



Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.



Gene wiki review

POLD1: Central mediator of DNA replication and repair, and implication in cancer and other pathologies



Emmanuelle Nicolas, Erica A. Golemis, Sanjeevani Arora *

Program in Molecular Therapeutics, Fox Chase Cancer Center, Philadelphia, PA 19111, USA

ARTICLE INFO

Article history:

Received 4 April 2016

Received in revised form 10 June 2016

Accepted 14 June 2016

Available online 16 June 2016

Keywords:

POLD1

p125

Polymerase delta

DNA damage response

Replication

MDPL syndrome

Hypermutation

ABSTRACT

The evolutionarily conserved human polymerase delta (*POLD1*) gene encodes the large p125 subunit which provides the essential catalytic activities of polymerase δ (Pol δ), mediated by 5'–3' DNA polymerase and 3'–5' exonuclease moieties. *POLD1* associates with three smaller subunits (POLD2, POLD3, POLD4), which together with Replication Factor C and Proliferating Nuclear Cell Antigen constitute the polymerase holoenzyme. Pol δ function is essential for replication, with a primary role as the replicase for the lagging strand. Pol δ also has an important proofreading ability conferred by the exonuclease activity, which is critical for ensuring replicative fidelity, but also serves to repair DNA lesions arising as a result of exposure to mutagens. Pol δ has been shown to be important for multiple forms of DNA repair, including nucleotide excision repair, double strand break repair, base excision repair, and mismatch repair. A growing number of studies in the past decade have linked germline and sporadic mutations in *POLD1* and the other subunits of Pol δ with human pathologies. Mutations in Pol δ in mice and humans lead to genomic instability, mutator phenotype and tumorigenesis. The advent of genome sequencing techniques has identified damaging mutations in the proofreading domain of *POLD1* as the underlying cause of some inherited cancers, and suggested that mutations in *POLD1* may influence therapeutic management. In addition, mutations in *POLD1* have been identified in the developmental disorders of mandibular hypoplasia, deafness, progeroid features and lipodystrophy and atypical Werner syndrome, while changes in expression or activity of *POLD1* have been linked to senescence and aging. Intriguingly, some recent evidence suggests that *POLD1* function may also be altered in diabetes. We provide an overview of critical Pol δ activities in the context of these pathologic conditions.

© 2016 Elsevier B.V. All rights reserved.

Contents

1. Introduction	129
2. POLD1 gene	129
3. p125 protein	130
4. p125 expression and regulation	132
4.1. Expression	132
4.2. Post-translational modification	133
4.3. Altered protein interactions	133
5. Pol δ function in DNA replication	133
6. Pol δ function in DNA repair processes	134
7. POLD1 in human disease	135

Abbreviations: ATR, Ataxia Telangiectasia and Rad3-related protein; AWS, atypical Werner syndrome; BER, base excision repair; BIR, break-induced recombination; bMMRD, biallelic mismatch repair deficiency; CIA, cytosolic iron–sulfur protein assembly; CRC, colorectal cancer; DSB, double strand break; EDMC, endometrial cancer; ISC, iron sulfur cluster; lncRNA, long non-coding RNA; LS, Lynch syndrome; MDPL, mandibular hypoplasia, deafness, progeroid features and lipodystrophy; miR, microRNA; MMEJ, microhomology-mediated end joining; MMR, mismatch repair; ncRNA, non-coding RNA; NER, nucleotide excision repair; NHEJ, nonhomologous end joining; NoDS, nucleolar detention sequence; OMIM, Online Mendelian Inheritance in Man; PCNA, proliferating cell nuclear antigen; PIP, PCNA-interacting protein; PKA, protein kinase A; Pol α , polymerase alpha; POLD1, human polymerase delta subunit 1; Pol δ , polymerase delta; Pol ϵ , polymerase epsilon; PRMT7, protein arginine methyltransferase 7; RFC, Replication Factor C; sLS, suspected cases of Lynch syndrome; Sp1, specificity protein 1; Sp3, specificity protein 3; SSB, single-strand break; TCGA, The Cancer Genome Atlas; TLS, translesion synthesis; WS, Werner syndrome.

* Corresponding author at: Fox Chase Cancer Center, 333 Cottman Ave., Philadelphia, PA 19111, USA.

E-mail address: sanjeevani.arora@fccc.edu (S. Arora).

7.1. POLD1 in MDPL (mandibular hypoplasia, deafness, progeroid features, and lipodystrophy) and atypical Werner Syndrome	135
7.2. POLD1 in cancer	136
8. Conclusions and future directions	137
Acknowledgments	138
References	138

1. Introduction

Replication of DNA is a fundamental property of all living organisms, from single celled prokaryotes through metazoans. The core protein machinery that mediates replication of nuclear DNA in humans is evolutionarily ancient, with polymerases (pols) alpha (α), delta (δ), and epsilon (ϵ) conserved from humans through yeast (Pol1-3) (Makarova et al., 2014). Pol α functions as a DNA primase, while Pol ϵ synthesizes the leading strand and most current studies indicate that Pol δ predominantly synthesizes the lagging strand. Single-celled eukaryotes often undergo very rapid replication cycles, with complete cell doubling occurring within 90 min, and can exist in both haploid and diploid forms. These constraints mandate a replicative process of high integrity under tight time constraints, which is addressed in part by the presence of robust proofreading functions encoded within the δ and ϵ polymerases. In more complex multicellular organisms with long lifespans, the integration of replicative activity with these proofreading functions is increasingly appreciated as essential for normal development, and as contributing to protection against disorders of aging.

Over the past decade, a growing body of information on the genomic and transcriptomic landscape of human diseases has implicated defects in the proofreading/exonuclease and DNA replicative function of δ and ϵ polymerases in developmental disorders and cancer; in response to therapeutics; and more speculatively, in the process of aging and metabolic disorders. In this review, we focus primarily on POLD1, the catalytic subunit of Pol δ , based on a number of recent studies that have emphasized key roles for this protein in human disease. We first summarize the basic gene and protein structures, and biological activities of POLD1 in the context of the polymerase holoenzyme. We then discuss evidence indicating that germline lesions in POLD1 are the etiologic basis of two genetic diseases: mandibular hypoplasia, deafness, progeroid features and lipodystrophy (MDPL) and a subtype of Werner syndrome not linked to the canonical WRN gene. We also discuss evidence linking defects in POLD1 to inherited risk for cancer, providing supporting evidence from mouse models and cell culture experiments. Finally, we discuss several studies that potentially link changes in POLD1 function to additional biological syndromes.

In the present review, the main emphasis is on the regulation of the POLD1 gene, its protein product, and functions in relationship to defects

that have been observed in patients. On other complementary aspects of the process of the replication, we direct the reader to recent reviews listed in Table 1.

2. POLD1 gene

The POLD1 gene, also known as CDC2, MDPL, POLD, and CRCS10, is located on chromosome 19 (Chung et al., 1991) at q13.3–q13.4 (Kemper et al., 1992) and is approximately 34 kb long. The major transcript (NM_002691.3) has 27 exons, which translate into a 1107 amino acid protein called the p125 subunit or A unit of Pol δ (Fig. 1A). A longer isoform with a 26 amino acid in-frame insertion after amino acid 592 (NP_001295561.1) is also reported in multiple databases, although at present no publication addresses the biological activity of this protein. A pseudogene (LOC100422453) is located on chromosome 6.

As is the case for many housekeeping genes, the POLD1 promoter is GC rich and does not contain a TATA box (Chang et al., 1995). Transcription of POLD1 is regulated during the cell cycle, with the highest level of expression observed in late G1/S phase when cellular DNA is replicated in preparation for mitosis. Fig. 1B summarizes elements of the promoter that have been studied. Several defined elements in the POLD1 promoter connect expression of this gene to the activity of proteins that regulate the cell cycle. Two 11-bp direct repeats which can be bound by the transcription factors specificity protein 1 (Sp1) and specificity protein 3 (Sp3), and an E2F-like sequence also located immediately upstream of the major transcription site, are involved in the induction of POLD1 by serum stimulation (Zhao and Chang, 1997). An Sp1 site is located between the two halves of a p53 site. By competing for binding to these sequences p53 (encoded by the TP53 tumor suppressor) represses Sp1-stimulated POLD1 promoter activity (Li and Lee, 2001). Competitive displacement of Sp1 by p53 from POLD1 promoter is proposed as the mechanism for the inhibition of POLD1 expression upon cadmium treatment (Antoniali et al., 2015).

A cell cycle element/cell cycle gene homology region (CDE/CHR), known to be important for transcription in G2/M (Muller et al., 2014), is located within 50 bp downstream of the start site (Song et al., 2009). Mutations in this element affect the regulation of the POLD1 promoter by E2F1 and p21 (Song et al., 2009). A recent study by Fischer and colleagues confirmed earlier observations of p53 repression of POLD1,

Table 1

List of references for recent reviews on replication, to guide further study.

Topic	References
Replication initiation and nuclear organization	Marks et al. (2016)
Asymmetry of nuclear DNA replication—fidelity in replication of the leading and lagging strands	Lujan et al. (2016b)
Role of polymerase epsilon in DNA replication and genome stability	Henninger and Pursell (2014)
Quality of nucleotide pools and accuracy of DNA replication	Waisertreiger et al. (2012)
Regulation of deoxynucleotide metabolism in cancer	Kohnken et al. (2015)
Correction of the rare mismatches that escape proofreading by mismatch repair	Kunkel and Erie (2015)
Translesion synthesis and damage tolerance	Hedglin and Benkovic (2015); Jansen et al. (2015b)
Checkpoint signal at the replication block-Chk1 activation	Gonzalez Besteiro and Gottifredi (2015)
Oncogene-induced replication stress	Hills and Diffley (2014)
Replication stress response	Berti and Vindigni (2016); Munoz and Mendez (2016); Roos et al. (2016)
Transcription as a source of replication stress	Gaillard and Aguilera (2016)
Replication stress and cancer therapy	Kotsantis et al. (2015)
Ribonucleotide incorporation during DNA replication	Lujan et al. (2016b)
Ribonucleotide triggered DNA damage and cellular response to damage	Wallace and Williams (2014)
Long range coordination and regulation of replication and repair events by Fe–S clusters	Fuss et al. (2015)
Role of PCNA in DNA replication and repair	Park et al. (2015)

and suggested that p53 does not directly bind to the CDE/CHR sites but causes indirect p21-dependent activation of a p107/DP/E2F4 DREAM complex, which binds at the CDE/CHR sites to inhibit transcription (Fischer et al., 2016). Similar to the promoters of other genes involved in the control of cell proliferation, a (−502, +66) cloned promoter of *POLD1* was more active in cancer cells than in normal fibroblasts (Kashkin et al., 2015). The *POLD1* promoter also contains a response element (RTMAAYA; Chen et al., 2016) for Forkhead family proteins. Overexpression of the microRNA (miR) miR-155 results in downregulation of *POLD1* mRNA and protein expression that correlates with suppression of the forkhead protein FOXO3a, a known miR-155 target (Czochor et al., 2016).

3. p125 protein

A recent review has thoroughly reviewed the historical milestones in the description of DNA polymerases, summarized in a useful discovery timeline diagram (Friedberg, 2006). Byrnes et al. first reported the discovery of a third DNA polymerase in mammalian cells, designating this entity polymerase delta (Byrnes et al., 1976). Further collective work elucidated this entity as a four-subunit enzyme for which activity could be reconstituted in vitro (Xie et al., 2002). The protein product of *POLD1* is the p125 catalytic subunit of Pol δ . p125 forms a heterotetramer with three smaller subunits encoded by the *POLD3* (p66), *POLD2* (p50) and *POLD4* (p12) genes. p50 serves as a scaffold by interacting with all other subunits (Zhou et al., 2012). During replication, the heterotetramer associates with Replication Factor C (RFC) that functions as a clamp loader, and Proliferating Cell Nuclear Antigen (PCNA) that functions as a molecular sliding clamp and processivity factor (Hindges and Hubscher,

1997). Fig. 2 provides a basic schematic of interactions during replication and repair.

Similar to Pol α and ϵ , Pol δ uses a common B-family fold: the shared and unique structural elements of these replicative polymerases have been recently reviewed in (Doublet and Zahn, 2014). The review compiled data from various crystal structures including the structure for Pol3, the yeast homolog of Pol δ (Swan et al., 2009). Proofreading of incorporation errors requires not only the exonuclease activity but also a switch of the nascent primer terminus between the polymerase and exonuclease sites (Jin et al., 2005) by a mechanism involving a conserved extended beta-hairpin loop (Hogg et al., 2007). The evolution of the beta hairpin structure in the family of B polymerases to meet specific needs was recently described in Darmawan et al. (2015). An alignment of the sequences coding for the exonuclease domain from phi29, RB69, T4, and Pol δ and ϵ in *Saccharomyces cerevisiae* and human showing high conservation is found in Henninger and Pursell (2014). Because of its high degree of evolutionary conservation, bacteriophage RB69 polymerase gp43 has proven a good model for understanding the high fidelity of B family replicative polymerases (Xia and Konigsberg, 2014). The close relationship between the B polymerases of eukaryotes, archaea, and those of NCLDV (nucleo-cytoplasmic large DNA viruses) has been hypothesized to support the process of evolutionary transfer (Takemura et al., 2015).

Fig. 3 summarizes some important conserved motifs and amino acids that are found in p125. Residues 4–19 form a nuclear localization signal. The exonuclease and polymerase catalytic domains are encompassed by amino acids 304–533 and 579–974, respectively. The exonuclease domain is of the DEDDy type in the classification of exonuclease superfamilies proposed by Zuo and Deutscher (2001);

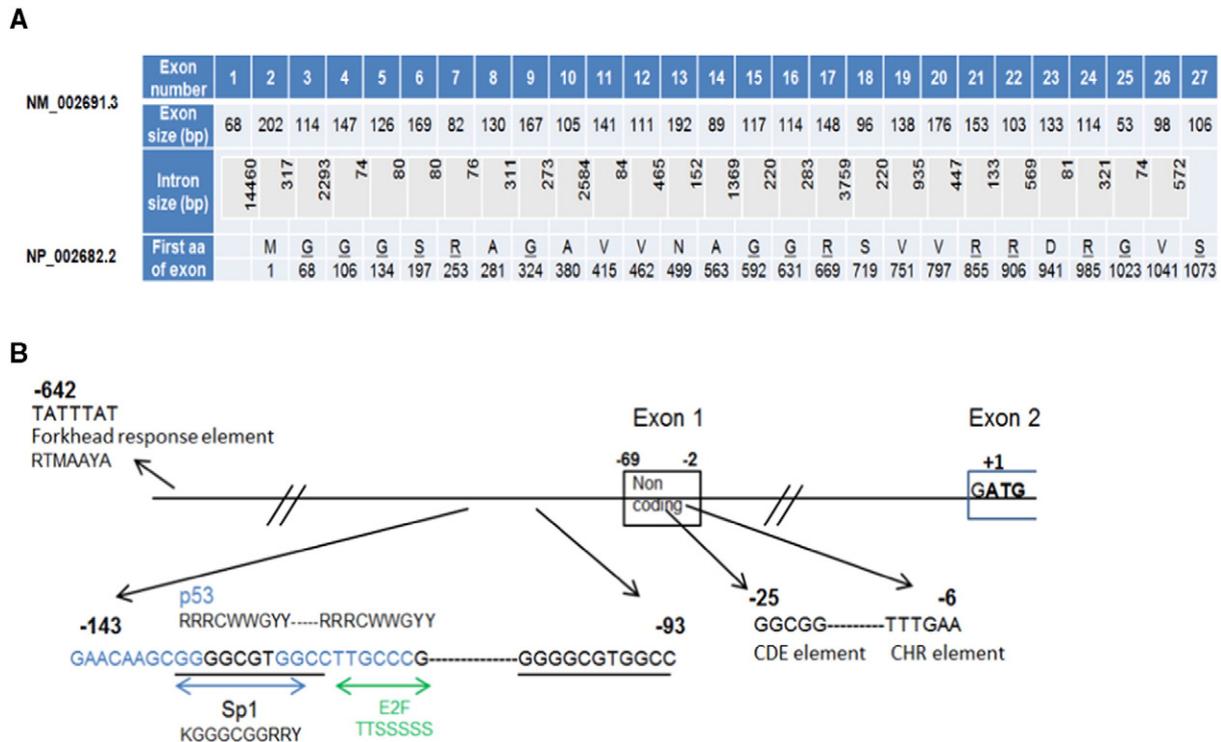


Fig. 1. *POLD1* gene and promoter structure. A. Splicing structure. Schematic representation of the number and sizes of exons and introns of transcript NM_002691.3. The information was extracted from GeneTable on the NCBI website (http://www.ncbi.nlm.nih.gov/gene/5424?report=gene_table). The coding sequence is from nucleotides 70 to 3393 of the spliced mRNA. The encoded protein NP_002682.2 has 1107 amino acids. The first amino acid encoded by each exon is indicated. The residues overlapping a splice site are underlined. B. Schematic representation of the *POLD1* promoter structure. The two 11 bp repeats (underlined) were identified by Zhao and Chang (1997). The p53 binding site (in blue) has 17 of 20 nucleotides matching the canonical site; a 5 bp spacer harbors a Sp1 binding site between its two halves (black double arrow). This site was identified as functional in Li and Lee (2001). An E2F binding site overlaps the 3' end of the motif (green double arrow). The CDE/CHR element, important for cell cycle regulation, was identified and functionally analyzed in Song et al. (2009). The forkhead response element may be involved in the regulation of expression by miR-155 (Czochor et al., 2016).

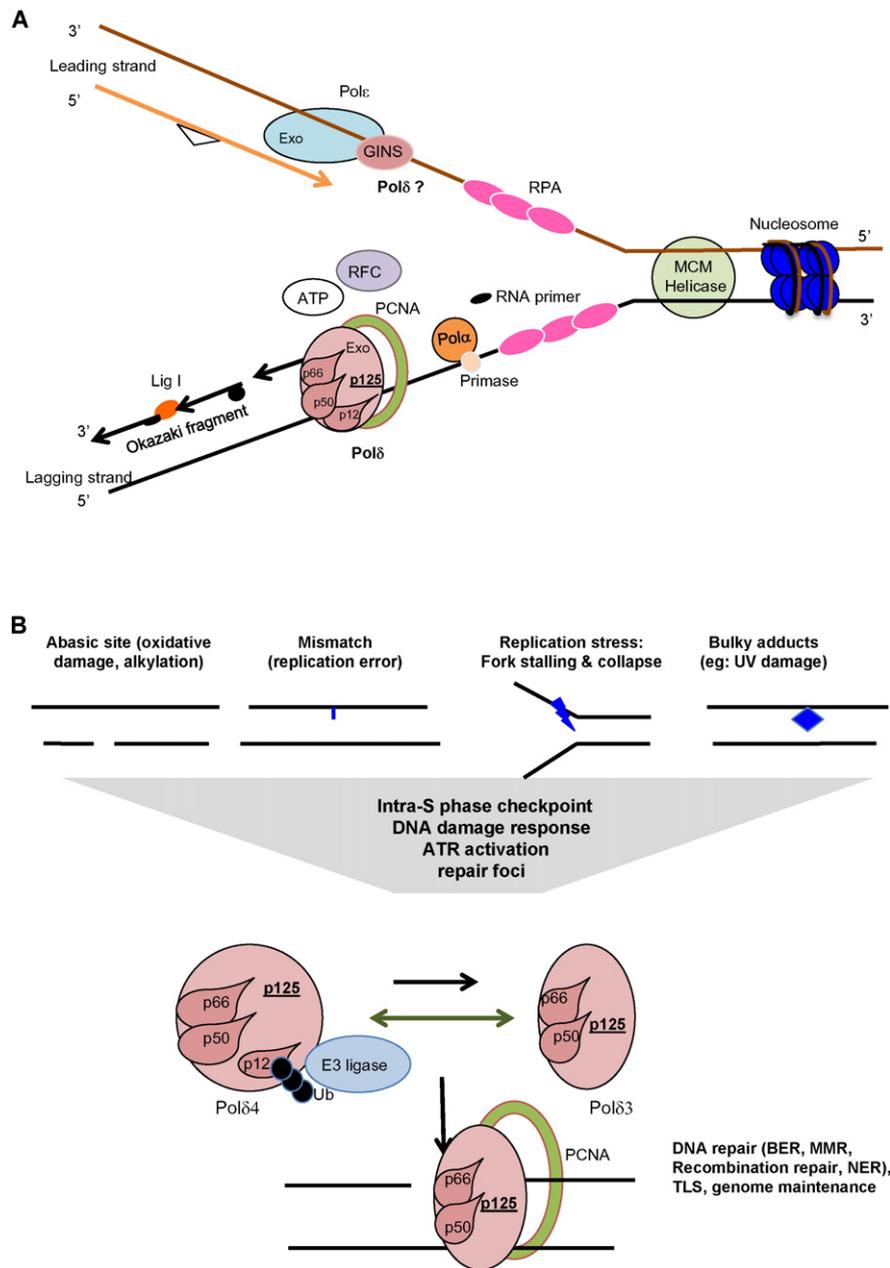


Fig. 2. A simplified view of the function of Pol δ at the DNA replication fork and in response to damaged DNA. **A.** The Pol δ complex (p125, p66, p50 and p12) associates with replication forks. Exo marks the exonuclease domain of p125 and Pol ϵ . The MCM helicase (light lime green) drives the replication fork forward. The single-stranded regions are coated with the single-stranded binding protein, replication protein A (RPA) (pink). Pol α is bound to a primase, which initiates synthesis of lagging strand (black line) by producing an RNA primer which is then elongated first by Pol α , then by Pol δ . Pol ϵ is positioned on the leading strand (orange line). GINS (go-ichi-ni-san comprising of four related subunits of the complex Sld5, Psf1, Psf2 and Psf3) (Lujan et al., 2016a, 2016b) interacts with Pol ϵ to initiate DNA synthesis. Some data suggest a role of the Pol δ complex in the leading strand synthesis. Both polymerases use PCNA (proliferating cell nuclear antigen; green rings) as a sliding clamp. The RFC (replication factor C) complex in conjunction with RPA loads PCNA onto the DNA. PCNA-loading typically requires ATP, although ATP-independent mechanisms have been suggested (Burgers and Yoder, 1993; Chen et al., 2009). As replication progresses, nucleosomes are displaced and single-stranded DNA is bound by RPA. As the lagging strand is synthesized in short fragments, Okazaki fragments, ligases (ligase I) are used to seal gaps. Replication errors created by the polymerases (indicated as open black triangle on the newly synthesized leading strand), can be corrected by post-replication mismatch repair (MMR). As recently reviewed by Johansson and Dixon (2013) and discussed by others (Shamoo and Steitz, 1999), it has been difficult to isolate intact replisomes. Work from Georgescu et al. suggests that the eukaryotic replisome is asymmetric in its architecture with Pol ϵ on the leading strand and Pol δ on the lagging strand (Georgescu et al., 2014; Georgescu et al., 2015; Zhang and O'Donnell, 2016). Additional details on the replisome architecture, including relative positioning of Pol δ and PCNA, have been recently reviewed (Zhang and O'Donnell, 2016). **B.** Multiple forms of DNA damage can activate an intra-S phase checkpoint. This recruits and activates ATR to the site of DNA damage, triggering downstream DNA damage response signaling. During this process, Pol δ is recruited to repair foci. P12 is ubiquitinated (black circles, Ub) and degraded by the proteasome, which leads to the conversion of Pol δ 4 to the Pol δ 3 complex, which has altered catalytic activity. It is possible to rapidly exchange between the Pol δ 3 and Pol δ 4 complexes (two-way green arrow) (Lee et al., 2012).

also see Hansen et al. (2015): the 4 conserved acidic residues (DEDD) that serve as ligands for the two metal ions required for catalysis are D316 and E318 in the ExoI motif, D402 in the ExoII motif and D515 in the ExoIII motif. Y511 contributes to the specific YX(3)D pattern of

the ExoIII motif and makes the domain of “y” type (contrasting with “h” exonucleases, which use histidines). The ExoIV (SYTLNAVS) and V (HFLGEQKED) motifs, located between ExoII and ExoIII are conserved regions in orthologous sequences of the catalytic units of Pol δ and ϵ

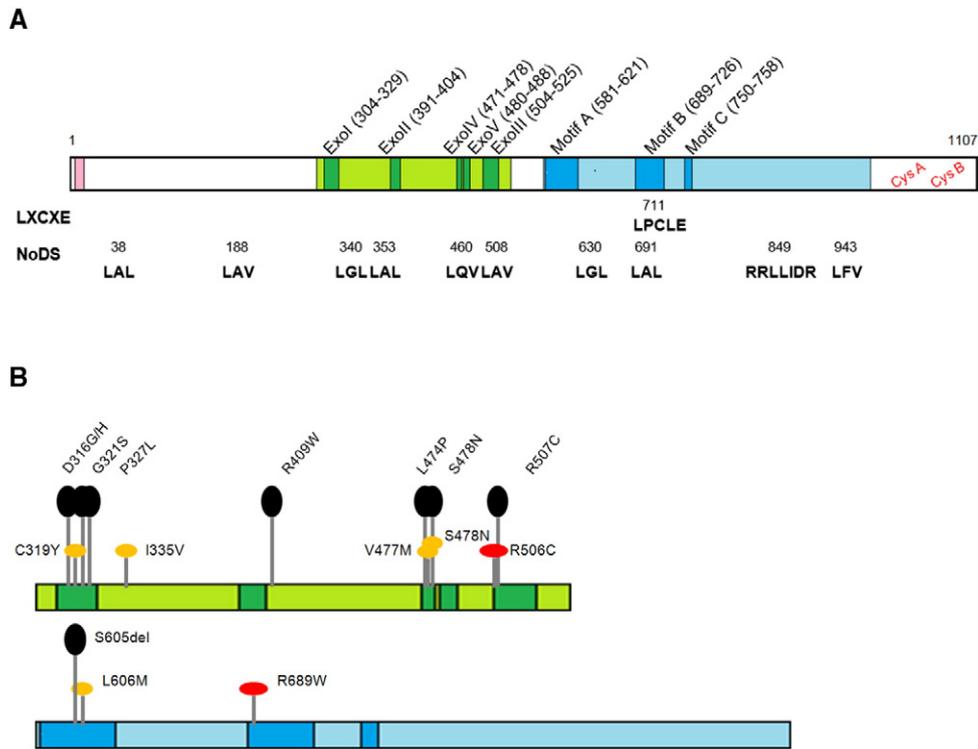


Fig. 3. p125/POLD1 protein structure. A. Schematic domain structure and motif sites in human p125/POLD1. The exonuclease and polymerase domains are shown in green and blue respectively. Conserved motifs are shown in darker shades. The ExoI–III motifs, and motifs A–C are highly conserved in the B-family of polymerases. ExoIV and V are conserved between Pol δ and Pol ϵ (Hansen et al., 2015). The LXCXE motif was reported by Krucher et al. (2000). The nucleolar detention sequence (NoDS) defined by the motif RR(I/L)XXXR and a least two hydrophobic triplets with leucine as first residue and leucine or valine as last residue is represented by the amino acid RRLIDR and nine hydrophobic triplets (Mekhail et al., 2007). In p125, this is represented by the amino acids 849–851 (RRL) and nine hydrophobic triplets (3 LAL, 2 LGL, 2 LAV, 1 LQV, 1 LFV starting at leucine residues 38, 188, 340, 353, 460, 508, 630, 691 and 943) (Mekhail et al., 2007; Audas et al., 2012). The cysteine motifs CysA and CysB located in C-terminal are a Zn-binding site and a Fe–S cluster respectively (Netz et al., 2012). The figure also shows the nuclear localization signal (NLS) in the amino-terminus (pink). B. Enlarged schematic of the exonuclease (green) and polymerase (blue) domains, representing mutations discussed in text. Black denotes germline mutations, orange denotes somatic mutations and red denotes mutations detected in human cell lines.

(Hansen et al., 2015). In the polymerase domain, two catalytic aspartates from motif A (DXLYPS, D602) and motif C (DTDS, D757) contact Ca^{2+} at the active site. In human p125, tyrosine Y701 occupies a position equivalent of Y567 in the RB69 bacteriophage ortholog, and provides a conserved bulky residue important for the exclusion of ribonucleotides during DNA synthesis, also referred to as a “steric gate” (Brown and Suo, 2011; Cerritelli and Crouch, 2016).

Residues 711 to 715 encode a LXCXE motif (Guiley et al., 2015), which has been reported to mediate p125 binding to pRB during the G1 phase of cell cycle (Krucher et al., 2000), but has otherwise been little studied. Residues 806 to 809 encode a highly conserved KKRY motif that is important for stabilizing the primer terminus in the polymerase active site (Hogg et al., 2007). A complex nucleolar detention sequence (NoDS), which mobilizes p125 to specific intranuclear compartments in a non-coding RNA (ncRNA)-mediated process is also present, represented by small sequence motifs dispersed throughout the protein coding region (Mekhail et al., 2007; Audas et al., 2012; Lam and Trinkle-Mulcahy, 2015). Two conserved cysteine-rich metal-binding motifs (CysA and CysB) are located in the C-terminal domain between amino acids 1012 and 1083. CysA Zn-binding motif is important for PCNA binding, while CysB coordinates an [4Fe–4S] cluster with roles in recruitment of accessory subunits (Netz et al., 2012, and discussed in Fuss et al., 2015). The Fe–S cluster is added through cytosolic iron–sulfur protein assembly (CIA), which requires the function of the mitochondrial iron sulfur cluster (ISC) assembly machinery (Paul and Lill, 2015). The maturation process is mediated by the core targeting complex CIA1–CIA2B/FAM96B–MMS19 that interacts with the apoprotein and assures specific Fe–S cluster insertion (Gari et al., 2012; Stehling et al., 2012).

4. p125 expression and regulation

p125 is ubiquitously expressed in all tissues. The expression and activity of p125 are controlled by multiple mechanisms, which include control of synthesis and stability, post-translational modification, and protein–protein interactions.

4.1. Expression

Decreased p125 expression has been observed in senescent human skin fibroblasts (Takahashi et al., 2005) and in the lymphocytes from elderly subjects when compared to younger ones (Wang et al., 2012). In response to DNA damage, p125 mRNA expression is epigenetically regulated by changes in the activity of protein arginine methyltransferase 7 (PRMT7) and the BRG1-based hSWI/SNF chromatin remodeling complex. PRMT7 typically methylates arginine 3 of histones H2A and H4 to repress the *POLD1* promoter. PRMT7 knockdown caused a decrease in methylation and derepression of various target DNA repair genes including *POLD1* that resulted in increased p125 and an enhanced cellular resistance to DNA-damaging agents that only *POLD1* knockdown was able to reverse (Karkhanis et al., 2012). p125 expression is also controlled indirectly by miR-155-dependent transcriptional regulation (Czochor et al., 2016), and changes in the activity of the transcription factors such as p53 that bind the *POLD1* promoter. p125 was recently identified as a target of a long ncRNA (lncRNA) PVT1 that has been proposed to have oncogenic function (Cui et al., 2016), with reduced expression of PVT1 decreasing *POLD1* gene expression (Wu et al., 2016).

4.2. Post-translational modification

p125 has been described as a substrate for cyclin-dependent kinases but no major effect of the phosphorylation has been reported (Wu et al., 1998). Some regulation by phosphorylation is found at the holoenzyme level. For example, phosphorylation of S458 located in the PCNA-interacting protein (PIP) binding motif of the p125 partner p50 by protein kinase A (PKA) decreases the affinity of the Pol δ complex for PCNA (Rahmeh et al., 2012).

4.3. Altered protein interactions

The catalytic activity of p125 in the Pol δ holoenzyme is modulated by the *POLD4* product p12, which is the only subunit of the heterotetramer (p125, p68, p50, p12) that does not have a paralog in yeast. In response to DNA damage or replication stress caused by UV irradiation, methyl methanesulfonate, hydroxyurea or aphidicolin, p12 is degraded, shifting the holoenzyme from the heterotetramer Pol δ 4 (with p12) to the heterotrimer Pol δ 3 (without p12) (Zhang et al., 2007). The depletion of Pol δ 4 is regulated in part by ATR (Ataxia Telangiectasia and Rad3-related protein) (Zhang et al., 2007) and mediated by the E3 ligase RNF8 (Zhang et al., 2013b). Shifting to dominance of the less error-prone Pol δ 3 is marked by altered enzymatic activities such as a greater proofreading ability and a greater discrimination against mismatched primers and small lesions that are readily bypassed in a mutagenic manner by Pol δ 4 (Meng et al., 2009; Lee et al., 2014). It has been suggested that Pol δ 3 was an adaptation of Pol δ for repair following induction of damage (Meng et al., 2009; Lee et al., 2014). However, during normal cell cycle, Pol δ 3 is also present in S phase, when DNA replication occurs, due to partial degradation of p12 by the E3 ligase CRL4^{Cdt2} (Zhang et al., 2013a). CRL4^{Cdt2} also degrades p12 in response to UV damage (Zhang et al., 2013a), triggering an intra-S-phase checkpoint and inhibiting fork progression (Teraï et al., 2013). In recent findings, p12 has also been shown to be degraded by the protease μ -calpain that mediates calcium-triggered apoptosis, although the biological significance of this degradation for Pol δ 3-dependent activities requires more investigation (Fan et al., 2014; Zhang et al., 2016).

Following some biological stimuli that limit replication, p125 is sequestered in the nucleolus. For example, p125 is immobilized in the nucleolus in association with the non polyadenylated intergenic spacer IGS₂₈ that separates individual rDNA transcription units in response to acidosis, in a process mediated by the p125 NoDS domain (Audas et al., 2012). Transport to the nucleolus of the holoenzyme also involves a physical interaction between p50, encoded by *POLD2*, and the RecQ helicase WRN (Szekely et al., 2000). WRN redistributes from the nucleolus to the nucleoplasm in response to DNA damage or replication arrest (Karmakar and Bohr, 2005) by a mechanism regulated by SIRT1 (Lee et al., 2015), contributing to the release of Pol δ . WRNIP1, possibly joined by WRN, is recruited at stalled replication forks by ATM signaling (Kanu et al., 2015) where it increases the efficiency of Pol δ for DNA synthesis and repair, primarily by increasing the frequency of initiation (Tsurimoto et al., 2005). Perhaps relatedly, p125 has been reported to interact with the *Coronavirus* nsp13 replicase during viral infection, with the interaction inducing S phase arrest and ATR-dependent DNA damage response (Xu et al., 2011).

p125 has also been shown to interact with PDIP46/SKAR, mediating strong activation of p125 in response to signaling from S6 kinase (Wang et al., 2016). Some activated oncogenes have been reported to induce collapse of the replication fork (Boyer et al., 2016). p125 has been shown to interact directly with the oncogenic transcription factor LMO2, with the interaction mediated by tethering to DNA: the authors of this study hypothesized that protein–protein interaction with essential DNA replicative enzymes may be an additional mechanism of oncogene-induced DNA replication stress (Sincennes et al., 2016). The process of replicative stress is currently of considerable interest as a general feature

of oncogenic transformation, characterizing the early stages of oncogenesis, and exerting both a barrier to early tumor formation and a modulator of disease course and therapeutic response (Boyer et al., 2016): the role of Pol δ as a target in this process requires more study.

5. Pol δ function in DNA replication

High fidelity of replication relies on an accurate base selection by the replicative polymerases (α , δ and ϵ), exonucleolytic removal of mispaired nucleotides (proofreading) by the exonuclease activity of Pol δ and ϵ , and post-replicative surveillance and repair by the mismatch repair (MMR) system (Ganai and Johansson, 2016). This broad field cannot be comprehensively covered in this article: recent reviews addressing specific aspects of the replication process are noted in Table 1. A schematic of p125 associations and action during replication is provided in Fig. 2A. Wild type human Pol δ is highly accurate, catalyzing on average less than one substitution per 220,000 nucleotides polymerized, as determined in a forward mutation assay. The misincorporation error rate goes up 10-fold for an exonuclease-deficient form (Schmitt et al., 2009). As for all polymerases, the replication errors are generated at different rates depending on the base pair substitution and the local DNA sequence (Fortune et al., 2005; Schmitt et al., 2009; Korona et al., 2011). Errors that escape proofreading are subject to MMR with variable efficiency (Kunkel and Erie, 2015), bringing the estimated mutation rate range of the in vivo complete replication complex to a frequency of 10^{-7} to 10^{-9} . A few examples, such as the observation of very efficient repair of single-base indel mismatches in long homopolymers, support the idea that MMR is most efficient at correcting mismatches generated at high rates during replication and inefficiently proofread (Kunkel and Erie, 2015). Complementarity between the factors contributing to replication fidelity (base selectivity, proofreading and MMR) is also observed when considering that average proofreading and MMR are higher during lagging strand replication that has average lower base selectivity, whereas the opposite is observed for the leading strand replication (St Charles et al., 2015).

Replication fidelity has been extensively studied in yeast, with this work providing basic models for conceptualizing the behavior of human polymerases (reviewed in Skoneczna et al., 2015). The prevailing model for organization at the replication fork in humans is that after Pol α initiation of replication by RNA-primed DNA synthesis, Pol ϵ and Pol δ replicate the leading strand and lagging strand respectively with high fidelity (Nick McElhinny et al., 2008). The model has been recently challenged with suggestions that Pol δ is also involved in the leading strand, with the topic currently attracting much debate (Pavlov and Shcherbakova, 2010; Johnson et al., 2015; Stillman, 2015; Burgers et al., 2016; Lujan et al., 2016b). Understanding the division of labor between the three polymerases is important as it has consequences for the mutational landscape they may produce when defective. In humans, mutational processes shape the somatic genome. The replication fidelity of the leading and lagging strands depends on the unique error signature of polymerases δ and ϵ (Korona et al., 2011), but also on the balance between proofreading and MMR, and differences in the processing of ribonucleotides between the two strands (Lujan et al., 2016b; Lujan et al., 2016a). The single stranded DNA formed during DNA lagging strand synthesis is vulnerable to ss-DNA targeting mutagens, and is a preferred substrate for APOBEC mutations (Hoopes et al., 2016). Error-prone DNA synthesized by Pol α is retained in the mature lagging strand, as DNA-binding proteins that rapidly reassociate post-replication prevent Pol δ from fulfilling its displacement/repair task (Reijns et al., 2015).

While studies of yeast polymerases are highly informative, pathways that are identified through studies of the yeast replication machinery are not invariably conserved in human. As one example, the yeast Pol3 mutant R696W has been shown to promote its own infidelity by inducing accumulation of incomplete intermediates that trigger a checkpoint response that involves Dun1-dependent upregulation of ribonucleotide

reductase and a subsequent elevation of the dNTP pools. Elevated dNTP levels decrease the nucleotide selectivity, which facilitates the extension of mismatched primer termini resulting in more errors (Mertz et al., 2015). A similar mechanism with Dun1 involvement was described for a Pol ϵ mutant (Williams et al., 2015). Human DLD-1 cancer cells bear the *POLD1* R689W mutation that is comparable to the yeast variant R696W and is associated with a very high mutator effect (Flohr et al., 1999). While suggestions were made that some mutator phenotypes due to error-prone polymerases could be modulated by treatments that target the dNTP pools, Dun1 is not conserved from yeast to mammal, suggesting that additional pathways should be looked for (Sohl et al., 2015).

The conventional replication machinery cannot replicate the end of a linear chromosome because the synthesis of Okazaki fragments requires the attachment of RNA primers ahead of the lagging strand. This end replication problem is solved by the reverse transcriptase telomerase, which catalyzes addition of telomeric TG-rich repeats onto the ends of chromosomes. With a dedicated RNA molecule as part of the holoenzyme, the telomerase adds complementary RNA bases to the 3' end of the DNA strand. Once the 3' end of the lagging strand template is sufficiently elongated, the polymerases α and δ add the complementary nucleotides to the ends of the chromosomes (Diede and Gottschling, 1999). Human telomeres, which consist of 5 to 10 kb of TTAGGG repeats, are coated with proteins and exhibit complex secondary structures such as hairpins and G-quadruplex. These pose a challenge for various polymerases including Pol δ used to replicate the telomeric G-rich lagging strand, and several translesion polymerases (discussed below) are potentially recruited in case of blockage (Edwards et al., 2014).

6. Pol δ function in DNA repair processes

The role of Pol δ in genome maintenance involves an active role in DNA repair processes, with this role particularly relevant to defects in *POLD1* being associated with human disease (Fig. 2B). During replication, encountering aberrant DNA structures that include misincorporated bases or other defects causes the polymerase complex to pause, and activation of exonuclease functions. Exonuclease function can also be activated if intracellular pools of dNTP are present at high concentrations, or with nucleotide imbalances (Reha-Krantz, 2010). The process of switching between polymerase and exonuclease domains is an important determinant of proofreading (and hence fidelity) and the kinetic mechanisms for both Pol δ (Meng et al., 2010) and Pol ϵ (Ganai et al., 2015), has been shown to conform to the model established for T7 DNA polymerase (Johnson, 1993; Kunkel and Bebenek, 2000).

Pol δ has long been known to participate in many categories of replication-coupled DNA events associated with repair, including translesion synthesis (TLS), Okazaki fragment maturation, nonhomologous end joining (NHEJ), break-induced recombination (BIR), nucleotide excision repair (NER), long patch base excision repair (BER) and mismatch repair (MMR) (reviewed in Prindle and Loeb, 2012). These DNA repair processes are highly specialized and deal with varied forms of DNA damage. Briefly, DSBs are the most lethal form of DNA damage, corrected by two major DNA repair processes: homologous recombination (HR) and NHEJ. BIR is the repair of one-ended DSBs via strand invasion into the homologous DNA duplex and replication to the chromosome end. NER deals with bulky, helix-distorting DNA adducts, while BER and MMR correct non-helix distorting damaged or incorrect bases in the DNA, respectively. More recent studies have identified a cooperative role of Pol δ with Pol η in microhomology-mediated end joining (MMEJ) (Meyer et al., 2015).

A study evaluating mutations of the human *POLD1* exonuclease domain (D402 and D515, in the ExoII and ExoIII motifs) using oligonucleotide templates suggested that these residues determine the efficiency of bypass of lesions such as 8-oxoguanine and abasic sites (Fazlieva

et al., 2009). If normal mechanisms of lesion bypass are restricted, TLS is a component of the evolutionarily conserved *RAD6/RAD18* mechanism of damage tolerance that allows cells to overcome unrepaired lesions that would interfere with the progression of the replication fork. Because TLS is potentially mutagenic, the process is tightly regulated. In part, it utilizes non-processive polymerases of low fidelity. These include four polymerases from the Y-family: Pol η (POLH), REV1, Pol κ (POLK) and Pol ι (POLI); one B-family polymerase: Pol ζ (REV3L), and two polymerases from the A-family: Pol ν (POLN) and Pol θ (POLQ) (reviewed in Saugar et al., 2014). During TLS, p125 is exchanged for a catalytic unit able to replicate over the lesion, in either error-prone or error-free mode, with participation of the accessory subunits p50 and p66 (Baranovskiy et al., 2012; Baldeck et al., 2015), and of the Fe-S clusters in the flexible C-terminal domains of the catalytic units being exchanged (Baranovskiy et al., 2012; Fuss et al., 2015). The polymerase switching depends in part on the monoubiquitination of the lysine residue 164 of the sliding clamp PCNA in the stalled holoenzyme. After nucleotide incorporation opposite the damage, the switch is reversed, likely because of the low affinity of TLS polymerase for PCNA (Hedglin and Benkovic, 2015), leading to substitution of Pol δ .

As noted above, DNA damage response signaling arising from some forms of DNA damage (alkylating damage, replication stress, oxidative damage and UV light-bulky damage) leads to a rapid degradation of p12 and transformation of the holoenzyme from the heterotetramer Pol δ 4 to the heterotrimer Pol δ 3, with Pol δ 3 having a more discriminative polymerase, and increased exonuclease activity (Meng et al., 2009; Lee et al., 2014) (Fig. 2B). Pol δ 3 has been located at replication forks that have been stalled by the bulky lesions cyclobutane pyrimidine dimers (CPDs) that are caused by UV irradiation, and is likely responsible for repair by TLS involving Pol δ and Pol η (Chea et al., 2012). As noted above, Pol δ 3 production depends on RNF8-induced p12 degradation, and RNF8 has been identified as an organizer for repair of double-strand breaks (DSBs) suggesting that RNF8-dependent production of Pol δ 3 is for processes involving homologous recombination (Lee et al., 2014). Pol δ 3/Fen1 performed better than Pol δ 4/Fen1 in a strand displacement/flap cleavage assay suggesting that Pol δ 3 is also highly adapted for the Pol α error editing during Okazaki fragment maturation (Lin et al., 2013).

Numerous studies in yeast models have documented accumulation of DNA damage and reduced viability associated with impairment or loss of *POLD1* function. The requirement of the *POLD1* ortholog Pol3 in controlling DNA damage has been established through many studies involving mutants, either natural or engineered. In a recent interesting study, an analysis of Pol3 DNA repair mutants by Johnson et al. led to the conclusion that Pol δ supports replication on both leading and lagging strands, but that the former activity is obscured because of more efficient repair of mismatches on the leading strand, because of differential mismatch removal (Johnson et al., 2015). However, as pointed out by in comments on this study, genetic studies that require the use of strains in which selective repair pathways are knocked-out and in which compensatory repair pathways may be activated as a result of the mutation, inform only about enzyme behavior in the conditions of the mutant background (Stillman, 2015; Zhang and O'Donnell, 2016). Further careful studies contrasting genetic and biochemical properties of the mutant polymerases are necessary. Synthetic interaction between accuracy, proofreading and MMR in haploid strains has been studied by Herr et al. (2014) who also described lethal synergy in proofreading and accuracy, with possible suppression by antimutator alleles, in diploid strains. Synergistic increases in mutation rates in double mutants deficient in proofreading and MMR or accuracy and MMR have previously been described (Morrison et al., 1993; Li et al., 2005). Proofreading-defective MMR-defective double mutants have an increase rate of reversion to the wild type due to the failure of MMR to repair proofreading errors. These findings in yeast are similar to patterns emerging in analysis of cancers with mutations in *POLD1*, discussed below.

Pol δ is important in the post-incision step of NER and has been shown to be recruited via RFC in a PCNA-independent manner (Overmeer et al., 2010). The exonuclease activity of POLD1 complements BER and single-strand break (SSB) repair by dealing with lesions that they are unable to repair. In *in vitro* experiments utilizing HeLa cell extracts and Pol δ exonuclease mutant (D400A) mouse embryonic fibroblasts, it was shown that the 3'-5' exonuclease activity of POLD1 is critical in excising lesions in close proximity to a SSB (Parsons et al., 2007). The exonuclease and polymerase activity (POLD1) of Pol δ is also critical in BER and MMR (Blank et al., 1994; Longley et al., 1997). In the yeast *Saccharomyces pombe*, it was recently shown that HR mechanisms could restart stalled replication forks by utilizing Pol δ strand synthesis activity (POLD1), albeit in a highly error-prone process (Miyabe et al., 2015). In the yeast *S. cerevisiae*, Pol δ has 3 subunits: Pol3/cdc2, catalytic subunit (125 kDa, D1 in human), Pol31/Hys2 (55 kDa, D2 in human) and Pol32 (40 kDa, D3 in human). In *S. cerevisiae*, Pol3 is essential in maintaining Pol δ stability (based on a C-terminal domain interaction with Pol31). A Pol3 mutant (*Pol3-ct*) was proficient in strand invasion and synthesis of a short tract of DNA but unable to perform extensive DNA synthesis required to complete BIR, with a requirement for POLD3 in BIR also demonstrated in human cells (Smith et al., 2009; Costantino et al., 2014). In addition, deletion of just the four C-terminal residues in Pol3 (*Pol3-ct*) leads to defects in HR and BIR repair mechanisms (Brocas et al., 2010).

In the HEK293 kidney cell line, depletion of p125 blocked cell cycle at G1 and G2/M phases, indicative of DNA damage checkpoint responses, and increased oxidative DNA damage (Song et al., 2015). p125 depletion in a panel of colorectal cancer cell lines (DLD-1, RKO, SW480 and LS513) increased sensitivity to chemical inhibition of the DNA damage checkpoint kinases ATR and CHK1, or genetic deficiency of CHK1 (Hocke et al., 2016). Indirect reduction of p125 expression based on miR-155 overexpression promoted genomic instability, with a mutation pattern that is distinct from that of MMR deficiency (Czochor et al., 2016); however, as miRs typically regulate multiple mRNAs, this may reflect the combined activity of multiple miR-155 targets.

7. POLD1 in human disease

7.1. POLD1 in MDPL (mandibular hypoplasia, deafness, progeroid features, and lipodystrophy) and atypical Werner Syndrome

Germline mutations in *POLD1* have been found in multiple patients with MDPL (#615381 in the Online Mendelian Inheritance in Man (OMIM) database) (Weedon et al., 2013; Pelosini et al., 2014; Reinier et al., 2015). MDPL syndrome is a very rare disease characterized by a complex phenotype including progressive lipodystrophy with lack of subcutaneous adipose tissue, mandibular hypoplasia, deafness and progeroid features (Weedon et al., 2013; Reinier et al., 2015). Five unrelated patients diagnosed with MDPL were found to have identical heterozygous c.1812_1814delCTC p.Ser605del (rs398122386) de novo variants. S605 is located in the highly conserved motif A of the polymerase active site (Fig. 3); the Ser605del mutant, lacking this amino acid, was shown to be able to bind DNA but not catalyze polymerization. A sixth patient carried the p.R507C mutation (Pelosini et al., 2014). Although it has not been functionally characterized, R507 is localized in the highly conserved ExoIII domain (Fig. 3B).

In cell line-based studies, a damaging mutation affecting the adjacent residue R506 has been identified in the DLD-1 and HCT-15 human colon cancer cell lines (da Costa et al., 1995). It is not clear whether these represent two independent occurrences of this mutation, or a single isolation, as some studies using DNA fingerprinting and karyotyping suggest that both cell lines arise from the same individual (contrast Tibbetts et al. (1977) and Dexter and Hager (1980) with Vermeulen et al. (1998)). Exome analysis of the HCT-15 cell line maintained in the NCI-60 panel, retrieved from CellMiner (<http://discover.nci.nih.gov/cellminer/>), confirms that it carries the mutation R506H;

this is likely a rare variant associated with cancer pathogenesis, as the Exome Aggregation Consortium database (<http://exac.broadinstitute.org/>) indicates that R506H is represented only once in 115744 alleles examined from the general population. In addition to R506H, DLD-1 has been reported to also contain G10V, R689W, and S746I mutations, which may contribute to the mutator phenotype (Flohr et al., 1999). HCT-15 and DLD-1 are also MSH6-deficient due to frameshift mutations in both alleles (Papadopoulos et al., 1995), further enhancing the mutator phenotype of this model.

As with MDPL, progeria (Swahari and Nakamura, 2016) is a hallmark of Werner syndrome (WS) (#277700 in OMIM) (Oshima et al., 2016). In classical WS, which affects 1 in 200,000 individuals in the United States and has a significantly higher frequency of cases in Japan, patients have multiple symptoms associated with premature aging, and a propensity to atherosclerosis and some types of cancer (Oshima et al., 2016). Most patients with WS have damaging mutations in a RecQ DNA helicase, WRN. Patients who present with symptoms similar to Werner but lack mutations in the *WRN* gene are classified as having atypical Werner syndrome (AWS). Screening of a subset of AWS patients identified the same Ser605del and R507C mutations identified in MDPL, causing these patients to be reclassified as MDPL. Two of the patients with the Ser605del mutation were related (mother and son), and offered the first example of vertical transmission in a segmental form of progeria (Lessel et al., 2015).

At present, the molecular basis of the effect of these mutations is not known. The authors of these studies have speculated that defective p125 arising from these mutations could lead to a higher incidence of stalled replication forks, increasing the prevalence of genomic instability, cell cycle checkpoint response, cell senescence and cell death. Interactions between Pol δ and the WRN protein (Szekely et al., 2000) increase the processivity of Pol δ in a PCNA-independent manner (Kamath-Loeb et al., 2012), impacting both DNA replication and DNA repair. The WRN and Pol δ complex contributes to replication fidelity by having an enhanced ability to hydrolyze structures such as bubbles, four-way junctions and D-loops (Kamath-Loeb et al., 2012). Hence, disruptions of these interactions may contribute to disease phenotype. As pointed out by Oshima and colleagues, mutations in other genes of the replication machinery have been found in AWS patients (Oshima et al., 2016). These include mutations in SPRTN, an adaptor that binds the TLS polymerase Pol η , and in the dNTP pool regulator SAMDH1. Damaging p125 lesions have been convincingly linked to cancer risk, as described below. The mother of one of patient with Ser605del, who also had features of MDPL, died at the age of 34 of ovarian cancer (Lessel et al., 2015). A patient with R507C developed cancer at age 62 (Lessel et al., 2015). However, metaphase spreads prepared from lymphoblastoid cell lines originating from three Ser605del or R507C patients did not show genomic abnormalities when tested under basal conditions or under mitomycin C or aphidicolin stress. It is possible that chromosomal instability occurs in other somatic cell types (Lessel et al., 2015). Alternatively, non-enzymatic effects similar to those identified for WRN (Su et al., 2014), altered interactions with lamins, or epigenetic effects might be involved.

Mutations in lamins are known to cause nuclear envelope-related lipodystrophies with phenotypes reminiscent of MDPL and WS (Guenantin et al., 2014). In HeLa cells in G₁/S arrest or early S-phase, the three replicative polymerases (α , δ and ϵ) are associated with the same nucleoprotein complexes containing lamins. In late S, however, only Pol ϵ is associated with the nuclear matrix through lamins, Pol δ being associated with components of the replication complexes and fulfilling post-replicative tasks such as translesion synthesis (Vaara et al., 2012). The CIA2B/FAM96B protein important for incorporation of the Fe-S cluster in p125 during protein maturation, also has been reported to associate with prelamin A (Xiong et al., 2013). Yet another mechanism for POLD1 action in MDPL has been proposed based on studies of plants, which are considered an interesting model for replication because of the high degree of conservation of the replication machinery

and the high tolerance of plants for missense mutations in components of this machinery (Iglesias and Cerdan, 2016). In plants, studies of the thermosensitive mutant *gis5* (representing the A707V in *POLD1*, corresponding to A692 in human p125, disrupting a hydrophobic triplet of the nuclear detention signal, Fig. 3A) led to the conclusion that Pol δ epigenetically influenced gene expression in the *SEP3* locus, and the suggestion that *POLD1* regulation of a subset of master gene regulators would explain the tissue specificity produced by Ser605del in humans (Iglesias et al., 2015).

7.2. *POLD1* in cancer

A growing number of proteins involved in the control of DNA repair have been shown to have pathogenic changes associated with cancer. Reports of mutations in *POLD1* in human cell lines date back to the 1990s (da Costa et al., 1995; Flohr et al., 1999), and a large number have now been catalogued (Preston et al., 2010). In 1999, Popanda et al. reported the R648Q mutation in *POLD1* in Novikoff rat hepatoma cells (equivalent to R652Q in human *POLD1*) that are characterized by several stable chromosomal rearrangements. The resulting enzyme had abnormal catalytic properties, including altered binding properties, higher rate of extension of mismatches and insertion of the wrong nucleotides, when compared to the equivalent from regenerating normal liver (Popanda et al., 1999). Subsequently, germline mutations in the exonuclease (proofreading) domains of Pol δ and Pol ϵ DNA polymerases were reported to be associated with oligo-adenomatous polyposis, early-onset colorectal cancer (CRC) and endometrial cancer (EDMC). Many of these are presented in detail in a recent review (Rayner et al., 2016).

The description of an association of germline mutations in *POLD1* and *POLE* that cluster to areas coding for the proofreading function has been a recent important development in the field of hereditary colorectal cancer (CRC) syndromes (Palles et al., 2013; Heitzer and Tomlinson, 2014; Shinbrot et al., 2014; Valle et al., 2014). It has been coined “polymerase proofreading associated polyposis” (PPAP) (Briggs and Tomlinson, 2013). Bellido and colleagues have enumerated the clinical characteristics leading to suspicion of PPAP (Bellido et al., 2016). Approximately 30% of CRCs are familial in nature; ~10% of these have defined genetic predispositions, which notably include Lynch syndrome (LS), characterized by microsatellite instability (MSI) due to MMR defects (*MSH2*, *MLH1*, *MSH6* and *PMS2*) and accounts for ~3% of CRC cases. The contributions of *POLD1* and *POLE* genetic defects to early-onset of familial colorectal cancer have been estimated at 0.2 and 0.6% respectively (Chubb et al., 2015). Germline *POLD1* variants that have been strongly linked to risk of cancer are depicted in Fig. 3B. Families bearing the p.S478N variant in *POLD1*/p125 develop microsatellite stable, chromosomal unstable colorectal adenocarcinoma and/or oligopolyposis with high penetrance and dominant inheritance (Palles et al., 2013; Valle et al., 2014). We have described this variant in a patient with familial colorectal cancer, but not bearing mutations in canonical genes associated with risk (Arora et al., 2015). A *POLD1* p.L474P proofreading domain variant has also been identified in a patient with hereditary non-polyposis colorectal cancer and deemed pathogenic by evidence from co-segregation, in silico predictions of functionality and functional assay in yeast. This study led to the suggestion that the acronym PPAP is somewhat misleading, as the patient had a non-polyposis form of colorectal cancer (Valle et al., 2014). These *POLD1* mutations predispose to endometrial tumors and may also predispose to breast and brain tumors (Palles et al., 2013; Valle et al., 2014; Bellido et al., 2016). More cases would need to be evaluated to show an elevated risk for brain and breast tumors. Routine genetic screening of the proofreading domains of *POLE* and *POLD1*, by inclusion of the genes in test panels or by exome sequencing, has been recommended (Valle et al., 2014; Chubb et al., 2015; Bellido et al., 2016). Guidelines for the management of the *POLE/POLD1* mutation carriers include colonoscopies every 1–2 years, gastroduodenoscopies

every 3 years starting at age 20–25, consideration for possible brain tumors, and endometrial cancer screening beginning at age 40 for *POLD1* female carriers (Bellido et al., 2016).

While somatic mutations in *POLE* have been reported to be present in many cancers, including colorectal and endometrial (Church et al., 2013; Hoang et al., 2015; Wong et al., 2015), target similar domains as those mutated in germline predisposing variants, and cause extremely hypermutable tumors, the case for somatic mutations in *POLD1* is not so clear. In an analysis of 62 suspected cases of Lynch syndrome (sLS) in which patients lacked the classical MMR germline mutations, nine tumors with ultramutated phenotype were found to carry germline ($n = 2$) or somatic ($n = 7$) mutations in the exonuclease domain of *POLE* or *POLD1*. The somatic variants in *POLD1* were p.S478N, p.V477M and p.I335V, while the germline variant was p.G321S. Eight of the nine tumors showed microsatellite instability. Six of the nine tumors with *POLE/POLD1* defects also had somatic mutations in MMR genes likely to affect function. The faulty somatic proofreading pathway was presented as the initiating event leading to somatic defects in MMR (Jansen et al., 2015a). The study supported the proposal that colon and endometrial cancers with MMR deficiency may arise from somatic, rather than germline mutations (Haraldsdottir et al., 2014).

Exome-sequencing of 224 colorectal carcinomas by The Cancer Genome Atlas (TCGA) showed that 35 of these carcinomas were hypermutated. Of these hypermutated carcinomas, ~77% exhibited microsatellite instability as expected. However the remaining hypermutated carcinomas, including the cases with the highest mutations rates, had somatic MMR and Pol ϵ mutations (Cancer Genome Atlas, 2012). Similarly, other studies have been reported with endometrial tumors that are microsatellite stable but have Pol ϵ mutations and highly elevated somatic mutation rates (Henninger and Pursell, 2014). These studies also showed that the Pol ϵ mutant tumors had at least one mutation in an MMR gene and thus support the emerging paradigm suggested by Jansen et al. (2015a). Somatic mutations identified by TCGA and in other studies as affecting *POLD1* in cancer are shown in Fig. 4. The data indicate specific association of *POLD1* mutations with hypermutated cancers with a strong bias towards mutation in hypermutated colorectal, stomach, lung, and uterine cancers.

When *POLD1* or *POLE* proofreading disabling somatic mutations occur in pediatric patients with biallelic mismatch repair deficiency (bMMRD), the double hit on the two pathways in charge of safeguarding the DNA results in an ultra-hypermutated phenotype, with a mutation burden among the highest ever documented (Schlesner and Eils, 2015; Shlien et al., 2015; Waterfall and Meltzer, 2015). The two *POLD1* somatic mutations observed by Shlien and colleagues were C319Y in the Exo1 motif and L606M, resulting in forms of the protein previously described to incorporate 7-fold more ribonucleotides into DNA than the un-mutated form (Clausen et al., 2013). The bMMRD/*POLD1* cancers exhibited many C>A (especially in CCN, with a particular enrichment at CCT) and C>T mutations, as well as an excess of T>A and T>C, when compared to bMMRD/*POLE* cancers (Schlesner and Eils, 2015). The characterization of these signatures is important as they may manifest as distinct damaging mutations in oncogenes or tumor suppressors genes. Examples of specific mutations affecting *PI3KCA*, *PTEN*, *APC*, *MSH6*, *FBXW7* and *TP53* associated with mutations in *POLE* have been recently reviewed (Rayner et al., 2016). To our knowledge, no such robust association has been described for *POLD1*, although one study has suggested possible association with specific alleles of *BRAF* and *KRAS*, and with *APC* mutations (Palles et al., 2013).

Hypermutated and ultra-hypermutated phenotypes are important to recognize as they open new avenues for cancer patient management and therapy. Such phenotypes can impact immune response targeting drugs that exploit the fact that hypermutated tumors produce immunogenic proteins not normally present in the body (Howitt et al., 2015; van Gool et al., 2015), as well as DNA damaging drugs, by leading tumors to exceed the upper limit of a tolerable mutation load (Roberts and Gordenin, 2014; Khanna, 2015; Roos et al., 2016). Two siblings with

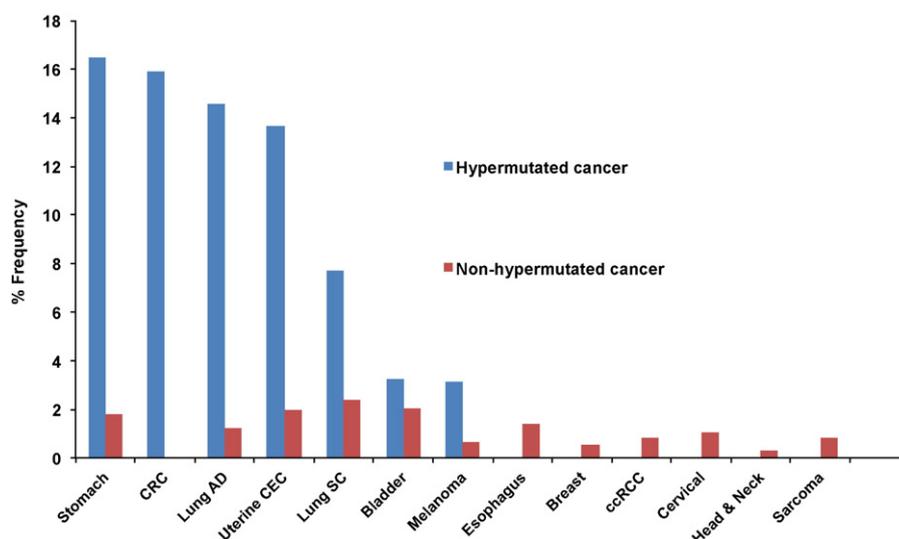


Fig. 4. Frequency of somatic mutations in different cancers extracted from cancer studies in the TCGA (The Cancer Genome Atlas) (data retrieval date March 30th 2016). Abbreviations used on the graph are CRC, colorectal cancer; lung AD, lung adenocarcinoma; lung SC, lung squamous carcinoma; ccRCC, clear cell renal cell carcinoma; uterine CEC, uterine corpus endometrial carcinoma.

recurrent biallelic bMMRD-glioblastoma multiform were recently reported to respond durably to a therapeutic trial with the anti-programmed death-1 inhibitor nivolumab. Both had somatic mutations in *POLE* (P436H in one, S461P in the other) (Bouffet et al., 2016). High mutation burden is not limited to bMMRD cancers, but is also observed in subsets of cancers including melanomas or lung cancers (Rizvi et al., 2015).

In mouse models, different cancer phenotypes were conferred by defective *Polδ* and *Polε* proofreading. Mouse models of *Polε* develop tumors from intestinal epithelial cells, histiocytes, and non-thymic lymphocytes (Albertson et al., 2009). Thymic lymphomas and skin squamous cell neoplasms, characteristics of *Polδ* deficiency, were seen in most of the double deficient animals by six months of age, indicating that deficient *Polε* proofreading accelerated these types of tumors (Albertson et al., 2009). Studies using mouse models have also begun to explore the functional role of specific *POLD1* mutations. Heterozygous mutations of a residue in the active site of the polymerase domain (L604K or G) or of a residue in the exonuclease domain associated with a proofreading defect (D400A) were tumorigenic in mice, while complete knockout or deficiency in proofreading and MMR functions caused embryonic lethality (Goldsby et al., 2001; Goldsby et al., 2002; Venkatesan et al., 2007; Albertson et al., 2009; Uchimura et al., 2009, and reviewed in Prindle and Loeb, 2012). While both L604K and L604G resulted in a mutator phenotype at the nucleotide and chromosomal level in MEFs, only L604K accelerated tumorigenesis and reduced life span in vivo (Venkatesan et al., 2007). Biochemical studies of the equivalent substitutions in human p125 (L606G, L606K) indicated that L606G was extremely error prone while L606K was extremely accurate in incorporating nucleotides but had an impaired ability to bypass DNA adducts (Schmitt et al., 2010). The impairment in lesion bypass, likely to induce stalling at the replication fork and cause DNA breaks, is thought to be the cause of the more aggressive tumor presentation observed in the L604K mice. Homozygous exonuclease-deficient D400A (*Pold1*^{exo/exo}) mice bred for several generations without artificial selection had an elevated rate of single nucleotide variations (17.2-fold) and in-frame deletions (8.6-fold) relative to wild type mice, as estimated by whole genome sequencing of derived cell lines. This was likely close to the upper limit for practical maintenance of inbred mice. D400A *Polδ* mice presented abnormal phenotypes 4.1 times more often than controls. The complex altered phenotypes varied between independent breeding lines and included human-audible vocalizations, shortened limbs and tail, and diluted

coat color, with the most striking difference being in reduced reproductive capacity, associated with defects at the blastocyst stage associated with peri-implantation lethality (Uchimura et al., 2015).

8. Conclusions and future directions

The integration of high throughput sequencing technologies with functional studies probing protein function have assigned *POLD1* an important role at the junction of DNA replication with repair and the maintenance of genome integrity. In the near future, it may be possible to exploit this information in several ways to improve clinical care. For instance, as mutation of the DNA repair protein BRCA1 predicts tumor sensitivity to specific DNA-damaging agents (O'Connor, 2015), identification of damaging mutations in *POLD1* may be used to optimize administration of DNA damaging chemoradiation and targeted therapies.

With the more frequent use of genomic technologies in the clinic, we may identify novel or rare mutations in *POLD1* and its interacting partners that further inform understanding of disease. In the future, genome-editing technologies like the CRISPR-Cas9 nucleases may be used to therapeutically correct *POLD1* mutations in patients, reducing cancer risk. While unwanted off-target effects have limited these nucleases (Hsu et al., 2014), modifications of the Cas9 enzyme have abrogated many genome-wide off-target effects (Kleinstiver et al., 2015).

Interestingly, although much less developed than evidence of *POLD1* involvement in MDPL or cancer, some provocative papers have suggested that altered expression or function of p125 may be relevant in additional pathogenic contexts. Some of the phenotypes of MDPL, such as lipodystrophy, have been linked to diabetes and insulin resistance (Vatier et al., 2013). Although there has been no direct investigation of functional relationships, studies of changing gene expression in a rat model of diabetes progression identified a decrease in *POLD1* expression at the onset of hyperglycemia (Zhou et al., 2011). *Polδ* activity can be promoted by activity of glycolytic enzymes including lactate dehydrogenase and 3-phosphoglycerate kinase (Popanda et al., 1998). The *POLD1* R648Q mutation identified in Novikoff rat hepatoma cells and described above as linked to defects in repair also modulated the stimulation of the *Polδ* by lactate dehydrogenase (Popanda et al., 1998). This is interesting, as the processes of glycolysis are altered in diabetes and in tumor development, a number of glycolytic enzymes have been identified at the replication fork, and depletion of specific proteins that mediate glycolysis and the tricarboxylic acid (TCA) cycle resulted in impairment of DNA synthesis (Konieczna et al., 2015a, 2015b). MDPL and

atypical Werner syndrome are associated with features of premature aging; studies of normal aging have more broadly connected decline in exonuclease function of polymerases associated or autonomous proofreaders such as TREX1 with clinical diseases associated with aging (Mason and Cox, 2012). The pro-oncogenic miR-155, noted above as a regulator of *POLD1* expression, has recently been found to be elevated in expression in obesity-induced inflammation in adipocytes (Karkeni et al., 2016), and in inflammation-associated senescence (Olivieri et al., 2015). While at present the functional linkages between *POLD1*, inflammation, diabetes, and aging remain speculative, investigation of these topics may reveal deep connections that improve clinical practice.

Acknowledgments

The authors were supported by NCI Core Grant P30 CA006927 (to Fox Chase Cancer Center), NCI CA191425 (to EAG); and a Board of Directors Fellowship from Fox Chase Cancer Center (to SA). This review and a corresponding Gene Wiki article were written as part of the Gene Wiki Review series—a series resulting from a collaboration between the journal GENE and the Gene Wiki Initiative. The Gene Wiki Initiative is supported by National Institutes of Health (GM083924). Additional support for Gene Wiki Reviews is provided by Elsevier, the publisher of GENE. The corresponding Gene Wiki entries for this review can be found here: <https://en.wikipedia.org/wiki/POLD1>

References

- Albertson, T.M., Ogawa, M., Bugni, J.M., Hays, L.E., Chen, Y., Wang, Y., Treuting, P.M., Heddle, J.A., Goldsby, R.E., Preston, B.D., 2009. DNA polymerase epsilon and delta proofreading suppress discrete mutator and cancer phenotypes in mice. *Proc. Natl. Acad. Sci. U. S. A.* 106, 17101–17104.
- Antoniali, G., Marcuzzi, F., Casarano, E., Tell, G., 2015. Cadmium treatment suppresses DNA polymerase delta catalytic subunit gene expression by acting on the p53 and Sp1 regulatory axis. *DNA Repair (Amst)* 35, 90–105.
- Arora, S., Yan, H., Cho, I., Fan, H.Y., Luo, B., Gai, X., Bodian, D.L., Vockley, J.G., Zhou, Y., Handorf, E.A., Egleston, B.L., Andrade, M.D., Nicolas, E., Serebriiskii, I.G., Yen, T.J., Hall, M.J., Golemis, E.A., Enders, G.H., 2015. Genetic variants that predispose to DNA double-strand breaks in lymphocytes from a subset of patients with familial colorectal carcinomas. *Gastroenterology* 149, 1872–1883, e9.
- Audas, T.E., Jacob, M.D., Lee, S., 2012. Immobilization of proteins in the nucleolus by ribosomal intergenic spacer noncoding RNA. *Mol. Cell* 45, 147–157.
- Baldeck, N., Janel-Bintz, R., Wagner, J., Tissier, A., Fuchs, R.P., Burkovics, P., Haracska, L., Despras, E., Bichara, M., Chatton, B., Cordonnier, A.M., 2015. FF483-484 motif of human Poleta mediates its interaction with the POLD2 subunit of Poldelta and contributes to DNA damage tolerance. *Nucleic Acids Res.* 43, 2116–21125.
- Baranovskiy, A.G., Lada, A.G., Siebler, H.M., Zhang, Y., Pavlov, Y.I., Tahirov, T.H., 2012. DNA polymerase delta and zeta switch by sharing accessory subunits of DNA polymerase delta. *J. Biol. Chem.* 287, 17281–17287.
- Bellido, F., Pineda, M., Aiza, G., Valdes-Mas, R., Navarro, M., Puente, D.A., Pons, T., Gonzalez, S., Iglesias, S., Darder, E., Pinol, V., Soto, J.L., Valencia, A., Blanco, I., Urioste, M., Brunet, J., Lazaro, C., Capella, G., Puente, X.S., Valle, L., 2016. POLE and POLD1 mutations in 529 kindred with familial colorectal cancer and/or polyposis: review of reported cases and recommendations for genetic testing and surveillance. *Genitourin. Med.* 18, 325–332.
- Berti, M., Vindigni, A., 2016. Replication stress: getting back on track. *Nat. Struct. Mol. Biol.* 23, 103–109.
- Blank, A., Kim, B., Loeb, L.A., 1994. DNA polymerase delta is required for base excision repair of DNA methylation damage in *Saccharomyces cerevisiae*. *Proc. Natl. Acad. Sci. U. S. A.* 91, 9047–9051.
- Boufflet, E., Larouche, V., Campbell, B.B., Merico, D., de Borja, R., Aronson, M., Durmo, C., Krueger, J., Cabric, V., Ramaswamy, V., Zhukova, N., Mason, G., Farah, R., Afzal, S., Yalon, M., Rechavi, G., Magimaiarajan, V., Walsh, M.F., Constantini, S., Dvir, R., Elhasid, R., Reddy, A., Osborn, M., Sullivan, M., Hansford, J., Dodgshun, A., Klauber-Demore, N., Peterson, L., Patel, S., Lindhorst, S., Atkinson, J., Cohen, Z., Laframboise, R., Dirks, P., Taylor, M., Malkin, D., Albrecht, S., Dudley, R.W., Jabado, N., Hawkins, C.E., Shlien, A., Tabori, U., 2016. Immune checkpoint inhibition for hypermutant glioblastoma multiforme resulting from germline biallelic mismatch repair deficiency. *J. Clin. Oncol.*
- Boyer, A.S., Walter, D., Sorensen, C.S., 2016. DNA replication and cancer: from dysfunctional replication origin activities to therapeutic opportunities. *Semin. Cancer Biol.*
- Briggs, S., Tomlinson, I., 2013. Germline and somatic polymerase epsilon and delta mutations define a new class of hypermutated colorectal and endometrial cancers. *J. Pathol.* 230, 148–153.
- Brocas, C., Charbonnier, J.B., Dherin, C., Gangloff, S., Maloisel, L., 2010. Stable interactions between DNA polymerase delta catalytic and structural subunits are essential for efficient DNA repair. *DNA Repair (Amst)* 9, 1098–1111.
- Brown, J.A., Suo, Z., 2011. Unlocking the sugar “steric gate” of DNA polymerases. *Biochemistry* 50, 1135–1142.
- Burgers, P.M., Yoder, B.L., 1993. ATP-independent loading of the proliferating cell nuclear antigen requires DNA ends. *J. Biol. Chem.* 268, 19923–19926.
- Burgers, P.M., Gordin, D., Kunkel, T.A., 2016. Who is leading the replication fork, pol epsilon or pol delta? *Mol. Cell* 61, 492–493.
- Byrnes, J.J., Downey, K.M., Black, V.L., So, A.G., 1976. A new mammalian DNA polymerase with 3′ to 5′ exonuclease activity: DNA polymerase delta. *Biochemistry* 15, 2817–2823.
- Cancer Genome Atlas, N., 2012. Comprehensive molecular characterization of human colon and rectal cancer. *Nature* 487, 330–337.
- Cerritelli, S.M., Crouch, R.J., 2016. The balancing act of ribonucleotides in DNA. *Trends Biochem. Sci.*
- Chang, L.S., Zhao, L., Zhu, L., Chen, M.L., Lee, M.Y., 1995. Structure of the gene for the catalytic subunit of human DNA polymerase delta (*POLD1*). *Genomics* 28, 411–419.
- Chea, J., Zhang, S., Zhao, H., Zhang, Z., Lee, E.Y., Darzynkiewicz, Z., Lee, M.Y., 2012. Spatio-temporal recruitment of human DNA polymerase delta to sites of UV damage. *Cell Cycle* 11, 2885–2895.
- Chen, S., Levin, M.K., Sakato, M., Zhou, Y., Hingorani, M.M., 2009. Mechanism of ATP-driven PCNA clamp loading by *S. cerevisiae* RFC. *J. Mol. Biol.* 388, 431–442.
- Chen, X., Ji, Z., Webber, A., Sharrocks, A.D., 2016. Genome-wide binding studies reveal DNA binding specificity mechanisms and functional interplay amongst Forkhead transcription factors. *Nucleic Acids Res.* 44, 1566–1578.
- Chubb, D., Broderick, P., Frampton, M., Kinnersley, B., Sherborne, A., Penegar, S., Lloyd, A., Ma, Y.P., Dobbins, S.E., Houlston, R.S., 2015. Genetic diagnosis of high-penetrance susceptibility for colorectal cancer (CRC) is achievable for a high proportion of familial CRC by exome sequencing. *J. Clin. Oncol.* 33, 426–432.
- Chung, D.W., Zhang, J.A., Tan, C.K., Davie, E.W., So, A.G., Downey, K.M., 1991. Primary structure of the catalytic subunit of human DNA polymerase delta and chromosomal location of the gene. *Proc. Natl. Acad. Sci. U. S. A.* 88, 11197–11201.
- Church, D.N., Briggs, S.E., Palles, C., Domingo, E., Kearsey, S.J., Grimes, J.M., Gorman, M., Martin, L., Howarth, K.M., Hodgson, S.V., Kaur, K., Taylor, J., Tomlinson, I.P., 2013. DNA polymerase epsilon and delta exonuclease domain mutations in endometrial cancer. *Hum. Mol. Genet.* 22, 2820–2828.
- Clausen, A.R., Zhang, S., Burgers, P.M., Lee, M.Y., Kunkel, T.A., 2013. Ribonucleotide incorporation, proofreading and bypass by human DNA polymerase delta. *DNA Repair (Amst)* 12, 121–127.
- Costantino, L., Sotiropoulos, S.K., Rantala, J.K., Magin, S., Mladenov, E., Helleday, T., Haber, J.E., Iliakis, G., Kallioniemi, O.P., Halazonetis, T.D., 2014. Break-induced replication repair of damaged forks induces genomic duplications in human cells. *Science* 343, 88–91.
- Cui, M., You, L., Ren, X., Zhao, W., Liao, Q., Zhao, Y., 2016. Long non-coding RNA PVT1 and cancer. *Biochem. Biophys. Res. Commun.* 471, 10–14.
- Czochor, J.R., Sulikowski, P., Glazer, P.M., 2016. miR-155 over-expression promotes genomic instability by reducing high-fidelity polymerase delta expression and activating error-prone DSB repair. *Mol. Cancer Res.*
- da Costa, L.T., Liu, B., el-Deiry, W., Hamilton, S.R., Kinzler, K.W., Vogelstein, B., Markowitz, S., Willson, J.K., de la Chapelle, A., Downey, K.M., et al., 1995. Polymerase delta variants in RER colorectal tumours. *Nat. Genet.* 9, 10–11.
- Darmawan, H., Harrison, M., Reha-Krantz, L.J., 2015. DNA polymerase 3′ → 5′ exonuclease activity: Different roles of the beta hairpin structure in family-B DNA polymerases. *DNA Repair (Amst)* 29, 36–46.
- Dexter, D.L., Hager, J.C., 1980. Maturation-induction of tumor cells using a human colon carcinoma model. *Cancer* 45, 1178–1184.
- Diede, S.J., Gottschling, D.E., 1999. Telomerase-mediated telomere addition in vivo requires DNA primase and DNA polymerases alpha and delta. *Cell* 99, 723–733.
- Doublet, S., Zahn, K.E., 2014. Structural insights into eukaryotic DNA replication. *Front. Microbiol.* 5, 444.
- Edwards, D.N., Machwe, A., Wang, Z., Orren, D.K., 2014. Intramolecular telomeric G-quadruplexes dramatically inhibit DNA synthesis by replicative and translesion polymerases, revealing their potential to lead to genetic change. *PLoS One* 9, e80664.
- Fan, X., Zhang, Q., You, C., Qian, Y., Gao, J., Liu, P., Chen, H., Song, H., Chen, Y., Chen, K., Zhou, Y., 2014. Proteolysis of the human DNA polymerase delta smallest subunit p12 by mu-calpain in calcium-triggered apoptotic HeLa cells. *PLoS One* 9, e93642.
- Fazlieva, R., Spittle, C.S., Morrissey, D., Hayashi, H., Yan, H., Matsumoto, Y., 2009. Proofreading exonuclease activity of human DNA polymerase delta and its effects on lesion-bypass DNA synthesis. *Nucleic Acids Res.* 37, 2854–2866.
- Fischer, M., Quaas, M., Steiner, L., Engeland, K., 2016. The p53–p21–DREAM-CDE/CHR pathway regulates G2/M cell cycle genes. *Nucleic Acids Res.* 44, 164–174.
- Flohr, T., Dai, J.C., Buttner, J., Popanda, O., Hagemuller, E., Thielmann, H.W., 1999. Detection of mutations in the DNA polymerase delta gene of human sporadic colorectal cancers and colon cancer cell lines. *Int. J. Cancer* 80, 919–929.
- Fortune, J.M., Pavlov, Y.I., Welch, C.M., Johansson, E., Burgers, P.M., Kunkel, T.A., 2005. *Saccharomyces cerevisiae* DNA polymerase delta: high fidelity for base substitutions but lower fidelity for single- and multi-base deletions. *J. Biol. Chem.* 280, 29980–29987.
- Friedberg, E.C., 2006. The eureka enzyme: the discovery of DNA polymerase. *Nat. Rev. Mol. Cell Biol.* 7, 143–147.
- Fuss, J.O., Tsai, C.L., Ishida, J.P., Tainer, J.A., 2015. Emerging critical roles of Fe–S clusters in DNA replication and repair. *Biochim. Biophys. Acta* 1853, 1253–1271.
- Gaillard, H., Aguilera, A., 2016. Transcription as a threat to genome integrity. *Annu. Rev. Biochem.*
- Ganai, R.A., Johansson, E., 2016. DNA replication—a matter of fidelity. *Mol. Cell* 62, 745–755.
- Ganai, R.A., Bylund, G.O., Johansson, E., 2015. Switching between polymerase and exonuclease sites in DNA polymerase epsilon. *Nucleic Acids Res.* 43, 932–942.
- Gari, K., Leon Ortiz, A.M., Borel, V., Flynn, H., Skehel, J.M., Boulton, S.J., 2012. MMS19 links cytoplasmic iron–sulfur cluster assembly to DNA metabolism. *Science* 337, 243–245.

- Georgescu, R.E., Langston, L., Yao, N.Y., Yurieva, O., Zhang, D., Finkelstein, J., Agarwal, T., O'Donnell, M.E., 2014. Mechanism of asymmetric polymerase assembly at the eukaryotic replication fork. *Nat. Struct. Mol. Biol.* 21, 664–670.
- Georgescu, R.E., Schauer, G.D., Yao, N.Y., Langston, L.D., Yurieva, O., Zhang, D., Finkelstein, J., O'Donnell, M.E., 2015. Reconstitution of a eukaryotic replisome reveals suppression mechanisms that define leading/lagging strand operation. *Elife* 4, e04988.
- Goldsby, R.E., Lawrence, N.A., Hays, L.E., Olmsted, E.A., Chen, X., Singh, M., Preston, B.D., 2001. Defective DNA polymerase-delta proofreading causes cancer susceptibility in mice. *Nat. Med.* 7, 638–639.
- Goldsby, R.E., Hays, L.E., Chen, X., Olmsted, E.A., Slayton, W.B., Spangrude, G.J., Preston, B.D., 2002. High incidence of epithelial cancers in mice deficient for DNA polymerase delta proofreading. *Proc. Natl. Acad. Sci. U. S. A.* 99, 15560–15565.
- Gonzalez Besteiro, M.A., Gottifredi, V., 2015. The fork and the kinase: a DNA replication tale from a CHK1 perspective. *Mutat. Res. Rev. Mutat. Res.* 763, 168–180.
- Guenant, A.C., Briand, N., Bidault, G., Afonso, P., Bereziat, V., Vatie, C., Lascos, O., Caron-Debarle, M., Capeau, J., Vigouroux, C., 2014. Nuclear envelope-related lipodystrophies. *Semin. Cell Dev. Biol.* 29, 148–157.
- Guiley, K.Z., Liban, T.J., Felthousen, J.G., Ramanan, P., Litovchick, L., Rubin, S.M., 2015. Structural mechanisms of DREAM complex assembly and regulation. *Genes Dev.* 29, 961–974.
- Hansen, M.F., Johansen, J., Bjornevoll, I., Sylvander, A.E., Steinsbekk, K.S., Saetrom, P., Sandvik, A.K., Drablos, F., Sjrursen, W., 2015. A novel POLE mutation associated with cancers of colon, pancreas, ovaries and small intestine. *Familial Cancer* 14, 437–448.
- Haraldsdottir, S., Hampel, H., Tomsic, J., Frankel, W.L., Pearlman, R., de la Chapelle, A., Pritchard, C.C., 2014. Colon and endometrial cancers with mismatch repair deficiency can arise from somatic, rather than germline, mutations. *Gastroenterology* 147, 1308–1316.e1.
- Hedglin, M., Benkovic, S.J., 2015. Regulation of Rad6/Rad18 activity during DNA damage replication. *Annu. Rev. Biophys.* 44, 207–228.
- Heitzer, E., Tomlinson, I., 2014. Replicative DNA polymerase mutations in cancer. *Curr. Opin. Genet. Dev.* 24, 107–113.
- Henninger, E.E., Pursell, Z.F., 2014. DNA polymerase epsilon and its roles in genome stability. *IUBMB Life* 66, 339–351.
- Herr, A.J., Kennedy, S.R., Knowels, G.M., Schultz, E.M., Preston, B.D., 2014. DNA replication error-induced extinction of diploid yeast. *Genetics* 196, 677–691.
- Hills, S.A., Diffley, J.F., 2014. DNA replication and oncogene-induced replicative stress. *Curr. Biol.* 24, R435–R444.
- Hindges, R., Hubscher, U., 1997. DNA polymerase delta, an essential enzyme for DNA transactions. *Biol. Chem.* 378, 345–362.
- Hoang, L.N., McConechy, M.K., Kobel, M., Anglesio, M., Senz, J., Maassen, M., Kommos, S., Meng, B., Postovit, L., Kelemen, L.E., Staebler, A., Brucker, S., Kramer, B., McAlpine, J.N., Gilks, C.B., Huntsman, D.G., Lee, C.H., 2015. Polymerase epsilon exonuclease domain mutations in ovarian endometrioid carcinoma. *Int. J. Gynecol. Cancer* 25, 1187–1193.
- Hocke, S., Guo, Y., Job, A., Orth, M., Ziesch, A., Lauber, K., De Toni, E.N., Gress, T.M., Herbst, A., Goke, B., Gallmeier, E., 2016. A synthetic lethal screen identifies ATR-inhibition as a novel therapeutic approach for POLD1-deficient cancers. *Oncotarget*.
- Hogg, M., Aller, P., Konigsberg, W., Wallace, S.S., Double, 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.
- Hoopes, J.L., Cortez, L.M., Mertz, T.M., Malc, E.P., Mieczkowski, P.A., Roberts, S.A., 2016. APOBEC3A and APOBEC3B preferentially deaminate the lagging strand template during DNA replication. *Cell Rep.* 14, 1273–1282.
- Howitt, B.E., Shukla, S.A., Sholl, L.M., Ritterhouse, L.L., Watkins, J.C., Rodig, S., Stover, E., Strickland, K.C., D'Andrea, A.D., Wu, C.J., Matulonis, U.A., Konstantinopoulos, P.A., 2015. Association of polymerase epsilon-mutated and microsatellite-unstable endometrial cancers with neoantigen load, number of tumor-infiltrating lymphocytes, and expression of PD-1 and PD-L1. *JAMA Oncol.* 1, 1319–1323.
- Hsu, P.D., Lander, E.S., Zhang, F., 2014. Development and applications of CRISPR-Cas9 for genome engineering. *Cell* 157, 1262–1278.
- Iglesias, F.M., Cerdan, P.D., 2016. Maintaining epigenetic inheritance during DNA replication in plants. *Front. Plant Sci.* 7, 38.
- Iglesias, F.M., Bruera, N.A., Dergan-Dylon, S., Marino-Buslje, C., Lorenzi, H., Mateos, J.L., Turck, F., Coupland, G., Cerdan, P.D., 2015. The arabidopsis DNA polymerase delta has a role in the deposition of transcriptionally active epigenetic marks, development and flowering. *PLoS Genet.* 11, e1004975.
- Jansen, A.M., van Wezel, T., van den Akker, B.E., Ventayol Garcia, M., Ruano, D., Tops, C.M., Wagner, A., Letteboer, T.G., Gomez-Garcia, E.B., Devilee, P., Wijnen, J.T., Hes, F.J., Morreau, H., 2015a. Combined mismatch repair and POLE/POLD1 defects explain unresolved suspected Lynch syndrome cancers. *Eur. J. Hum. Genet.*
- Jansen, J.G., Tsaalbi-Shtylik, A., de Wind, N., 2015b. Roles of mutagenic translesion synthesis in mammalian genome stability, health and disease. *DNA Repair (Amst)* 29, 56–64.
- Jin, Y.H., Garg, P., Stith, C.M., Al-Refai, H., Sterling, J.F., Murray, L.J., Kunkel, T.A., Resnick, M.A., Burgers, P.M., Gordenin, D.A., 2005. The multiple biological roles of the 3' → 5' exonuclease of *Saccharomyces cerevisiae* DNA polymerase delta require switching between the polymerase and exonuclease domains. *Mol. Cell Biol.* 25, 461–471.
- Johansson, E., Dixon, N., 2013. Replicative DNA polymerases. *Cold Spring Harb. Perspect. Biol.* 5.
- Johnson, K.A., 1993. Conformational coupling in DNA polymerase fidelity. *Annu. Rev. Biochem.* 62, 685–713.
- Johnson, R.E., Klassen, R., Prakash, L., Prakash, S., 2015. A major role of DNA polymerase delta in replication of both the leading and lagging DNA strands. *Mol. Cell Biol.* 35, 163–175.
- Kamath-Loeb, A.S., Shen, J.C., Schmitt, M.W., Loeb, L.A., 2012. The Werner syndrome exonuclease facilitates DNA degradation and high fidelity DNA polymerization by human DNA polymerase delta. *J. Biol. Chem.* 287, 12480–12490.
- Kanu, N., Zhang, T., Burrell, R.A., Chakraborty, A., Cronshaw, J., Costa, C.D., Gronroos, E., Pemberton, H.N., Anderton, E., Gonzalez, L., Sabbioneda, S., Ulrich, H.D., Swanton, C., Behrens, A., 2015. RAD18, WRNIP1 and ATMIN promote ATM signalling in response to replication stress. *Oncogene*.
- Karkeni, E., Astier, J., Toumiaire, F., El Abed, M., Romier, B., Gouranton, E., Lin, W., Borel, P., Salles, J., Walrand, S., Ye, J., Landrier, J.F., 2016. Obesity-associated inflammation induces microRNA-155 expression in adipocytes and adipose tissue: outcome on adipocyte function. *J. Clin. Endocrinol. Metab.* (jc20153410).
- Karkhanis, V., Wang, L., Tae, S., Hu, Y.J., Imbalzano, A.N., Sif, S., 2012. Protein arginine methyltransferase 7 regulates cellular response to DNA damage by methylating promoter histones H2A and H4 of the polymerase delta catalytic subunit gene, POLD1. *J. Biol. Chem.* 287, 29801–29814.
- Karmakar, P., Bohr, V.A., 2005. Cellular dynamics and modulation of WRN protein is DNA damage specific. *Mech. Ageing Dev.* 126, 1146–1158.
- Kashkin, K., Chernov, I., Stukacheva, E., Monastyrskaya, G., Uspenskaya, N., Kopantzev, E., Sverdlov, E., 2015. Cancer specificity of promoters of the genes controlling cell proliferation. *J. Cell. Biochem.* 116, 299–309.
- Kemper, R.R., Ahn, E.R., Zhang, P., Lee, M.Y., Rabin, M., 1992. Human DNA polymerase delta gene maps to region 19q13.3–q13.4 by in situ hybridization. *Genomics* 14, 205–206.
- Khanna, A., 2015. DNA damage in cancer therapeutics: a boon or a curse? *Cancer Res.* 75, 2133–2138.
- Kleinstiver, B.P., Prew, M.S., Tsai, S.Q., Topkar, V.V., Nguyen, N.T., Zheng, Z., Gonzales, A.P., Li, Z., Peterson, R.T., Yeh, J.R., Aryee, M.J., Joung, J.K., 2015. Engineered CRISPR-Cas9 nucleases with altered PAM specificities. *Nature* 523, 481–485.
- Kohnken, R., Kodigepalli, K.M., Wu, L., 2015. Regulation of deoxynucleotide metabolism in cancer: novel mechanisms and therapeutic implications. *Mol. Cancer* 14, 176.
- Konicznca, A., Szczepanska, A., Sawiuk, K., Lyzen, R., Wegrzyn, G., 2015a. Enzymes of the central carbon metabolism: are they linkers between transcription, DNA replication, and carcinogenesis? *Med. Hypotheses* 84, 58–67.
- Konicznca, A., Szczepanska, A., Sawiuk, K., Wegrzyn, G., Lyzen, R., 2015b. Effects of partial silencing of genes coding for enzymes involved in glycolysis and tricarboxylic acid cycle on the entrance of human fibroblasts to the S phase. *BMC Cell Biol.* 16, 16.
- Korona, D.A., Lecompte, K.G., Pursell, Z.F., 2011. The high fidelity and unique error signature of human DNA polymerase epsilon. *Nucleic Acids Res.* 39, 1763–1773.
- Kotsantis, P., Jones, R.M., Higgs, M.R., Petermann, E., 2015. Cancer therapy and replication stress: forks on the road to perdition. *Adv. Clin. Chem.* 69, 91–138.
- Krucher, N.A., Zygmunt, A., Mazloum, N., Tamrakar, S., Ludlow, J.W., Lee, M.Y., 2000. Interaction of the retinoblastoma protein (pRb) with the catalytic subunit of DNA polymerase delta (p125). *Oncogene* 19, 5464–5470.
- Kunkel, T.A., Bebenek, K., 2000. DNA replication fidelity. *Annu. Rev. Biochem.* 69, 497–529.
- Kunkel, T.A., Erie, D.A., 2015. Eukaryotic mismatch repair in relation to DNA replication. *Annu. Rev. Genet.* 49, 291–313.
- Lam, Y.W., Trinkle-Mulcahy, L., 2015. New Insights Into Nucleolar Structure and Function. *F1000Prime Rep* 7, p. 48.
- Lee, M.Y., Zhang, S., Lin, S.H., Chea, J., Wang, X., LeRoy, C., Wong, A., Zhang, Z., Lee, E.Y., 2012. Regulation of human DNA polymerase delta in the cellular responses to DNA damage. *Environ. Mol. Mutagen.* 53, 683–698.
- Lee, M.Y., Zhang, S., Lin, S.H., Wang, X., Darzynkiewicz, Z., Zhang, Z., Lee, E.Y., 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.
- Lee, S.Y., Lee, H., Kim, E.S., Park, S., Lee, J., Ahn, B., 2015. WRN translocation from nucleolus to nucleoplasm is regulated by SIRT1 and required for DNA repair and the development of chemoresistance. *Mutat. Res.* 774, 40–48.
- Lessel, D., Hisama, F.M., Szakson, K., Saha, B., Sanjuanolo, A.B., Salbert, B.A., Steele, P.D., Baldwin, J., Brown, W.T., Piussan, C., Plauchu, H., Szilvassy, J., Horkay, E., Hogel, J., Martin, G.M., Herr, A.J., Oshima, J., Kubisch, C., 2015. POLD1 germline mutations in patients initially diagnosed with Werner syndrome. *Hum. Mutat.* 36, 1070–1079.
- Li, B., Lee, M.Y., 2001. Transcriptional regulation of the human DNA polymerase delta catalytic subunit gene POLD1 by p53 tumor suppressor and Sp1. *J. Biol. Chem.* 276, 29729–29739.
- Li, L., Murphy, K.M., Kanevets, U., Reha-Krantz, L.J., 2005. Sensitivity to phosphonoacetic acid: a new phenotype to probe DNA polymerase delta in *Saccharomyces cerevisiae*. *Genetics* 170, 569–580.
- Lin, S.H., Wang, X., Zhang, S., Zhang, Z., Lee, E.Y., Lee, M.Y., 2013. Dynamics of enzymatic interactions during short flap human Okazaki fragment processing by two forms of human DNA polymerase delta. *DNA Repair (Amst)* 12, 922–935.
- Longley, M.J., Pierce, A.J., Modrich, P., 1997. DNA polymerase delta is required for human mismatch repair in vitro. *J. Biol. Chem.* 272, 10917–10921.
- Lujan, S.A., Williams, J.S., Kunkel, T.A., 2016a. DNA polymerases divide the labor of genome replication. *Trends Cell Biol.*
- Lujan, S.A., Williams, J.S., Kunkel, T.A., 2016b. Eukaryotic genome instability in light of asymmetric DNA replication. *Crit. Rev. Biochem. Mol. Biol.* 51, 43–52.
- Makarova, K.S., Krupovic, M., Koonin, E.V., 2014. Evolution of replicative DNA polymerases in archaea and their contributions to the eukaryotic replication machinery. *Front. Microbiol.* 5, 354.
- Marks, A.B., Smith, O.K., Aladjem, M.I., 2016. Replication origins: determinants or consequences of nuclear organization? *Curr. Opin. Genet. Dev.* 37, 67–75.
- Mason, P.A., Cox, L.S., 2012. The role of DNA exonucleases in protecting genome stability and their impact on ageing. *Age (Dordr.)* 34, 1317–1340.
- Mekhalil, K., Rivero-Lopez, L., Al-Masri, A., Brandon, C., Khacho, M., Lee, S., 2007. Identification of a common subnuclear localization signal. *Mol. Biol. Cell* 18, 3966–3977.
- Meng, X., Zhou, Y., Zhang, S., Lee, E.Y., Frick, D.N., Lee, M.Y., 2009. DNA damage alters DNA polymerase delta to a form that exhibits increased discrimination against modified template bases and mismatched primers. *Nucleic Acids Res.* 37, 647–657.

- Meng, X., Zhou, Y., Lee, E.Y., Lee, M.Y., Frick, D.N., 2010. The p12 subunit of human polymerase delta modulates the rate and fidelity of DNA synthesis. *Biochemistry* 49, 3545–3554.
- Mertz, T.M., Sharma, S., Chabes, A., 2015. Colon cancer-associated mutator DNA polymerase delta variant causes expansion of dNTP pools increasing its own infidelity. 112, E2467–E2476.
- Meyer, D., Fu, B.X., Heyer, W.D., 2015. DNA polymerases delta and lambda cooperate in repairing double-strand breaks by microhomology-mediated end-joining in *Saccharomyces cerevisiae*. *Proc. Natl. Acad. Sci. U. S. A.* 112, E6907–E6916.
- Miyabe, I., Mizuno, K., Keszthelyi, A., Daigaku, Y., Skouteri, M., Mohebi, S., Kunkel, T.A., Murray, J.M., Carr, A.M., 2015. Polymerase delta replicates both strands after homologous recombination-dependent fork restart. *Nat. Struct. Mol. Biol.* 22, 932–938.
- Morrison, A., Johnson, A.L., Johnston, L.H., Sugino, A., 1993. Pathway correcting DNA replication errors in *Saccharomyces cerevisiae*. *EMBO J.* 12, 1467–1473.
- Muller, G.A., Wintsche, A., Stangner, K., Prohaska, S.J., Stadler, P.F., Engeland, K., 2014. The CHR site: definition and genome-wide identification of a cell cycle transcriptional element. *Nucleic Acids Res.* 42, 10331–10350.
- Munoz, S., Mendez, J., 2016. DNA replication stress: from molecular mechanisms to human disease. *Chromosoma*.
- Netz, D.J., Stith, C.M., Stumpff, M., Kopf, G., Vogel, D., Genau, H.M., Stodola, J.L., Lill, R., Burgers, P.M., Pierik, A.J., 2012. Eukaryotic DNA polymerases require an iron-sulfur cluster for the formation of active complexes. *Nat. Chem. Biol.* 8, 125–132.
- Nick McElhinny, S.A., Gordenin, D.A., Stith, C.M., Burgers, P.M., Kunkel, T.A., 2008. Division of labor at the eukaryotic replication fork. *Mol. Cell* 30, 137–144.
- O'Connor, M.J., 2015. Targeting the DNA damage response in cancer. *Mol. Cell* 60, 547–560.
- Olivieri, F., Albertini, M.C., Orciani, M., Ceka, A., Cricca, M., Procopio, A.D., Bonafe, M., 2015. DNA damage response (DDR) and senescence: shuttled inflamma-miRNAs on the stage of inflamm-aging. *Oncotarget* 6, 35509–35521.
- Oshima, J., Sidorova, J.M., Monnat Jr., R.J., 2016. Werner syndrome: clinical features, pathogenesis and potential therapeutic interventions. *Ageing Res. Rev.*
- Overmeer, R.M., Gourdin, A.M., Giglia-Mari, A., Kool, H., Houtsmuller, A.B., Siegal, G., Fouteri, M.I., Mullenders, L.H., Vermeulen, W., 2010. Replication factor C recruits DNA polymerase delta to sites of nucleotide excision repair but is not required for PCNA recruitment. *Mol. Cell. Biol.* 30, 4828–4839.
- Palles, C., Cazier, J.B., Howarth, K.M., Domingo, E., Jones, A.M., Broderick, P., Kemp, Z., Spain, S.L., Guarino, E., Salguero, I., Sherborne, A., Chubb, D., Carvajal-Carmona, L.G., Ma, Y., Kaur, K., Dobbins, S., Barclay, E., Gorman, M., Martin, L., Kovac, M.B., Humphray, S., Lucassen, A., Holmes, C.C., Bentley, D., Donnelly, P., Taylor, J., Petridis, C., Roylance, R., Sawyer, E.J., Kerr, D.J., Clark, S., Grimes, J., Kearsey, S.E., Thomas, H.J., McVean, G., Houlston, R.S., Tomlinson, I., 2013. Germline mutations affecting the proofreading domains of POLE and POLD1 predispose to colorectal adenomas and carcinomas. *Nat. Genet.* 45, 136–144.
- Papadopoulos, N., Nicolaidis, N.C., Liu, B., Parsons, R., Lengauer, C., Palombo, F., D'Arrigo, A., Markowitz, S., Willson, J.K., Kinzler, K.W., et al., 1995. Mutations of GTBP in genetically unstable cells. *Science* 268, 1915–1917.
- Park, S.Y., Jeong, M.S., Han, C.W., Yu, H.S., Jang, S.B., 2015. Structural and functional insight into proliferating cell nuclear antigen. *J. Microbiol. Biotechnol.*
- Parsons, J.L., Preston, B.D., O'Connor, T.R., Dianov, G.L., 2007. DNA polymerase delta-dependent repair of DNA single strand breaks containing 3'-end proximal lesions. *Nucleic Acids Res.* 35, 1054–1063.
- Paul, V.D., Lill, R., 2015. Biogenesis of cytosolic and nuclear iron-sulfur proteins and their role in genome stability. *Biochim. Biophys. Acta* 1853, 1528–1539.
- Pavlov, Y.I., Shcherbakova, P.V., 2010. DNA polymerases at the eukaryotic fork—20 years later. *Mutat. Res.* 685, 45–53.
- Pelosini, C., Martinelli, S., Ceccarini, G., Magno, S., Barone, I., Basolo, A., Fierabracci, P., Vitti, P., Maffei, M., Santini, F., 2014. Identification of a novel mutation in the polymerase delta 1 (POLD1) gene in a lipodystrophic patient affected by mandibular hypoplasia, deafness, progeroid features (MDPL) syndrome. *Metabolism* 63, 1385–1389.
- Popanda, O., Fox, G., Thielmann, H.W., 1998. Modulation of DNA polymerases alpha, delta and epsilon by lactate dehydrogenase and 3-phosphoglycerate kinase. *Biochim. Biophys. Acta* 1397, 102–117.
- Popanda, O., Flohr, T., Fox, G., Thielmann, H.W., 1999. A mutation detected in DNA polymerase delta cDNA from Novikoff hepatoma cells correlates with abnormal catalytic properties of the enzyme. *J. Cancer Res. Clin. Oncol.* 125, 598–608.
- Preston, B.D., Albertson, T.M., Herr, A.J., 2010. DNA replication fidelity and cancer. *Semin. Cancer Biol.* 20, 281–293.
- Prindle, M.J., Loeb, L.A., 2012. DNA polymerase delta in DNA replication and genome maintenance. *Environ. Mol. Mutagen.* 53, 666–682.
- Rahmeh, A.A., Zhou, Y., Xie, B., Li, H., Lee, E.Y., Lee, M.Y., 2012. Phosphorylation of the p68 subunit of Pol delta acts as a molecular switch to regulate its interaction with PCNA. *Biochemistry* 51, 416–424.
- Rayner, E., van Gool, I.C., Palles, C., Kearsey, S.E., Bosse, T., Tomlinson, I., Church, D.N., 2016. A panoply of errors: polymerase proofreading domain mutations in cancer. *Nat. Rev. Cancer* 16, 71–81.
- Reha-Krantz, L.J., 2010. DNA polymerase proofreading: multiple roles maintain genome stability. *Biochim. Biophys. Acta* 1804, 1049–1063.
- Reijns, M.A., Kemp, H., Ding, J., de Proce, S.M., Jackson, A.P., Taylor, M.S., 2015. Lagging-strand replication shapes the mutational landscape of the genome. *Nature* 518, 502–506.
- Reinier, F., Zoledziewska, M., Hanna, D., Smith, J.D., Valentini, M., Zara, I., Berutti, R., Sanna, S., Oppo, M., Cusano, R., Satta, R., Montesu, M.A., Jones, C., Cerimele, D., Nickerson, D.A., Angius, A., Cucca, F., Cottoni, F., Crisponi, L., 2015. Mandibular hypoplasia, deafness, progeroid features and lipodystrophy (MDPL) syndrome in the context of inherited lipodystrophies. *Metabolism* 64, 1530–1540.
- Rizvi, N.A., Hellmann, M.D., Snyder, A., Kvistborg, P., Makarov, V., Havel, J.J., Lee, W., Yuan, J., Wong, P., Ho, T.S., Miller, M.L., Reikhtman, N., Moreira, A.L., Ibrahim, F., Bruggeman, C., Gasmí, B., Zappasodi, R., Maeda, Y., Sander, C., Garon, E.B., Merghoub, T., Wolchok, J.D., Schumacher, T.N., Chan, T.A., 2015. Cancer immunology. Mutational landscape determines sensitivity to PD-1 blockade in non-small cell lung cancer. *Science* 348, 124–128.
- Roberts, S.A., Gordenin, D.A., 2014. Hypermutation in human cancer genomes: footprints and mechanisms. *Nat. Rev. Cancer* 14, 786–800.
- Roos, W.P., Thomas, A.D., Kaina, B., 2016. DNA damage and the balance between survival and death in cancer biology. *Nat. Rev. Cancer* 16, 20–33.
- Saugar, I., Ortiz-Bazan, M.A., Tercero, J.A., 2014. Tolerating DNA damage during eukaryotic chromosome replication. *Exp. Cell Res.* 329, 170–177.
- Schlesner, M., Eils, R., 2015. Hypermutation takes the driver's seat. *Genome Med.* 7, 31.
- Schmitt, M.W., Matsumoto, Y., Loeb, L.A., 2009. High fidelity and lesion bypass capability of human DNA polymerase delta. *Biochimie* 91, 1163–1172.
- Schmitt, M.W., Venkatesan, R.N., Pillaire, M.J., Hoffmann, J.S., Sidorova, J.M., Loeb, L.A., 2010. Active site mutations in mammalian DNA polymerase delta alter accuracy and replication fork progression. *J. Biol. Chem.* 285, 32264–32272.
- Shamoo, Y., Steitz, T.A., 1999. Building a replisome from interacting pieces: sliding clamp complex to a peptide from DNA polymerase and a polymerase editing complex. *Cell* 99, 155–166.
- Shinbrot, E., Henninger, E.E., Weinhold, N., Covington, K.R., Goksenin, A.Y., Schultz, N., Chao, H., Doddapaneni, H., Muzny, D.M., Gibbs, R.A., Sander, C., Pursell, Z.F., Wheeler, D.A., 2014. Exonuclease mutations in DNA polymerase epsilon reveal replication strand specific mutation patterns and human origins of replication. *Genome Res.* 24, 1740–1750.
- Shlien, A., Campbell, B.B., de Borja, R., Alexandrov, L.B., Merico, D., Wedge, D., Van Loo, P., Tarpey, P.S., Coupland, P., Behjati, S., Pollett, A., Lipman, T., Heidari, A., Deshmukh, S., Avizur, N., Meier, B., Gerstung, M., Hong, Y., Merino, D.M., Ramakrishna, M., Remke, M., Arnold, R., Panigrahi, G.B., Thakkar, N.P., Hodel, K.P., Henninger, E.E., Goksenin, A.Y., Bakry, D., Charames, G.S., Druker, H., Lerner-Ellis, J., Mistry, M., Dvir, R., Grant, R., Elhasid, R., Farah, R., Taylor, G.P., Nathan, P.C., Alexander, S., Ben-Shachar, S., Ling, S.C., Gallinger, S., Constantini, S., Dirks, P., Huang, A., Scherer, S.W., Grundy, R.G., Durno, C., Aronson, M., Gartner, A., Meyn, M.S., Taylor, M.D., Pursell, Z.F., Pearson, C.E., Malkin, D., Futreal, P.A., Stratton, M.R., Bouffet, E., Hawkins, C., Campbell, P.J., Tabori, U., Biallelic Mismatch Repair Deficiency, C., 2015. Combined hereditary and somatic mutations of replication error repair genes result in rapid onset of ultra-hypermutated cancers. *Nat. Genet.* 47, 257–262.
- Sincennes, M.C., Humbert, M., Grondin, B., Lisi, V., Veiga, D.F., Haman, A., Cazaux, C., Mashtalir, N., Affar, E.B., Verreault, A., Hoang, T., 2016. The LMO2 oncogene regulates DNA replication in hematopoietic cells. *Proc. Natl. Acad. Sci. U. S. A.*
- Skoneczna, A., Kaniak, A., Skoneczny, M., 2015. Genetic instability in budding and fission yeast—sources and mechanisms. *FEMS Microbiol. Rev.* 39, 917–967.
- Smith, C.E., Lam, A.F., Symington, L.S., 2009. Aberrant double-strand break repair resulting in half crossovers in mutants defective for Rad51 or the DNA polymerase delta complex. *Mol. Cell. Biol.* 29, 1432–1441.
- Sohl, C.D., Ray, S., Sweasy, J.B., 2015. Pools and Pols: mechanism of a mutator phenotype. *Proc. Natl. Acad. Sci. U. S. A.* 112, 5864–5865.
- Song, N., Zhu, X., Shi, L., An, J., Wu, Y., Sang, J., 2009. Identification and functional analysis of a CDE/CHR element in the POLD1 promoter. *Sci. China C Life Sci.* 52, 551–559.
- Song, J., Hong, P., Liu, C., Zhang, Y., Wang, J., Wang, P., 2015. Human POLD1 modulates cell cycle progression and DNA damage repair. *BMC Biochem.* 16, 14.
- St Charles, J.A., Liberti, S.E., Williams, J.S., Lujan, S.A., Kunkel, T.A., 2015. Quantifying the contributions of base selectivity, proofreading and mismatch repair to nuclear DNA replication in *Saccharomyces cerevisiae*. *DNA Repair (Amst)* 31, 41–51.
- Stehling, O., Vashisht, A.A., Mascarenhas, J., Jonsson, Z.O., Sharma, T., Netz, D.J., Pierik, A.J., Wohlschlegel, J.A., Lill, R., 2012. MMS19 assembles iron-sulfur proteins required for DNA metabolism and genomic integrity. *Science* 337, 195–199.
- Stillman, B., 2015. Reconsidering DNA polymerases at the replication fork in eukaryotes. *Mol. Cell* 59, 139–141.
- Su, F., Mukherjee, S., Yang, Y., Mori, E., Bhattacharya, S., Kobayashi, J., Yannone, S.M., Chen, D.J., Asaithamby, A., 2014. Nonenzymatic role for WRN in preserving nascent DNA strands after replication stress. *Cell Rep.* 9, 1387–1401.
- Swahari, V., Nakamura, A., 2016. Speeding up the clock: the past, present and future of progeria. *Develop. Growth Differ.* 58, 116–130.
- Swan, M.K., Johnson, R.E., Prakash, L., Prakash, S., Aggarwal, A.K., 2009. Structural basis of high-fidelity DNA synthesis by yeast DNA polymerase delta. *Nat. Struct. Mol. Biol.* 16, 979–986.
- Szekely, A.M., Chen, Y.H., Zhang, C., Oshima, J., Weissman, S.M., 2000. Werner protein recruits DNA polymerase delta to the nucleolus. *Proc. Natl. Acad. Sci. U. S. A.* 97, 11365–11370.
- Takahashi, Y., Moriwaki, S., Sugiyama, Y., Endo, Y., Yamazaki, K., Mori, T., Takigawa, M., Inoue, S., 2005. Decreased gene expression responsible for post-ultraviolet DNA repair synthesis in aging: a possible mechanism of age-related reduction in DNA repair capacity. *J. Invest. Dermatol.* 124, 435–442.
- Takemura, M., Yokobori, S., Ogata, H., 2015. Evolution of eukaryotic DNA polymerases via interaction between cells and large DNA viruses. *J. Mol. Evol.* 81, 24–33.
- Terai, K., Shibata, E., Abbas, T., Dutta, A., 2013. Degradation of p12 subunit by CRL4Cdt2 E3 ligase inhibits fork progression after DNA damage. *J. Biol. Chem.* 288, 30509–30514.
- Tibbetts, L.M., Chu, M.Y., Hager, J.C., Dexter, D.L., Calabresi, P., 1977. Chemotherapy of cell-line-derived human colon carcinomas in mice immunosuppressed with antithymocyte serum. *Cancer* 40, 2651–2659.
- Tsurimoto, T., Shinozaki, A., Yano, M., Seki, M., Enomoto, T., 2005. Human Werner helicase interacting protein 1 (WRNIP1) functions as a novel modulator for DNA polymerase delta. *Genes Cells* 10, 13–22.
- Uchimura, A., Hidaka, Y., Hirabayashi, T., Hirabayashi, M., Yagi, T., 2009. DNA polymerase delta is required for early mammalian embryogenesis. *PLoS One* 4, e4184.

- Uchimura, A., Higuchi, M., Minakuchi, Y., Ohno, M., Toyoda, A., Fujiyama, A., Miura, I., Wakana, S., Nishino, J., Yagi, T., 2015. Germline mutation rates and the long-term phenotypic effects of mutation accumulation in wild-type laboratory mice and mutator mice. *Genome Res.* 25, 1125–1134.
- Vaara, M., Itkonen, H., Hillukkala, T., Liu, Z., Nasheuer, H.P., Schaarschmidt, D., Pospiech, H., Syaaja, J.E., 2012. Segregation of replicative DNA polymerases during S phase: DNA polymerase epsilon, but not DNA polymerases alpha/delta, are associated with lamins throughout S phase in human cells. *J. Biol. Chem.* 287, 33327–33338.
- Valle, L., Hernandez-Illan, E., Bellido, F., Aiza, G., Castillejo, A., Castillejo, M.I., Navarro, M., Segui, N., Vargas, G., Guarinos, C., Juarez, M., Sanjuan, X., Iglesias, S., Alenda, C., Egoavil, C., Segura, A., Juan, M.J., Rodriguez-Soler, M., Brunet, J., Gonzalez, S., Jover, R., Lazaro, C., Capella, G., Pineda, M., Soto, J.L., Blanco, I., 2014. New insights into POLE and POLD1 germline mutations in familial colorectal cancer and polyposis. *Hum. Mol. Genet.* 23, 3506–3512.
- van Gool, I.C., Eggink, F.A., Freeman-Mills, L., Stelloo, E., Marchi, E., de Bruyn, M., Palles, C., Nout, R.A., de Kroon, C.D., Osse, E.M., Klenerman, P., Creutzberg, C.L., Tomlinson, I.P., Smit, V.T., Nijman, H.W., Bosse, T., Church, D.N., 2015. POLE proofreading mutations elicit an antitumor immune response in endometrial cancer. *Clin. Cancer Res.* 21, 3347–3355.
- Vatier, C., Bidault, G., Briand, N., Guenantin, A.C., Teyssieres, X., Lascols, O., Capeau, J., Vigouroux, C., 2013. What the genetics of lipodystrophy can teach us about insulin resistance and diabetes. *Curr. Diab. Rep.* 13, 757–767.
- Venkatesan, R.N., Treuting, P.M., Fuller, E.D., Goldsby, R.E., Norwood, T.H., Gooley, T.A., Ladiges, W.C., Preston, B.D., Loeb, L.A., 2007. Mutation at the polymerase active site of mouse DNA polymerase delta increases genomic instability and accelerates tumorigenesis. *Mol. Cell. Biol.* 27, 7669–7682.
- Vermeulen, S.J., Chen, T.R., Speleman, F., Nollet, F., Van Roy, F.M., Mareel, M.M., 1998. Did the four human cancer cell lines DLD-1, HCT-15, HCT-8, and HRT-18 originate from one and the same patient? *Cancer Genet. Cytogenet.* 107, 76–79.
- Waisertreiger, I.S., Liston, V.G., Menezes, M.R., Kim, H.M., Lobachev, K.S., Stepchenkova, E.I., Tahirov, T.H., Rogozin, I.B., Pavlov, Y.I., 2012. Modulation of mutagenesis in eukaryotes by DNA replication fork dynamics and quality of nucleotide pools. *Environ. Mol. Mutagen.* 53, 699–724.
- Wallace, B.D., Williams, R.S., 2014. Ribonucleotide triggered DNA damage and RNA–DNA damage responses. *RNA Biol.* 11, 1340–1346.
- Wang, J.L., Guo, H.L., Wang, P.C., Liu, C.G., 2012. Age-dependent down-regulation of DNA polymerase delta1 in human lymphocytes. *Mol. Cell. Biochem.* 371, 157–163.
- Wang, X., Zhang, S., Zheng, R., Yue, F., Lin, S.H., Rahmeh, A.A., Lee, E.Y., Zhang, Z., Lee, M.Y., 2016. PDIP46 (DNA polymerase delta interacting protein 46) is an activating factor for human DNA polymerase delta. *Oncotarget.*
- Waterfall, J.J., Meltzer, P.S., 2015. Avalanching mutations in biallelic mismatch repair deficiency syndrome. *Nat. Genet.* 47, 194–196.
- Weedon, M.N., Ellard, S., Prindle, M.J., Caswell, R., Lango Allen, H., Oram, R., Godbole, K., Yajnik, C.S., Sbraccia, P., Novelli, G., Turnpenny, P., McCann, E., Goh, K.J., Wang, Y., Fulford, J., McCulloch, L.J., Savage, D.B., O'Rahilly, S., Kos, K., Loeb, L.A., Semple, R.K., Hattersley, A.T., 2013. An in-frame deletion at the polymerase active site of POLD1 causes a multisystem disorder with lipodystrophy. *Nat. Genet.* 45, 947–950.
- Williams, L.N., Marjavaara, L., Knowels, G.M., Schultz, E.M., Fox, E.J., Chabes, A., Herr, A.J., 2015. dNTP pool levels modulate mutator phenotypes of error-prone DNA polymerase epsilon variants. *Proc. Natl. Acad. Sci. U. S. A.* 112, E2457–E2466.
- Wong, A., Kuick, C.H., Wong, W.L., Tham, J.M., Mansor, S., Loh, E., Jain, S., Vikas, N.N., Tan, S.H., Chan, S.H., Li, S.T., Chew, S.H., Hong, W., Ngeow, J., 2015. Mutation spectrum of POLE and POLD1 mutations in South East Asian women presenting with Grade 3 endometrioid endometrial carcinomas. *Gynecol. Oncol.*
- Wu, S.M., Zhang, P., Zeng, X.R., Zhang, S.J., Mo, J., Li, B.Q., Lee, M.Y., 1998. Characterization of the p125 subunit of human DNA polymerase delta and its deletion mutants. Interaction with cyclin-dependent kinase-cyclins. *J. Biol. Chem.* 273, 9561–9569.
- Wu, X., Ruan, L., Yang, Y., Mei, Q., 2016. Identification of crucial regulatory relationships between long non-coding RNAs and protein-coding genes in lung squamous cell carcinoma. *Mol. Cell. Probes.*
- Xia, S., Konigsberg, W.H., 2014. RB69 DNA polymerase structure, kinetics, and fidelity. *Biochemistry* 53, 2752–2767.
- Xie, B., Mazloun, N., Liu, L., Rahmeh, A., Li, H., Lee, M.Y., 2002. Reconstitution and characterization of the human DNA polymerase delta four-subunit holoenzyme. *Biochemistry* 41, 13133–13142.
- Xiong, X.D., Wang, J., Zheng, H., Jing, X., Liu, Z., Zhou, Z., Liu, X., 2013. Identification of FAM96B as a novel prelamin A binding partner. *Biochem. Biophys. Res. Commun.* 440, 20–24.
- Xu, L.H., Huang, M., Fang, S.G., Liu, D.X., 2011. Coronavirus infection induces DNA replication stress partly through interaction of its nonstructural protein 13 with the p125 subunit of DNA polymerase delta. *J. Biol. Chem.* 286, 39546–39559.
- Zhang, D., O'Donnell, M., 2016. The eukaryotic replication machine. *Enzyme* 39, 191–229.
- Zhang, S., Zhou, Y., Trusa, S., Meng, X., Lee, E.Y., Lee, M.Y., 2007. A novel DNA damage response: rapid degradation of the p12 subunit of dna polymerase delta. *J. Biol. Chem.* 282, 15330–15340.
- Zhang, S., Zhao, H., Darzynkiewicz, Z., Zhou, P., Zhang, Z., Lee, E.Y., Lee, M.Y., 2013a. A novel function of CRL4(Cdt2): regulation of the subunit structure of DNA polymerase delta in response to DNA damage and during the S phase. *J. Biol. Chem.* 288, 29550–29561.
- Zhang, S., Zhou, Y., Sarkeshik, A., Yates 3rd, J.R., Thomson, T.M., Zhang, Z., Lee, E.Y., Lee, M.Y., 2013b. Identification of RNF8 as a ubiquitin ligase involved in targeting the p12 subunit of DNA polymerase delta for degradation in response to DNA damage. *J. Biol. Chem.* 288, 2941–2950.
- Zhang, Q., Zhang, Q., Chen, H., Chen, Y., Zhou, Y., 2016. Multiple forms of human DNA polymerase delta sub-assembling in cellular DNA transactions. *Curr. Protein Pept. Sci.*
- Zhao, L., Chang, L.S., 1997. The human POLD1 gene. Identification of an upstream activator sequence, activation by Sp1 and Sp3, and cell cycle regulation. *J. Biol. Chem.* 272, 4869–4882.
- Zhou, H., Saito, S., Piao, G., Liu, Z.P., Wang, J., Horimoto, K., Chen, L., 2011. Network screening of Goto-Kakizaki rat liver microarray data during diabetic progression. *BMC Syst. Biol.* 5 (Suppl. 1), S16.
- Zhou, Y., Meng, X., Zhang, S., Lee, E.Y., Lee, M.Y., 2012. Characterization of human DNA polymerase delta and its subassemblies reconstituted by expression in the MultiBac system. *PLoS One* 7, e39156.
- Zuo, Y., Deutscher, M.P., 2001. Exoribonuclease superfamilies: structural analysis and phylogenetic distribution. *Nucleic Acids Res.* 29, 1017–1026.