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

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Abstract

The evolutionarily conserved human polymerase delta (POLD1) gene encodes the large p125 subunit which provides the essential catalytic activities of polymerase 6 (Polδ), mediated by 5′–3′ DNA polymerase and 3′–5′ exonuclease activities. POLD1 associates with three smaller subunits (POLD2, POLD3, POLD4), which together with Replication Factor C and Proliferating Nuclear Cell Antigen constitute the polymerase holoenzyme. Polδ function is essential for replication, with a primary role as the replicase for the lagging strand. Polδ 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. Polδ 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 Polδ with human pathologies. Mutations in Polδ 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 Polδ activities in the context of these pathologic conditions.
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 ε functions as a DNA primase, while Pol δ 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 δε 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 holozyme. 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 (Antonioli 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,
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 was proved 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 Hubsch, 1997). Fig. 2 provides a basic schematic of interactions during replication and repair.

Similar to Pol δ and ε, Polo uses a common B-family fold: the shared and unique structural elements of these replicative polymerases have been recently reviewed in (Doublie and Zahn, 2014). The review compiled data from various crystal structures including the structure for Pol3, the yeast homolog of Polo (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 Polo 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);
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. 2. A simplified view of the function of Polδ at the DNA replication fork and in response to damaged DNA. A. The Poli complex (p125, p66, p50 and p12) associates with replication forks. Exo marks the exonuclease domain of p125 and Poli. 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). Polα is bound to a primase, which initiates synthesis of lagging strand (black line) by producing an RNA primer which is then elongated first by Polo, then by Poli. Poli is positioned on the leading strand (orange line). GINS (go-i-chi-ni-san comprising of four related subunits of the complex Sld5, Psf1, Psf2 and Psf3) (Lujan et al., 2016a, 2016b) interacts with Poli to initiate DNA synthesis. Some data suggest a role of the Poli 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 Polδ on the leading strand and Poli 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 Poli 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, Poli is recruited to repair foci. P12 is ubiquitinated (black circles, Ub) and degraded by the proteasome, which leads to the conversion of Polδ4 to the Polδ3 complex, which has altered catalytic activity. It is possible to rapidly exchange between the Polδ3 and Polδ4 complexes (two-way green arrow) (Lee et al., 2012).
In the polymerase domain, two catalytic aspartates from motif A (DDXYLPS, D602) and motif C (DTD; 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 KKKY motif that is important for stabilizing the primer terminus in the polymerase active site (Hogg et al., 2007). A complex nuclear detachment 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$^2$S] 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 inversely (Krucher et al., 2000). p125 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 inversely (Krucher et al., 2000).
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 Polδ complex for PCNA (Rahmeh et al., 2012).

4.3. Altered protein interactions

The catalytic activity of p125 in the Polδ holoenzyme is modulated by the POLD4 product p12, which is the only subunit of the heterotrimer 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 heterotrimer Polδ4 (with p12) to the heterotrimer Polδ3 (without p12) (Zhang et al., 2007). The depletion of Polδ4 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 Polδ3 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 Polδ4 (Meng et al., 2009; Lee et al., 2014). It has been suggested that Polδ3 was an adaptation of Polδ for repair following induction of damage (Meng et al., 2009; Lee et al., 2014). However, during normal cell cycle, Polδ3 is also present in S phase, when DNA replication occurs, due to partial degradation of p12 by the E3 ligase RNF8 (Zhang et al., 2013a), CRL4 also degrades p12 in response to UV damage (Zhang et al., 2013a), triggering an intra-S-phase checkpoint and inhibiting fork progression (Terai 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 Polδ-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 IG548 that separates individual rDNA transcription units in response to acidosis, in a process mediated by the p125 Nod5 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 Polδ 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 PDP4/9SKAR, 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 LM2, 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 tumour formation and a modulator of disease course and therapeutic response (Boyer et al., 2016): the role of Polδ as a target in this process requires more study.

5. Polδ 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 Polδ and ε, and post-replicative surveillance and repair by the mismatch repair (MMR) system (Gaïani 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 Polδ 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 Polα initiation of replication by RNA-primed DNA synthesis, Polδ and Polε 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 Polδ 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 Polε is retained in the mature lagging strand, as DNA-binding proteins that rapidly reassociate post-replication prevent Polδ 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 R698W 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 Polε 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 linear chromosomes. 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 telomerases, 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 Polδ 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. Polδ function in DNA repair processes

The role of Polδ 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. 28). 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 (Rehab-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 Polε (Meng et al., 2010) and Polδ (Ganai et al., 2015), has been shown to conform to the model established for T7 DNA polymerase (Johnson, 1993; Kunkel and Bebenek, 2000).

Polδ 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 Polδ with Polα 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: Polθ (POLH), REV1, Polκ (POLK) and Polσ (POL); one B-family polymerase: Polç (REV3L), and two polymerases from the A-family: Polη (POLN) and Polζ (POLQ) (reviewed in Saugier 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 Polδ.

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 Polξ4 to the heterotrimer Polζ3, with Polζ3 having a more discriminating polymerase, and increased exonuclease activity (Meng et al., 2009; Lee et al., 2014) (Fig. 28). Polζ3 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 Polδ and Polζ (Chea et al., 2012). As noted above, Polζ3 production depends on RNFS-induced p12 degradation, and RNFS has been identified as an organizer for repair of double-strand breaks (DSBs) suggesting that RNFS-dependent production of Polζ3 is for processes involving homologous recombination (Lee et al., 2014). Polζ3/Fen1 performed better than Polξ4/Fen1 in a strand displacement/ﬂap cleavage assay suggesting that Polζ3 is also highly adapted for the Polξ 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 Polζ 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.
Polô 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 are unable to repair. In in vitro experiments utilizing HeLa cell extracts and Pol ô exonuclease mutant (Δ400A) 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 ô 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 ô strand synthesis activity (POLD1), albeit in a highly error-prone process (Miyabe et al., 2015). In the yeast S. cerevisiae, Polô 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, Polô is essential in maintaining Polô stability (based on a C-terminal domain interaction with Polô3). A Polô mutant (Polô3-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 Polô3 (Polô3-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 deletion 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 (mandibuloperiosteal dysplasia, 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; Pelosini et al., 2014; 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 G101V, R689W, and S746L mutations, which may contribute to the mutator phenotype (Flohr et al., 1999). HTC-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 Polô and the WRN protein (Szekely et al., 2000) increase the processivity of Polô in a PCNA-independent manner (Kamath-Loeb et al., 2012), impacting both DNA replication and DNA repair. The WRN and Polô 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 SPTN1, an adaptor that binds the TLS polymerase Polôś, and in the DNT pool regulator SMDH1. Damaging p125 lesions have been convincingly linked to cancer risk, as described below. The mother of one 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 lamin, or epigenetic effects might be involved.

Mutations in laminas are known to cause nuclear envelope-related lipodystrophies with phenotypes reminiscent of MDPL and WS (Guentinant 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 laminas. In late S, however, only Polô is associated with the nuclear matrix through laminas, Polô 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 gi5 (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 Pole epi-geneic 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 Polo and Polo: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 POLE and POLD1 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; Vallee 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; Vallee 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 (Vallee et al., 2014). These POLD1 mutations predispose to endometrial tumors and may also predispose to breast and brain tumors (Palles et al., 2013; Vallee 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 (Vallee 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; Huang 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 Polo mutations (Cancer Genome Atlas, 2012). Similarly, other studies have been reported with endometrial tumors that are microsatellite stable but have Polo mutations and highly elevated somatic mutation rates (Henninger and Pursell, 2014). These studies also showed that the Polo: 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 ExoI motif and L606M, resulting in forms of the protein previously described to incorporates 7-fold more nucleobases 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 PIK3CA, 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
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 Polδ 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 Polδ 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 (Goldsbly 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 (Pold1flox/flox) 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 Polε 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 (Kleinsteiver 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). Polε 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 Polβ 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 in exonuclease function of polymerases associated or autonomous aging; studies of normal aging have more broadly connected decline atypical Werner syndrome are associated with features of premature aging (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.

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