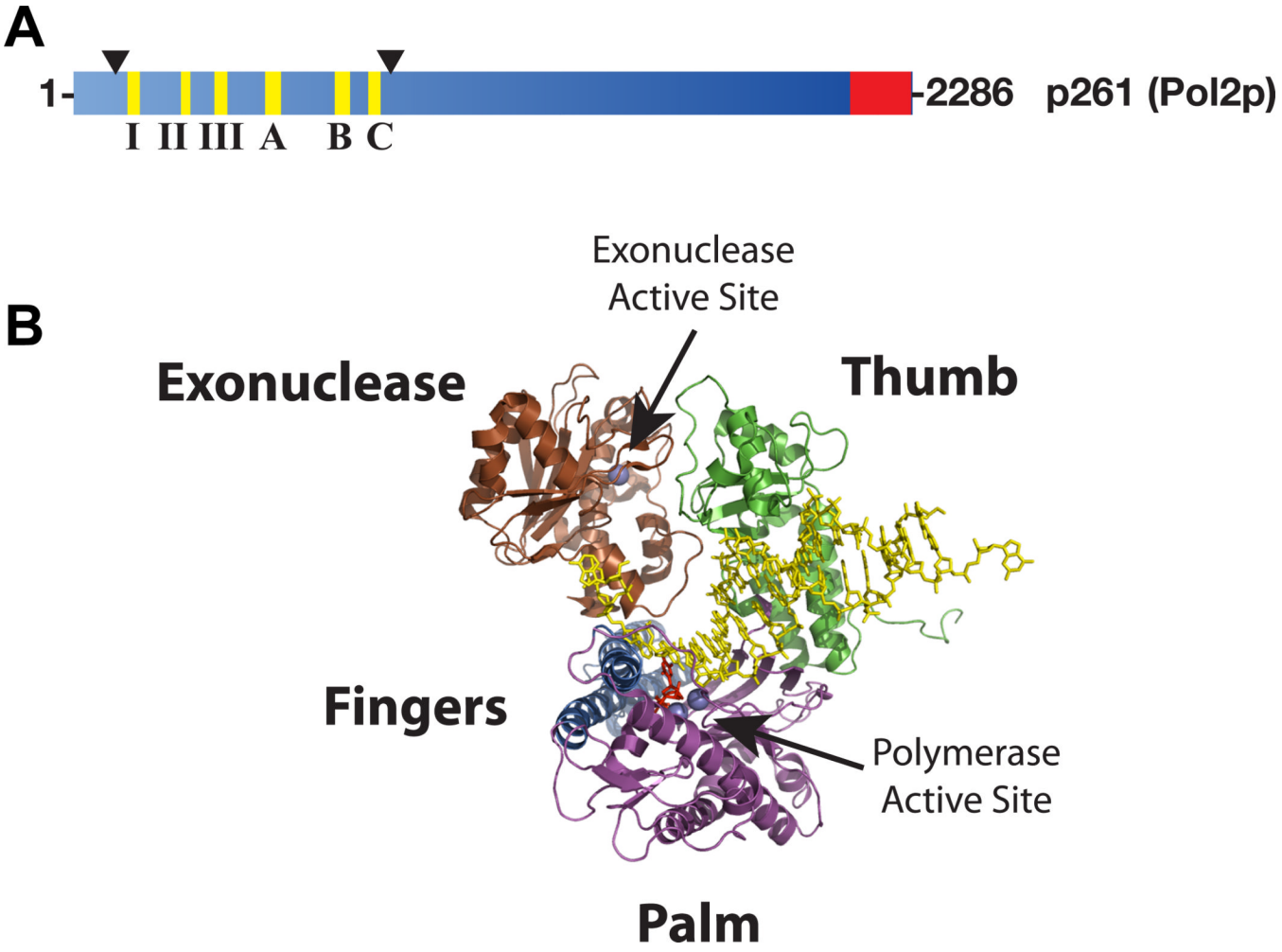


Figure 1. Pol ε: At the Intersection of Complex and Diverse Cellular Processes

Pol ε is involved in a wide array of diverse cellular processes. Its involvement is depicted here with the direction and size of the arrows meant to reflect the direction of influence and relative importance of its involvement in each process, respectively. By functioning as the leading strand polymerase in normal replication, a large arrow points from DNA replication towards Pol ε (see text in section E). However, some data suggest that, while important for normal replication, the essential function of Pol ε lies in its role as a checkpoint sensor during replication (see section F), influencing both replication itself as well as cell cycle progression. Thus large arrows point away from Pol ε toward these processes. Pol ε is also implicated in the repair of damaged DNA, though the degree to which it operates in

specialized repair pathways remains unclear (see sections I and J). Pol ε contributes to altering chromatin status, typically by promoting a silenced state either transiently or in a heritable, epigenetic manner (see sections G and H), thus arrows point from Pol ε toward these processes. Additionally, alterations to chromatin or epigenetic modifications may target Pol ε to these regions, thus arrows point from these processes toward Pol ε. The interaction of Pol ε with other factors (see section C) as well as the intrinsic fidelity and other biochemical properties of Pol ε (see section D) play important roles throughout each of these processes. Proper cell cycle progression, DNA damage repair and DNA replication, and possibly chromatin and epigenetic states as well, serve the overall goal of maintaining genome stability.



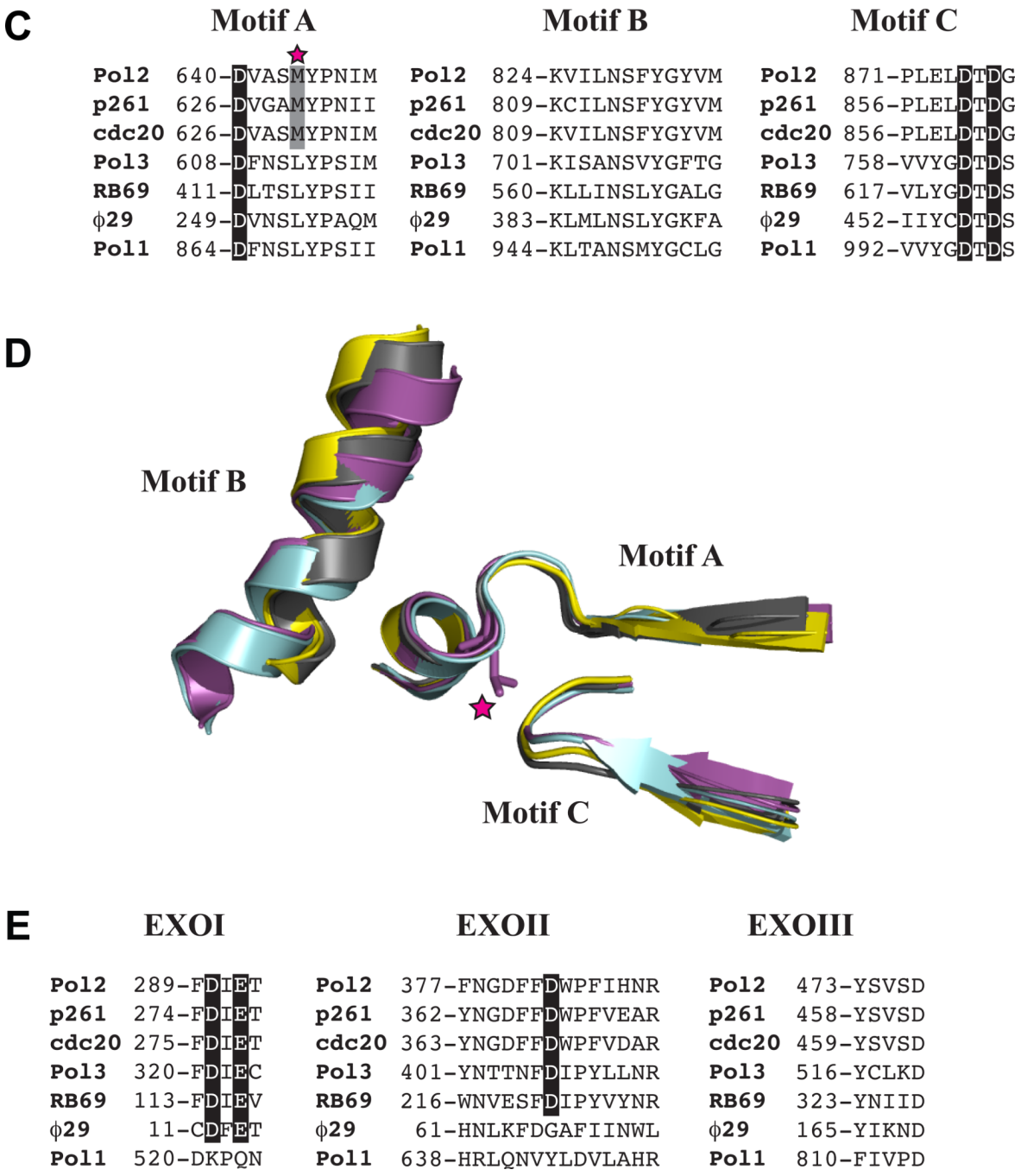


Figure 2. Pol ε Catalytic Subunit

(A) A schematic of the Pol ε catalytic subunit. Conserved motifs in the exonuclease and polymerase domains are shown in yellow, with the C-terminal protein-protein interaction region in red. DEAD-box cleavage sites in human Pol ε are shown as black arrows. (B) The structure of the Pol ε homologue RB69 DNA polymerase complexed with an incoming (correct) dTTP and primer-template DNA is shown using coordinates from PDB accession number 1IG9 (11). The fingers, palm, thumb, and exonuclease domains are shown in blue, purple, green, and brown, respectively. The duplex DNA is yellow and dTTP shown at the polymerase active site is red. The light blue spheres represent the divalent metal ions in the polymerase and exonuclease active sites. (C) Alignment of the amino acid sequences of

conserved polymerase motifs A, B, and C from Pol ε and other representative B family polymerases. Conserved catalytic aspartate residues are shown in the black boxes. The conserved motif A methionine that differs between Pol ε and the other B family polymerases is shown in a gray box with a magenta star. Pol2, p261, and cdc20 are Pol ε from *S. cerevisiae, H. sapiens*, and *S. pombe*, respectively. Pol1 and Pol3 are *S. cerevisiae* pols α and δ, respectively. RB69 and ϕ29 are bacteriophage DNA polymerases. (D) Ribbon diagram depicting an overlay of the structures of polymerase motifs A, B, and C from three B family DNA polymerases. Coordinates from PDB accession numbers 2PYL (ϕ29 pol, cyan), 1IG9 (RB69 pol, magenta), 1TGO (Tgo pol, gray), and 1QQC (D.tok pol, yellow) were used to align the structures with PyMol. The conserved Leu/Met that was altered to generate the mutator alleles described in the text is shown as a magenta star in the RB69 Pol structure. (E) Alignment of the amino acid sequences of conserved exonuclease motifs I, II, and III from Pol ε and other B family polymerases. Conserved catalytic carboxylates are shown in black boxes. DNA polymerases are as in (C).

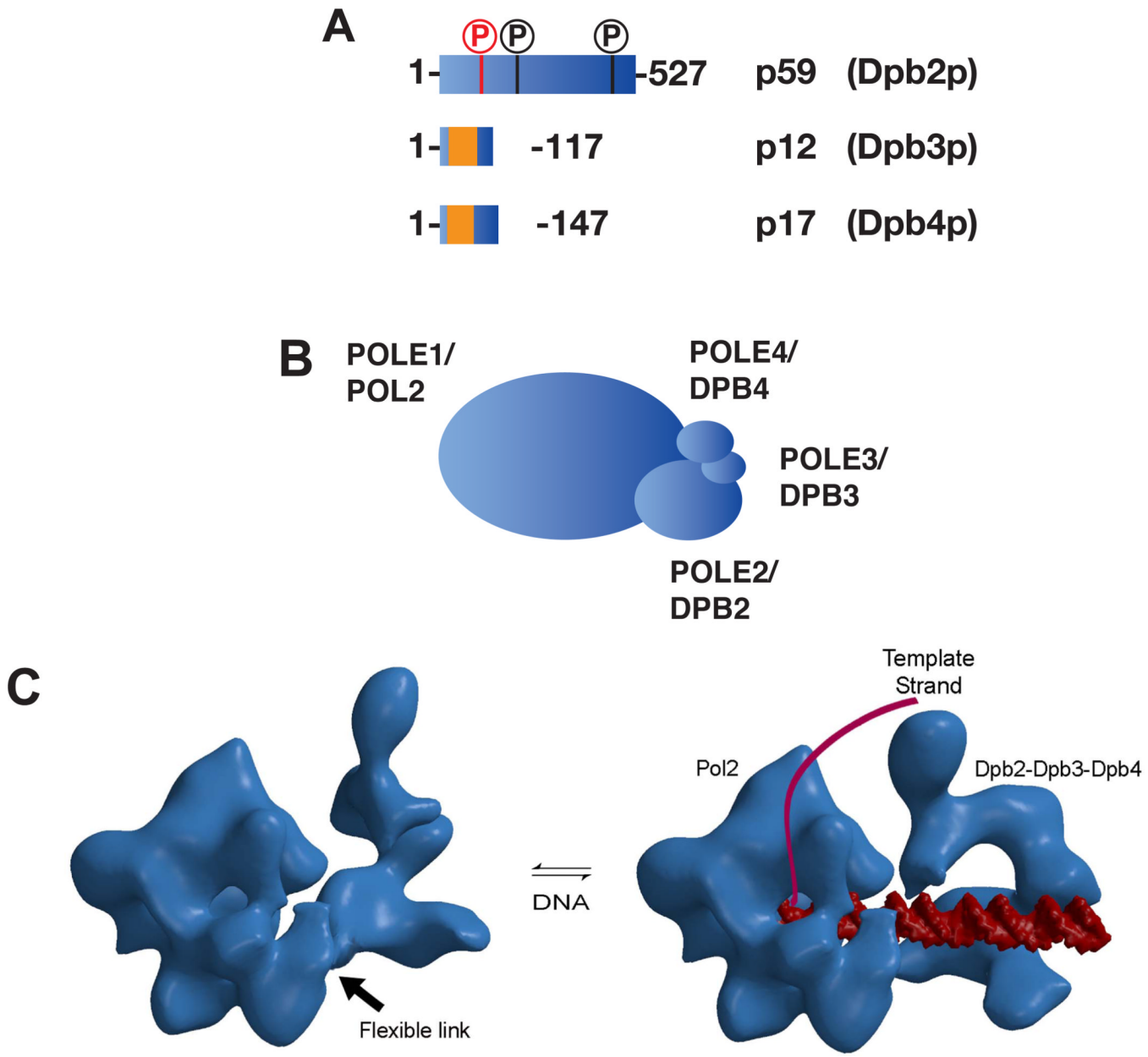


Figure 3. Pol ε Holoenzyme

(A) Schematic representation of each of the three Pol ε accessory subunits. Sites of known *in vitro* and potential *in vivo* phosphorylation (17) are shown as red and black circles, respectively. Histone-fold motifs are shown in orange. (B) Cartoon of four-subunit Pol ε holoenzyme. Each subunit is drawn approximately to scale, based on its predicted molecular weight. Human (and yeast) gene names are indicated next to each subunit. (C) Cryo-EM structure of four-subunit yeast Pol ε (from (23). The open conformation is shown on the left, while the right depicts a model of a closed conformation with duplex DNA bound. This image is reprinted from (23) with permission from the authors.

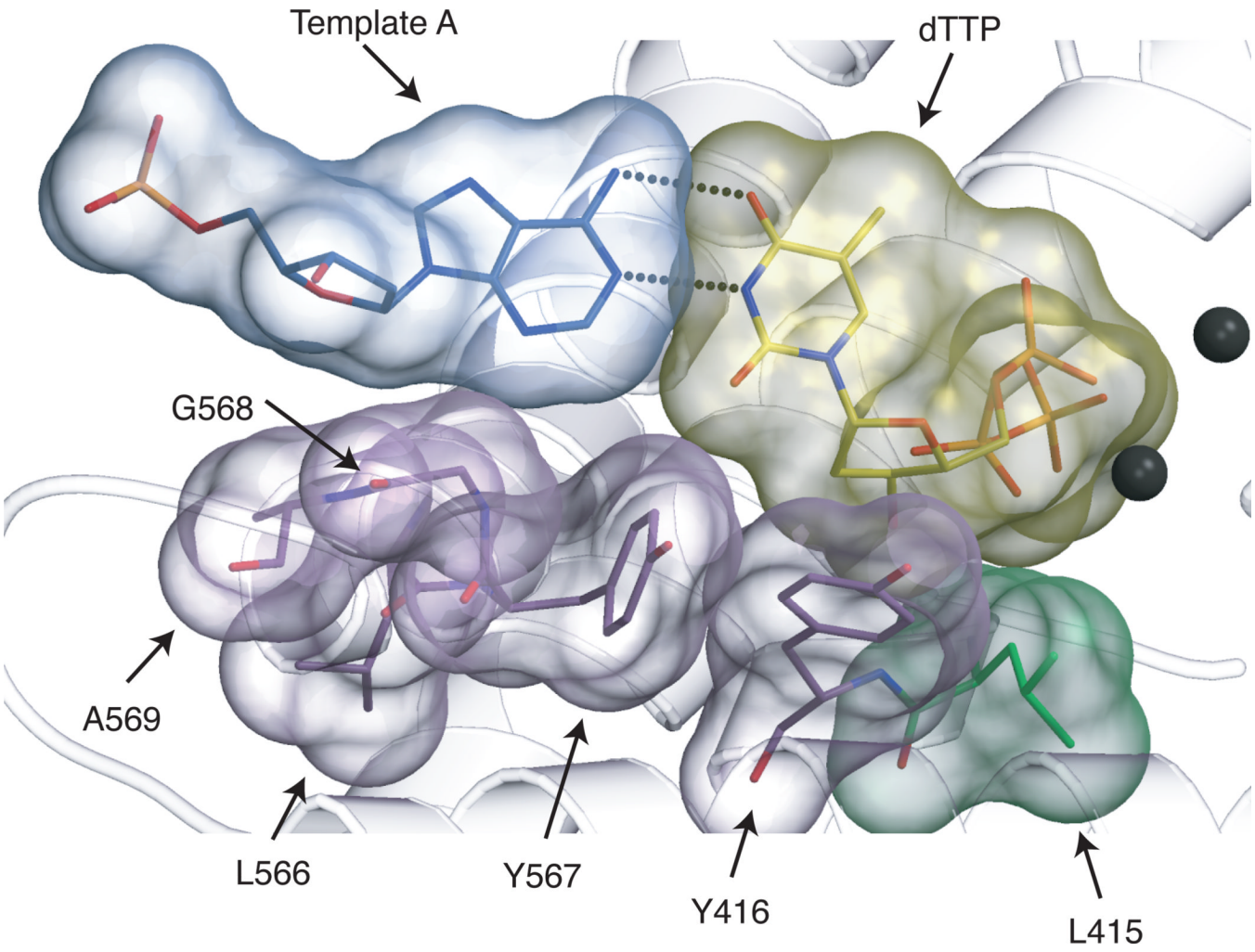


Figure 4. Nascent Base Pair Binding Pocket of A B Family Polymerase

Surface representation of the nascent base pair and several amino acids in RB69 Pol that form the DNA minor groove edge of the binding pocket at the polymerase active site (Adapted from (212) with the author's permission). Met644 in yeast Pol ε (in parentheses) aligns with Leu415 in RB69 Pol (green). The adjacent Tyr416 in RB69 Pol aligns with Tyr869 in yeast Pol α, which, when substituted with alanine, results in a mutator phenotype.

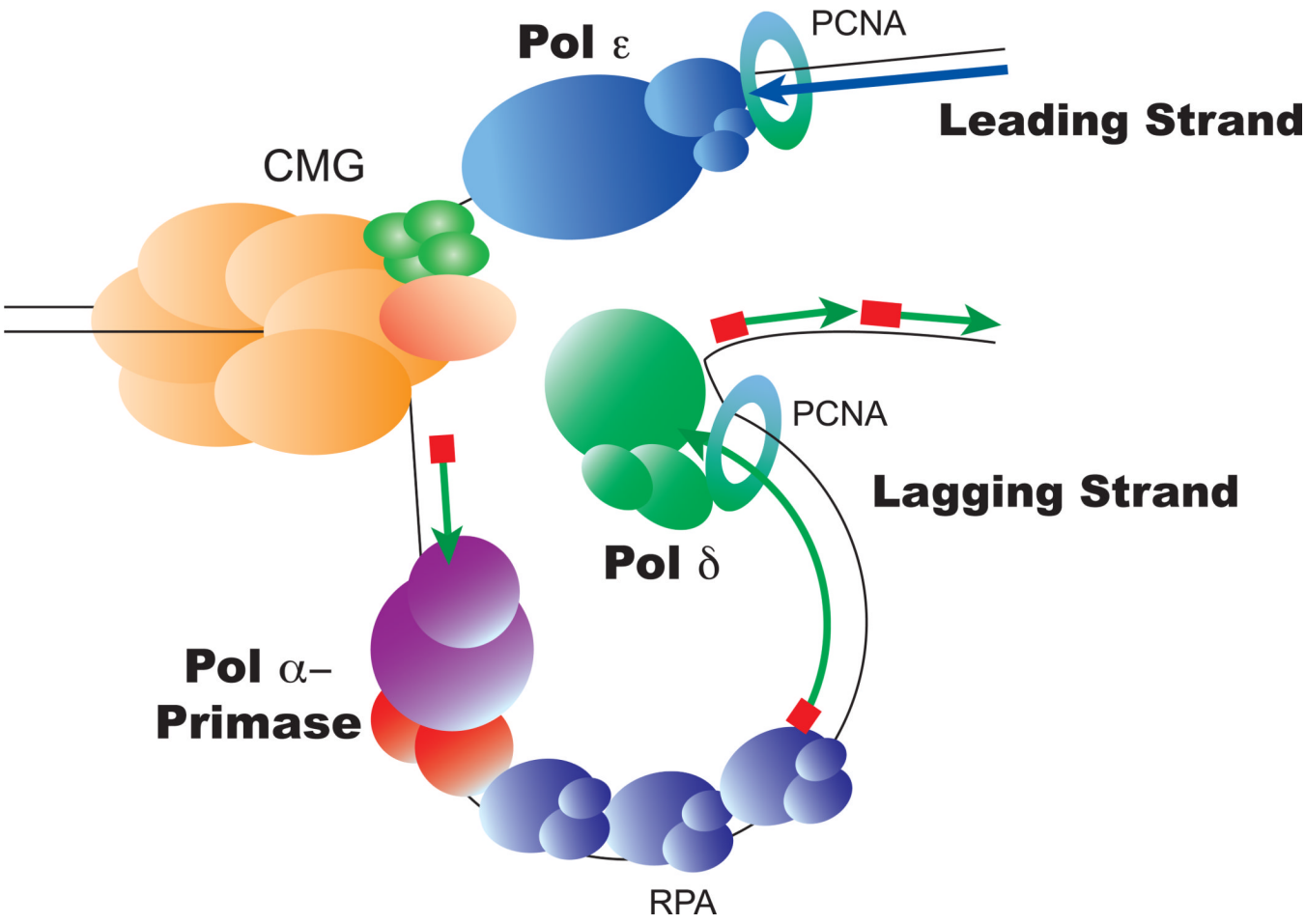


Figure 5. Model of A Eukaryotic Replication Fork

This model is based on the currently favored hypothesis that Pol ε is primarily responsible for leading strand synthesis, shown in blue, and Pol δ is primarily responsible for lagging strand synthesis, shown in green. Pol α-primase cooperates with Pol δ to conduct lagging strand synthesis, with the initiating RNA primers shown in red. RPA heterotrimers are shown in violet. The CMG replicative helicase is shown as a heterohexameric MCM complex (orange) associated with the GINS complex (green) and Cdc45 (light red).

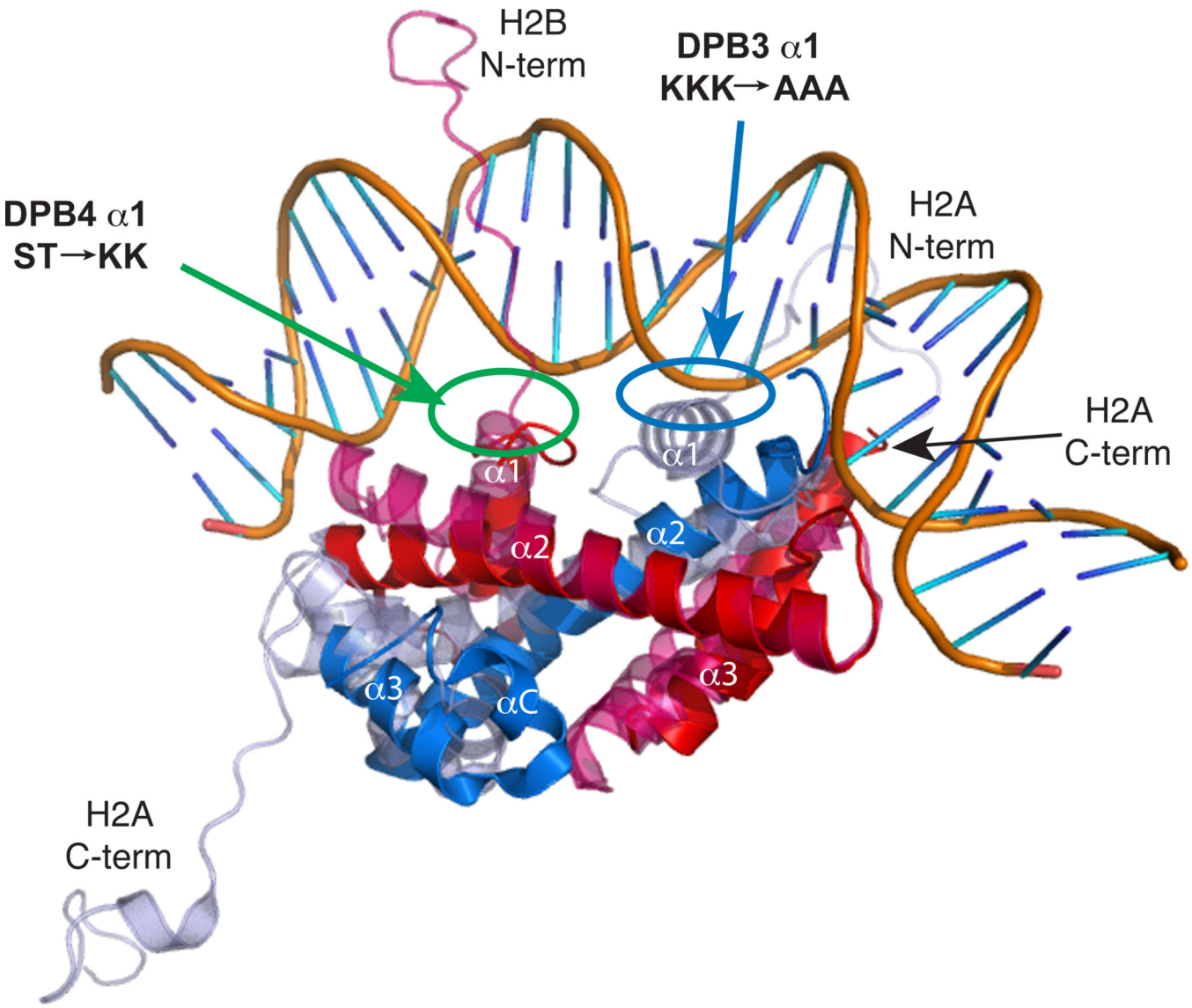


Figure 6. Model of Histone-Fold Subunits-DNA Interaction

Shown is a structural alignment of the heterodimer DmCHRAC-14/DmCHRAC-16 (shown in red/blue, respectively) from (138) with the DNA-bound heterodimer of histone H2A–H2B (magenta/light blue, respectively) from (213). DmCHRAC-14 is the same as DmPol ε-p17. The blue circle indicates where the KKK→AAA triple mutant of Dpb3 that results in loss of DNA-binding and telomeric silencing (52) maps to the structure. The green circle represents where the S/T→KK mutant in Dpb4 that partially suppresses the KKK→AAA Dpb3 mutant maps to in the structure. The α1, α2, α3, and αC helices on each HFM subunit are indicated. N- and C-terminal ends of histones are indicated.

Table 1

Pol ε Gene Names

Organism	Gene Name	Number of Amino Acid Residues	Predicted Molecular Weight (kDa)	Also Known As
S.cerevisiae	*POL2*	2222	255	
	DPB2	689	78	
	DPB3	201	23	
	DPB4	196	22	
H.sapiens	POLE1	2286	261	p261
	POLE2	527	59	p59
	POLE3	117	12	p12
	POLE4	147	17	p17/CHRAC-17
S.pombe	*cdc20*	2199	253	
	dpb2	594	67	
	dpb3	199	22	
	dpb4	210	24	

Table 2

Physical Interactions with Pol ε

Process	Name	Organism[a]	Reference
Replication			
	Dpb11p	Sc	(25)
	TopBP1	Hs	(33)
	PCNA	Hs	(20)
	Claspin	Xl	(206)
Repair			
	PCNA	Hs	(20)
	LigI	Hs	(159)
Other			
Sister Chromatid Cohesion	Trf4	Sc	(140)
Chromatin Remodeling	CHRAC (complex)[b]	Sc/Hs/Dm	(124,125,130)
Transcription/Repair?	RNAPII (complex)	Hs	(35)
Cell Cycle	Mdm2	Hs	(39)
Recombination	LigIII	Bt	(178)

[a]Bt: *B. taurus*; Dm: *D. melanogaster*; Hs: *H. sapiens*; Sc: *S. cerevisiae*; Xl: *X. laevis*

[b]Dpb4 (and its homologs) is a subunit of CHRAC. There is no evidence that Pol ε interacts with CHRAC directly.

(25) (33) (20) (206) (20) (159) (140) (124,125,130) (35) (39) (178)

Table 3

Fidelity of Yeast DNA Polymerase ε Compared to Other Yeast DNA Polymerases.

Enzyme	Family	Base Substitution Error Rate (x10-5)	Single-base Deletion Error Rate (x10-5)	Reference
Pol ε (exo−)	B	24.0	5.60	(57)
Pol ε (exo+)	B	≤2.0	≤0.05	(57)
Pol δ (exo−)	B	13.0	5.70	(207)
Pol δ (exo+)	B	≤1.3	1.30	(208)
Pol α	B	9.6	3.10	(209)
PolIV	X	320.0	360.00	(210)
Pol η	Y	950.0	93.00	(211)

(57) (57) (207) (208) (209) (210) (211)