Three DNA polymerases of the B family function at the replication fork in eukaryotic cells: DNA polymerases α, δ, and ε. DNA polymerase α, an heterotetramer composed of two primase subunits and two polymerase subunits, initiates replication. DNA polymerases δ and ε elongate the primers generated by pol α. The DNA polymerase from bacteriophage RB69 has served as a model for eukaryotic B family polymerases for some time. The recent crystal structures of pol δ, α, and ε revealed similarities but also a number of unexpected differences between the eukaryotic polymerases and their bacteriophage counterpart, and also among the three yeast polymerases. This review will focus on their shared structural elements as well as the features that are unique to each of these polymerases.

Keywords: DNA polymerase, B family, eukaryotic replication, fidelity, proofreading

OVERALL STRUCTURE OF B FAMILY POLYMERASES

All DNA polymerases share a common polymerase fold, which has been compared to a human right hand, composed of three subdomains: fingers, palm, and thumb (Steitz, 1999; Patel and Loeb, 2001). The palm, a highly conserved fold composed of four antiparallel β strands and two helices, harbors two strictly conserved catalytic aspartates located in motif A, DXXLYPS and motif C, DTDS (Delarue et al., 1990; Braithwaite and Ito, 1993). This RRM-like fold is shared by a very large group of enzymes, including DNA and RNA polymerases, reverse transcriptases, CRISPR polymerase, and even reverse (3′–5′) transferases such as Thg1 (Anantharaman et al., 2010; Hyde et al., 2010). In contrast, the thumb and fingers subdomains exhibit substantially more structural diversity (Steitz, 1999). The fingers undergo a conformational change upon binding DNA and the correct incoming nucleotide. This movement allows residues in the fingers subdomain to come in contact with the nucleotide in the nascent base pair. The thumb holds the DNA duplex during replication and plays a part in processivity (Doublié and Ellenberger, 1998; Doublié et al., 1999).

Eukaryotic DNA polymerases α, δ, and ε share homology with many archaeal, bacterial, bacteriophage, and viral polymerases (Delarue et al., 1990; Braithwaite and Ito, 1993; Franklin et al., 2001; Firbank et al., 2008; Wang and Yang, 2009). Koonin and collaborators contributed a detailed phylogenetic analysis of archaeal DNA polymerases and their relationship with eukaryotic polymerases in this issue of Frontiers in Microbiology dedicated to polymerases Makarova et al. (2014).

All B family polymerases are composed of five subdomains: the fingers, thumb, and palm (described above) constituting the core of the enzyme, as well as an exonuclease domain and an N-terminal domain (NTD) (Franklin et al., 2001; Xia and Konigsberg, 2014) (Figure 1; Table S1). The exonuclease domain carries a 3′–5′ proofreading activity, which removes misincorporated nucleotides. The exonuclease active site is located 40–45 Å away from the DNA polymerase active site. The NTD seems to be
Table 1 | Eukaryotic DNA polymerases are multi-subunit enzymes.

| Polymerase | Species | Function |
|------------|---------|----------|
| Catalytic or A-subunit | H. sapiens (POLA1 p180) | Catalytic subunit; polymerase activity; inactivated exonuclease |
| B-subunit | POLA2 (p70) | POL1 | Regulatory subunit |
| Primase small subunit | PRIM1 (p49) | PR1 | Primase |
| Primase large subunit | PRIM2 (p58) | PRI2 | Primase |

| Polymerase | Species | Function |
|------------|---------|----------|
| Catalytic or A-subunit | H. sapiens (POLD1 p125) | Catalytic subunit; has both polymerase and exonuclease activity |
| B-subunit | POLD2 (p50) | POLD3 (p66 or p88) | POL3 | Accessory subunit |
| C-subunit | POLD3 (p66 or p88) | Accessory subunit |
| D-subunit | POLD4 (p12) | – | Accessory subunit |

| Polymerase | Species | Function |
|------------|---------|----------|
| Catalytic or A-subunit | H. sapiens (POLE or POLE1) | Catalytic subunit; has both polymerase and exonuclease activity |
| B-subunit | POLE2 | Accessory subunit |
| C-subunit | POLE3 (p17; CHRAC17) | Accessory subunit |
| D-subunit | POLE4 (p12) | Accessory subunit |

DNA POLYMERASE α

The catalytic subunit of DNA polymerase α is composed of 1468 amino acids (Table 2). The protein construct designed for crystallization was truncated at the N- and C-termini (residues 349–1258) and therefore lacks the CTD and its [4Fe-4S] cluster (Figure 2). The construct was crystallized unliganded, in a binary complex with a DNA/RNA hybrid oligonucleotide, and in a ternary complex with DNA/RNA and incoming nucleotide (Perera et al., 2013) (Figure 1).

A MECHANISM OF DISENGAGEMENT OF THE POLYMERASE

The RNA/DNA oligonucleotide captured in the crystals adopts an A-form conformation, as expected. The thumb domain engages in multiple interactions with the RNA primer, both via hydrophobic contacts and polar interactions (Perera et al., 2013). Experiments in solution have shown that the extension of the RNA primer by pol α is limited to 10–12 nucleotides, which amounts to one turn of a helix. This observation led the authors to suggest a mechanism for termination of primer synthesis by pol α in which loss of specific interactions between the thumb and the RNA would trigger the polymerase to disengage from the DNA/RNA oligonucleotide, and allow a hand off to a replicative polymerase.

MOVEMENTS IN THE PALM DOMAIN MAY FACILITATE TRANSLATION OF POL α

Having crystallized the enzyme in three states (apo, binary, and ternary) allowed the authors to overlay all three structural models. Pol α is the only eukaryotic family B DNA polymerase for which all three states were captured in a crystal structure. The structural superposition revealed that, in addition to the well-documented movements of the fingers and thumb subdomains accompanying substrate binding and nucleotidyl transfer, the palm subdomain itself undergoes a structural rearrangement (Perera et al., 2013). The authors propose that the different conformations of the palm domain could facilitate translation of pol α along and beyond the RNA/DNA duplex. As mentioned above, loss of contacts to the RNA strand is predicted to trigger release of primer, which then becomes available for extension by pol δ or ε.

A DIFFERENT PROTEIN FOLD IN THE INACTIVATED EXONUCLEASE SUBDOMAIN

The proofreading activity is abolished in pol α, due to mutations in all four carboxylates (Asp114/Glu116/Asp222/Asp327 in RB69 gp43 correspond to Ser542/Gln544/Tyr644/Asn757 in a structure-based alignment) (Table 2). Moreover, the β-hairpin motif found in most polymerases of the B family (residues 246–267 in RB69 gp43) is replaced by a helical region in pol α (residues 667–676; 681–693) (Hogg et al., 2007). The β-hairpin is part of the exonuclease domain and has been shown in T4 and RB69 pols to participate in the partitioning of the DNA primer between the polymerase and the exonuclease active site (Reha-Krantz, 1988; Stocki et al., 1995; Hogg et al., 2007). In the absence of proofreading activity it is not surprising that this motif was not retained in pol α. Residues His 684 and Phe 685 of the helical region in pol α stack with a thymine and guanine base, respectively, at positions -3 and -2 in the unpaired 5’end of the template (Perera et al., 2013). Thus, in pol α the region corresponding to the β-hairpin motif adopts a different fold (helices vs. β strands) and a different function (stabilizing the unpaired region of the template strand rather than facilitating active site interactions with the RNA/DNA hybrid oligonucleotide).
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FIGURE 1 | Ternary complexes of polymerases α, δ, ε, and RB69 gp43 are illustrated from identical orientations for comparison. The thumb (green) and fingers (dark blue) domains grasp the duplex nucleic acid (primer shown in beige, template in orange) against the palm domain (red). The N-terminal domain appears in gold, adjacent to the 3′–5′ exonuclease domain (cyan).

(A) Polymerase α (PDBID 4FYD) binds an RNA/DNA hybrid, where the wide, shallow minor groove of A-form DNA is apparent near the thumb. The 3′–5′ exonuclease domain is devoid of activity. A helical region (magenta) in the inactivated exonuclease domain stabilizes the 5′ end of the template.

(B) Polymerase δ (PDBID 3IAY) harbors a large β hairpin motif (magenta), which is important in switching the primer strand from the polymerase active site to the exonuclease active site in the event of proofreading.

(C) Polymerase ε (PDBID 3IAY) wields a unique P-domain (purple), which endows the polymerase with increased processivity. Interestingly, the β hairpin motif is atrophied in pol ε.

(D) Conservation of the family B DNA polymerase fold, and domain organization, is evident when the model enzyme from bacteriophage RB69 gp43 (PDBID 2OZS) is viewed along with the three eukaryotic replicative polymerases. The domain delineation for each polymerase is given in Table S1. Figure was made with PyMOL (The PyMOL Molecular Graphics System, Version 1.5.0.4 Schrödinger, LLC.).

switching). Since pol α is devoid of proofreading activity the question arises as to whether the short oligonucleotides are corrected, and if so, by which DNA polymerase. It appears that proofreading of the primers synthesized by pol α is performed by pol δ (Pavlov et al., 2006a).

DNA POLYMERASE δ

Human pol δ is composed of four subunits whereas Saccharomyces cerevisiae has three (Gerik et al., 1998; Liu et al., 2000) (Table 1). In addition to its function in DNA replication pol δ has been shown to play a role in DNA repair and recombination (Hubscher...
et al., 2002; Lee et al., 2012; Tahirov, 2012). P12, the smallest subunit in human pol δ and also the subunit that is not seen in budding yeast, is degraded in response to DNA damage (Lee et al., 2014). The catalytic subunit of yeast pol δ (POL3) is composed of 1097 residues. The construct used for crystallization comprises residues 67–985 and thus lacks the CTD (Figure 1; Table 2).

A THIRD METAL ION IN THE POLYMERASE ACTIVE SITE
The palm domain contains three conserved carboxylates (Asp608, Asp762, and Asp764). The two catalytic aspartates, Asp608 and Asp764, contact two metal ions (Ca$^{2+}$) in the polymerase active site separated by 3.7 Å. Intriguingly a third metal was observed coordinated by the γ phosphate of the incoming nucleotide and Glu802, with Glu800 in the vicinity. Mutating both glutamates to alanine yielded a polymerase variant with reduced incorporation for both correct and incorrect nucleotides (Swan et al., 2009). At these amino acid positions, pol α and pol ε also have carboxylate residues (pol δ Glu800/Glu802 correspond to pol α Asp1033/Asp1035, and pol ε Glu945/Asp947). Whether these carboxylates play similar roles in pol α and ε remains to be investigated.

HIGH FIDELITY AND PROOFREADING
Human pol δ is a high-fidelity polymerase, catalyzing the nucleotidyl transfer reaction with an error frequency of 1 per 22,000 (Schmitt et al., 2009). Proofreading boosts the fidelity of the polymerase by a factor of 10–100 (McCulloch and Kunkel, 2008; Prindle et al., 2013). Pol δ harbors a polymerase and exonuclease active site, separated by about 45 Å (Swan et al., 2009). DNA polymerases with proofreading activity are able to sense misincorporated nucleotides by contacting the minor groove of base pairs beyond the insertion site. The protein interacts with universal hydrogen bond acceptors at the N3 and O2 positions of purines and pyrimidines, respectively (Seeman et al., 1976; Doublié et al., 1998; Franklin et al., 2001). These hydrogen bond contacts are preserved when the base pair adopts a Watson-Crick geometry and lost in the event of a mismatch. In RB69 gp43, the contacts extend to the first two base pairs beyond the nascent base pair (Franklin et al., 2001; Hogg et al., 2004, 2005). The contacts are much more extensive in pol δ, extending to five base pairs post-insertion (Swan et al., 2009), which could contribute to its high fidelity.

As mentioned above, the β-hairpin segment from the exonuclease domain plays a critical role in the partition of the DNA between polymerization and proofreading sites in T4 and RB69 pols (Stocki et al., 1995; Hogg et al., 2007). In RB69 gp43 the β-hairpin motif adopts different conformations, depending on whether the complex was obtained with undamaged DNA (Franklin et al., 2001; Zahn et al., 2007) or DNA containing a damage (Freisinger et al., 2004; Hogg et al., 2007). It was fully visualized contacting both the primer and template strands in a complex with thymine glycol (Aller et al., 2011). Similarly, the β hairpin in pol δ protrudes into the major groove of the DNA and acts as a wedge between double-stranded DNA and the single-stranded 5’ end of the template strand, which is stabilized by two aromatic residues Phe441 and Tyr446 (Figure 1) (Swan et al., 2009). The position of the β hairpin is consistent with a role in active site switching.

INTERDOMAIN CONTACTS AND FIDELITY
Mutations involved in cancer are mostly found in the exonuclease domain of pol δ and ε, emphasizing the critical role of proofreading in lowering the incidence of mutations (Church et al., 2013; Henninger and Pursell, 2014). One mutation in human colorectal cancer cells localizes to the fingers domain, R689W. The
| Polymerase name | Yeast gene | Conserved aspartates in polymerase active site | Conserved carboxylates in exonuclease active site | UNIPROT ID | Source | Iron sulfur clusters? | Number of amino acids | Protein construct crystallized | PDB ID code and reference |
|----------------|------------|---------------------------------------------|-----------------------------------------------|------------|--------|---------------------|---------------------|--------------------------|--------------------------|
| Pol α | POL1 | Asp864, Asp998, Asp969 | N/A | P13382 | S. cerevisiae | 1 [4Fe-4S] Cys1348,1353,1367,1372 | 1468 aa | 349–1258 | 4FYD 3.1 Å | Perera et al., 2013 |
| Pol δ | POL3 | Asp608, Asp764, Asp762 | Asp321, Glu323, Asp407, Asp520 | P15436 | S. cerevisiae | 1 [4Fe-4S] Cys1056,1059,1069,1074 | 1097 aa | 67–985 | 3IAY 2.0 Å | Swan et al., 2009 |
| Pol ε | POL2 | Asp640, Asp877, Asp875 | Asp290, Glu292, Asp383, Asp477 | P21951 | S. cerevisiae | 2 [4Fe-4S] Cys665,667,668,763 Cys2164,2167,2179,2181 | 2222 aa | 1-1228 1-1187 | 4M8O 2.2 Å 4PTF 2.8 Å | Jain et al., 2014a |
| RB69 gp43 | | Asp411, Asp623, Asp621 | Asp114, Glu116, Asp222, Asp327 | Q38087 | Bacteriophage RB69 | None | 903 aa | 1-803 | 1IG9 2.6 Å | Franklin et al., 2001 |
| E. coli Pol II | Asp419, Asp547, Asp545 | Asp156, Glu158, Asp229, Asp315 | P21189 | Escherichia coli | None | 783 aa | 1-780 | 3K57 2.08 Å | Wang and Yang, 2009 |
| Archaeal Tgo | Asp404, Asp642, Asp640 | Asp141, Glu143, Asp215, Asp335 | P56689 | Thermococcus gordonii | None | 773 aa | 1-757 | 2WVJ 2.78 Å | Firbank et al., 2008 |
analagous mutation in yeast (R696W) results in a mutator pheno-
type (Dae et al., 2010). A mutation in the vicinity of Arg696
in the highly conserved motif B of the fingers subdomain of
yeast pol δ (A699Q) also results in a mutator phenotype. This
region of the fingers is in close proximity to the NTD. Mutating
Met540 of the NTD to alanine abolishes the mutator pheno-
type of A699Q, illustrating that interactions between the fingers
and the NTD can affect the fidelity of the polymerase (Prindle
et al., 2013). Similarly in T4 and RB69 pols the NPL core motif,
which involves residues from the N-terminal and palm domains,
is in contact with the fingers domain and was shown to stabilize
polymerase-DNA complexes (Li et al., 2010).

DNA POLYMERASE ε
The catalytic subunit of DNA polymerase ε is the product of a
very large gene (2222 amino acids in yeast; 2286 in humans),
and is only third in size after polymerase ζ (also a member of
the B family) and pol β, a family A polymerase (3130 and 2590
amino acids, respectively, in humans) (Lange et al., 2011; Hogg
and Johansson, 2012) (Figure 1; Table 2). Pol ε is twice as large
as pol δ and is composed of two tandem polymerase/exonuclease
regions. The N-terminal segment harbors both polymerase and
proofreading activities whereas the C-terminal segment is inac-
tivated. The two exonuclease-polymerase modules are distant
related (Tahirov et al., 2009). Although the inactivated segment
is presumed to play a structural role during replication, two
groups were able to crystallize catalytically active pol ε constructs
(residues 1–1228; 1–1187) lacking the entire C-terminal module
(Hogg et al., 2014; Jain et al., 2014a). Both crystal structures were
of a ternary complex of the polymerase, DNA primer/template
and incoming nucleotide.

A NOVEL PROCESSIVITY DOMAIN EMANATING FROM THE PALM
DOMAIN
Pol ε differs from pol δ in that it does not require the DNA sliding
clamp PCNA for high processivity (Hogg and Johansson, 2012). The
palm domain of pol ε is substantially larger (380 residues)
that than of pol α or δ (175 and 203 residues, respectively). The
recent pol ε crystal structures revealed that insertions in the palm
domaincollectively form a new segment consisting of three β
strands and two helices (residues 533–555; 682–760) (Hogg et al.,
2014; Jain et al., 2014a) (Figure 1; Table S1). Deleting residues
690–751 resulted in a variant with decreased polymerase activity.
Moreover, mutating positively charged residues (His748, Arg749,
and Lys751) located in the vicinity of the phosphate backbone
affected the processivity of the enzyme (Hogg et al., 2014). The
extra domain originating from the palm was thus named the pro-
cessivity or P domain, after its function. The base of the P domain
harbors a metal binding site (see below) (Hogg et al., 2014; Jain
et al., 2014a,b).

AN IRON SULFUR CLUSTER WITHIN THE POLYMERASE DOMAIN
Unexpectedly solution studies revealed that the catalytic subunit
of yeast polymerase ε itself contains an [4Fe-4S] cluster within
its polymerase fold (Jain et al., 2014b), in addition to the [4Fe-
4S] cluster in the CTD (Figure 2; Table 2). The second [4Fe-4S]
cluster within pol ε suggests that this polymerase may be more
sensitive to oxidative stress (Jain et al., 2014b). The crystal struc-
tures of pol ε, however, did not reveal a [4Fe-4S] cluster in the
polymerase domain (Hogg et al., 2014; Jain et al., 2014a; Zahn and
Doublié, 2014). Two of the cysteines residues are disordered in
the structural models and the resulting metal binding site appears
to bind zinc (Hogg et al., 2014; Jain et al., 2014a). Substitution
of a [4Fe-4S] by a non-native zinc in metal-binding proteins is
not unusual (Netz et al., 2012) as [4Fe-4S] clusters are labile.
Visualizing the [4Fe-4S] within the polymerase domain of pol ε
may necessitate anaerobic conditions.

A SHORT β-HAIRPIN MOTIF IN THE EXONUCLEASE DOMAIN
In any DNA polymerase harboring both polymerase and exonu-
clease activities the bound DNA is in equilibrium between the
two active centers (Beechem et al., 1998). The concentration of
incoming nucleotide and the presence of a damaged base
or mispair are two factors that influence the transfer of DNA
from the polymerase active site to the proofreading active site.
Polymerases monitor the minor groove side of the newly formed
base pairs and interact with the universal H bond acceptors, O3,
and N2, as a way of checking for mismatches (Seeman et al., 1976;
Franklin et al., 2001). A unique feature of pol ε is the contact to
the major groove side of the nascent base pair via a residue from
the exonuclease domain, Tyr431. Further analysis is warranted
to elucidate the potential role of this tyrosine in the high fidelity
of pol ε.

In pol δ the β-hairpin segment inserts itself in the DNA and
acts as a wedge between single-stranded and double-stranded
DNA (Swan et al., 2009). In E. coli DNA Pol II, the insertion of a β
barrel shifts the position of the βhairpin in such a way that poly-
erization is favored over proofreading (Wang and Yang, 2009).
This modification presumably allows this polymerase to carry out
translesion synthesis extension. Since pol ε is an accurate DNA
polymerase the assumption before knowledge of the crystal struc-
ture would be that the β hairpin should be closer to that of pol δ
than that of E. coli Pol II. Surprisingly, the β-hairpin motif in pol ε
is truncated, too short to contact the DNA (Figure 1). Which pro-
tein motif, then, might be facilitating active site switching upon
sensing of a mispair? The P domain is a good candidate, because
of its contacts to both primer and template strands; residues from
the P domain could sense replication errors and thus may help
facilitate active site switching.

CONCLUSIONS
All three eukaryotic replicative DNA polymerases use a common
B-family fold, and each polymerase has incorporated modified
structural elements which are unique and tailored for each poly-
erase’s specific function (for example, the addition of the pro-
cessivity domain in pol ε, a processive polymerase that does not
use PCNA, or the modified region contacting the 5′end of
the template in pol α, a polymerase devoid of proofreading activity).
The fold of B family polymerases is well suited for high-fidelity,
replicative polymerases. But surprisingly, it is also used by transle-
sion polymerases. Eukaryotic pol ζ (or REV3L) is a 353 kDa
polymerase which functions in translesion synthesis and appears
to suppress tumorigenesis (Wittschieben et al., 2010; Lange et al.,
2011; Zahn et al., 2011; Hogg and Johansson, 2012; Sharma et al.,
The structure of E. coli Pol II revealed modifications in the NTD which affect the position of the β hairpin of the exonuclease domain, and thus partitioning of the DNA between the polymerization and proofreading sites (Wang and Yang, 2009). The structure of pol ζ may reveal similar adjustments, which alter the fold employed by high-fidelity, replicative polymerases to render the enzyme less faithful and able to perform translesion synthesis.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: http://www.frontiersin.org/Journal/10.3389/fmicb.2014.00444/abstract

REFERENCES

Aller, P., Duclos, S., Wallace, S. S., and Doublé, S. (2011). A crystallographic study of the role of sequence context in thymine glycol bypass by a replicative DNA polymerase serendipitously sheds light on the exonuclease complex. J. Mol. Biol. 412, 22–34. doi: 10.1016/j.jmb.2011.07.007

Anantharaman, V., Iyer, L. M., and Aravind, L. (2010). Presence of a classical βNTD which affect the position of the hairpin of the exonuclease domain, and thus partitioning of the DNA between the polymerase and proofreading sites (Wang and Yang, 2009). The mechanism of asymmetric polymerase assembly at the eukaryotic replication fork. Nat. Struct. Mol. Biol. 21, 664–670. doi: 10.1038/nsmb.2851

Georgescu, R. E., Langston, L., Yao, N. Y., Yurieva, O., Zhang, D., Finkelstein, J., et al. (2014). Mechanism of asymmetric polymerase assembly at the eukaryotic replication fork. Nat. Struct. Mol. Biol. 21, 664–670. doi: 10.1038/nsmb.2851

Gerik, K. J., Li, X., Pautz, A., and Burgers, P. M. (1998). Characterization of the two small subunits of Saccharomyces cerevisiae DNA polymerase delta. J. Biol. Chem. 273, 19747–19755. doi: 10.1074/jbc.273.31.19747

Henniger, E. E., and Pursell, Z. F. (2014). DNA polymerase epsilon and its roles in genome stability. IUBMB Life 66, 339–351. doi: 10.1002/iub.1276

Hogg, M., Aller, P., Konigsberg, W., Wallace, S. S., and Doublé, S. (2007). Structural and biochemical investigation of the role in proofreading of a beta hairpin loop found in the exonuclease domain of a replicative DNA polymerase of the B family. J. Biol. Chem. 282, 1432–1444. doi: 10.1074/jbc.M605675200

Hogg, M., and Johansson, E. (2012). DNA polymerase epsilon. Subcell Biochem. 62, 237–257. doi: 10.1007/978-94-007-4572-8_13

Hogg, M., Osterman, P., Bylund, G. O., Ganai, R. A., Lundstrom, E. B., Sauer-Eriksson, A. E., et al. (2014). Structural basis for processive DNA synthesis by yeast DNA polymerase varepsilon. Nat. Struct. Mol. Biol. 21, 49–55. doi: 10.1038/nsmb.2712

Hogg, M., Wallace, S. S., and Doublé, S. (2004). Crystallographic snapshots of a replicative DNA polymerase encountering an abasic site. EMBO J. 23, 1483–1493. doi: 10.1038/sj.emboj.7600150

Hogg, M., Wallace, S. S., and Doublé, S. (2005). Bumps in the road: how replicative DNA polymerases see DNA damage. Curr. Opin. Struct. Biol. 15, 86–93. doi: 10.1016/j.sbi.2005.01.014

Hubscher, U., Maga, G., and Spadari, S. (2002). Eukaryotic DNA polymerases. Annu. Rev. Biochem. 71, 133–163. doi: 10.1146/annurev.biochem.71.090501.150041

Hughes, M. B., Yee, A. M., Dawson, M., and Karam, J. (1987). Genetic mapping of the amino-terminal domain of bacteriophage T4 DNA polymerase. Genetics 115, 393–403.

Hyde, S. J., Eckenroth, B. E., Smith, B. A., Heintz, N. H., Jackman, J. E., et al. (2010). tRNA(His) guanylyltransferase (THG1), a unique 3′–5′ nucleotidyl transferase, shares unexpected structural homology with canonical 5′–3′ DNA polymerases. Proc. Natl. Acad. Sci. U.S.A. 107, 20305–20310. doi: 10.1073/pnas.1010436107

Jain, R., Rajashankar, K. R., Buku, A., Johnson, R. E., Prakash, L., Prakash, S., et al. (2014a). Crystal structure of yeast DNA polymerase epsilon catalytic domain. PLoS ONE 9:e89435. doi: 10.1371/journal.pone.0094835

Jain, R., Vanamee, E. S., Dzikovski, B. G., Buku, A., Johnson, R. E., Prakash, L., et al. (2014b). An iron-sulfur cluster in the polymerase domain of yeast DNA polymerase epsilon. J. Mol. Biol. 426, 301–308. doi: 10.1016/j.jmb.2013.10.015

Johnsson, E., and Macnell, S. A. (2010). The eukaryotic replicative DNA polymerases take shape. Trends Biochem. Sci. 35, 339–347. doi: 10.1016/j.tibs.2010.01.004

Kunkel, T. A. (2004). DNA replication fidelity. J. Biol. Chem. 279, 16895–16898. doi: 10.1074/jbc.R400006200

Kunkel, T. A. (2011). Balancing eukaryotic replication asymmetry with replication fidelity. Curr. Opin. Chem. Biol. 15, 620–626. doi: 10.1016/j.cob.2011.07.025

Kunkel, T. A., and Burgers, P. M. (2008). Dividing the workload at a eukaryotic replication fork. Trends Cell. Biol. 18, 521–527. doi: 10.1016/j.tcb.2008.08.003

Lange, S. S., Takata, K., and Wood, R. D. (2011). DNA polymerases and cancer. Nat. Rev. Cancer 11, 96–110. doi: 10.1038/nrc2998

Lee, M. Y., Zhang, S., Lin, S. H., Chea, J., Wang, X., Leroy, C., et al. (2012). Regulation of human DNA polymerase delta in the cellular responses to DNA damage. Environ. Mol. Mutagen. 53, 683–698. doi: 10.1002/em.21743

Lee, M. Y., Zhang, S., Lin, S. H., Wang, X., Darynkiewicz, Z., Zhang, Z., et al. (2014). The tail that wags the dog: p12, the smallest subunit of DNA polymerase delta, is degraded by ubiquitin ligases in response to DNA damage and during cell cycle progression. Cell Cycle 13, 23–31. doi: 10.4161/cc.27407

Lee, Y. S., Kennedy, W. D., and Yin, Y. W. (2009). Structural insight into processive human mitochondrial DNA synthesis and disease-related polymerase mutations. Cell 139, 312–324. doi: 10.1016/j.cell.2009.07.030

Li, V., Hogg, M., and Reha-Krantz, L. I. (2010). Identification of a new motif in family B DNA polymerases by mutational analyses of the bacteriophage T4 DNA polymerase. J. Mol. Biol. 400, 295–308. doi: 10.1016/j.jmb.2010.05.030

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Pursell, Z. F., Isoz, I., Lundstrom, E. B., Johansson, E., and Kunkel, T. A. (2007). Amino acid changes coded by bacteriophage T4 DNA polymerase function: identification of a fourth subunit of mammalian DNA polymerase delta. *Biochemistry* 288, 5572–5580. doi: 10.1021/j111.436410

Pursell, Z. E., Isot, I., Lundstrom, E. B., Johansson, E., and Kunkel, T. A. (2007). Yeast DNA polymerase epsilon participates in leading-strand DNA replication. *Science* 317, 127–130. doi: 10.1126/science.1144067

Reha-Krantz, L. I. (1988). Amino acid changes coded by bacteriophage T4 DNA polymerase mutant mutators. Relating structure to function. *J. Mol. Biol.* 202, 711–724. doi: 10.1016/0022-2836(88)90532-9

Sanchez Garcia, J., Gufo, L. F., Yang, X., Kearsey, S. E., and Macneill, S. A. (2004). The C-terminal zinc finger of the catalytic subunit of DNA polymerase delta is responsible for direct interaction with the B-subunit. *Nucleic Acids Res.* 32, 3005–3016. doi: 10.1093/nar/gkh623

Schmitt, M. W., Matsumoto, Y., and Loeb, L. A. (2009). High fidelity and lesion bypass capability of human DNA polymerase delta. *Biochimie* 91, 1163–1172. doi: 10.1016/j.bioch.2009.06.007

Seeman, N. C., Rosenberg, J. M., and Rich, A. (1976). Sequence-specific recognition of double helical nucleic acids by proteins. *Proc. Natl. Acad. Sci. U.S.A.* 73, 804–808. doi: 10.1073/pnas.73.3.804

Sharma, S., Helchowski, C. M., and Cann, C. E. (2013). The roles of DNA polymerase zeta and the Y family DNA polymerases in promoting or preventing genome instability. *Mutat. Res.* 743–744, 97–110. doi: 10.1016/j.mrfmm.2012.11.002

Steitz, T. A. (1999). DNA polymerases: structural diversity and common mechanisms. *J. Biol. Chem.* 274, 17395–17398. doi: 10.1074/jbc.C724.25.17395

Stocki, S. A., Nonay, R. L., and Reha-Krantz, L. J. (1995). Dynamics of bacteriophage T4 DNA polymerase function: identification of amino acid residues that affect switching between polymerase and 3′→5′ exonuclease activities. *J. Mol. Biol.* 254, 15–28. doi: 10.1006/jmbi.1995.0595

Swafford, M. K., Johnson, R. E., Prakash, L., Prakash, S., and Aggarwal, A. K. (2009). Structural basis of high-fidelity DNA synthesis by yeast DNA polymerase delta. *Nat. Struct. Mol. Biol.* 16, 979–986. doi: 10.1038/nsmb.1663

Tahirov, T. H. (2012). Structure and function of eukaryotic DNA polymerase delta. *Subcell Biochem.* 62, 217–236. doi: 10.1007/978-94-007-4572-8_12

Tahirov, T. H., Makarova, K. S., Rogozin, I. B., Pavlov, Y. I., and Koonin, E. V. (2009). Evolution of DNA polymerases: an inactivated polymerase-exonuclease module in Pol epsilon and a chimeric origin of eukaryotic polymerases from two classes of archaeal ancestors. *Biol. Direct* 4, 11. doi: 10.1186/1745-6150-4-11

Wang, E., and Yang, W. (2009). Structural insight into translesion synthesis by DNA Pol II. *Cell* 139, 1279–1289. doi: 10.1016/j.cell.2009.11.043

Wittschien, J. P., Patil, V., Ghusits, V., Robinson, L. J., Kasewitz, D. F., and Wood, R. D. (2010). Loss of DNA polymerase zeta enhances spontaneous tumorigenesis. *Cancer Res.* 70, 2770–2778. doi: 10.1158/0008-5472.CAN-09-4267

Xia, S., and Konigsberg, W. H. (2014). RB69 DNA polymerase structure, kinetics, and fidelity. *Biochemistry* 53, 2752–2767. doi: 10.1021/bi4014215

Zahn, K. E., Beltihali, H., Wallace, S. S., and Doublé, S. (2007). Caught bending the A-rule: crystal structures of translesion DNA synthesis with a non-natural nucleotide. *Biochemistry* 46, 10551–10561. doi: 10.1021/bi7008807

Zahn, K. E., and Doublé, S. (2014). Look Ma, no PCNA: how DNA polymerase epsilon synthesizes long stretches of DNA without a processivity factor. *Nat. Struct. Mol. Biol.* 21, 12–14. doi: 10.1038/nsmh.2749

Zahn, K. E., Wallace, S. S., and Doublé, S. (2011). DNA polymerases provide a canone of strategies for translesion synthesis past oxidatively generated lesions. *Curr. Opin. Struct. Biol.* 21, 358–369. doi: 10.1016/j.sbi.2011.03.008

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