DNA polymerases and cancer

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Abstract | There are 15 different DNA polymerases encoded in mammalian genomes, which are specialized for replication, repair or the tolerance of DNA damage. New evidence is emerging for lesion-specific and tissue-specific functions of DNA polymerases. Many point mutations that occur in cancer cells arise from the error-generating activities of DNA polymerases. However, the ability of some of these enzymes to bypass DNA damage may actually defend against chromosome instability in cells, and at least one DNA polymerase, Pol ζ, is a suppressor of spontaneous tumorigenesis. Because DNA polymerases can help cancer cells tolerate DNA damage, some of these enzymes might be viable targets for therapeutic strategies.

DNA damage
A term that encompasses the many types of chemical alterations that can change the structure of DNA. Damage can be caused by reactions that disrupt bonds in the nucleobases, the deoxyribose sugar ring, or the phosphate groups of DNA or by the addition of chemical moieties such as hydroxyl groups, methyl groups, or even bulkier groups derived from polycyclic molecules. Such additions are often referred to as DNA adducts. DNA damage is already plural, so DNA damages is incorrect, an alternative is DNA lesions.

DNA polymerases are enzymes that synthesize DNA. These proteins have an essential role in genome duplication, but they are also crucial for protecting the cell against the effects of DNA damage. In both normal and cancer cells, DNA is subjected to damage from many sources. Water-catalysed reactions and attack by reactive oxygen species (ROS) are inescapable and inflict continual damaging alterations to DNA. Other ubiquitous sources of lesions include naturally occurring ionizing radiation, ultraviolet (UV) radiation from the sun and reactive chemicals that are present in the environment or that arise as natural metabolites. The toxic and mutagenic consequences of such damage are minimized by distinct DNA repair pathways, including base excision repair (BER) and nucleotide excision repair (NER) (BOX 1). These repair mechanisms rely on a DNA polymerase to fill gaps in the DNA that are left by the removal of damaged bases. If the DNA damage is unrepaired, cells can often tolerate it by using a specialized DNA polymerase during DNA replication to insert a base opposite a lesion and bypass the damage, in a process called translesion DNA synthesis (TLS) (FIGS 1, 2). This process is responsible for many of the point mutations in cells[1] and is particularly relevant to the large increase in point mutations found in cancer genomes compared with normal tissues[2]. Another method by which cells tolerate DNA damage encountered at replication forks is template switching, which can be mediated by recombination and is primarily error-free[3]. Finally, breaks and gaps can arise in DNA, and repair mechanisms must be used, including homologous recombination (HR) and various non-homologous end joining (NHEJ) processes. DNA polymerases are also essential components of these pathways (BOX 1).

DNA strand breaks, particularly double-strand breaks (DSBs), seem to be formed more frequently in cancer cells than in normal cells. This is probably a consequence of the frequent disruption of normal controls on DNA replication in cancer cells[4]. For example, loss of p53 function, which is frequent in cancer cells, weakens a checkpoint control that would normally prohibit cells from initiating DNA replication when breaks are present. Many cancer cells exhibit a heightened basal level of activation of some responses to DNA breaks, accumulating HR and other repair proteins on chromatin. This may be caused by the expression of oncoproteins, which is thought to lead to recurrent initiation and collision of DNA replication forks, thereby resulting in increased numbers of DNA breaks[4].

Fifteen mammalian DNA polymerases have been identified (TABLE 1), some of which function in the replication of the genome, but most of which participate in DNA repair and TLS. In this Review, we describe how DNA polymerases are specialized for distinct cellular mechanisms, focusing on the roles of DNA polymerases in carcinogenesis. Insights into these roles are anticipated to provide new approaches for cancer prevention, diagnosis and treatment. Much background understanding of DNA polymerases has come from research carried out using the yeast *Saccharomyces cerevisiae*, providing knowledge that has proved to be predictive of the situation in other eukaryotes. Here, we primarily focus on DNA polymerases in vertebrates.

Replicative DNA polymerases

Genomic DNA in the nucleus is normally replicated accurately by DNA polymerase α (Pol α), Pol δ and Pol ε. Pol α initiates DNA synthesis on both the leading and lagging strands by providing an RNA primer

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At a glance

- Fifteen DNA polymerases are encoded in mammalian genomes. Some function in the replication of the genome, but most participate in specialized DNA repair and DNA damage tolerance processes. The activity of these DNA polymerases will affect the response of a cell to DNA-damaging carcinogens and chemotherapeutic agents.
- Some DNA polymerases catalyse DNA synthesis on damaged sites in DNA, helping cells tolerate DNA damage by translesion DNA synthesis (TLS). TLS polymerases are specialized for the bypass of different DNA adducts. Defects in Pol η (also known as POLH) are responsible for the variant type of xeroderma pigmentosum (XP-V).
- Pol ζ (the catalytic subunit of which is REV3L) and REV1 are required for nearly all damage-induced base change mutagenesis in mammalian cells. Reduction of their activities sensitizes cells, including tumour cells, to DNA-damaging agents. However, chromosome rearrangements and inflammation can increase in the absence of these proteins, promoting carcinogenesis.
- The expression of some genes encoding DNA polymerases may be altered in some cancers. In breast cancers, levels of *POLQ* (which encodes Pol θ) seem to be the most elevated compared with normal levels of expression. Comprehensive studies of DNA polymerase protein levels in cancer remain to be carried out.
- The inhibition of DNA polymerase activities could be useful as an adjuvant to DNA-damaging therapies, and inhibitors for some polymerases have been found. Pharmacologically effective inhibitors highly specific for a single DNA polymerase remain to be identified.
- Whole-genome analyses of cancers have not yet revealed cancer-associated alterations in DNA polymerase genes. It seems likely, however, that at least some cells in a tumour will have relevant alterations. Some DNA polymerases can be considered as tumour suppressors (Pol ζ, REV1, Pol η, Pol ι, Pol κ, Pol δ and Pol ε).

and synthesizing approximately 20–30 bases of DNA (that form part of the Okazaki fragments)[5]. Pol ε and Pol δ elongate these primers. In *S. cerevisiae*, Pol ε may be especially important for leading strand synthesis and Pol δ for lagging strand synthesis[6,7]. The base substitution error rates of Pol δ and Pol ε are approximately 10^{-5}, the lowest of all the characterized DNA polymerases[7], and when they do occasionally misincorporate a nucleotide it is usually removed by a 3′–5′ exonuclease associated with these DNA polymerases[8]. Errors that escape such proofreading can be corrected by the DNA mismatch repair (MMR) pathway (BOX 1), so that the spontaneous mutation rate during nuclear DNA replication is very low at less than 10^{-9} per base pair per cell division[9].

The proofreading 3′–5′ exonuclease activities of Pol δ and Pol ε are crucial for preventing mutations; cells from *Pold1* (which encodes the catalytic subunit of Pol δ) or *Pole* (which encodes the catalytic subunit of Pol ε) exonuclease-deficient (exo) mice have a tenfold increased frequency of mutagenesis, and these mutations have been shown to drive carcinogenesis (TABLE 2). *Pold1*$^{exo/exo}$ mice either die by 8 months of age from thymic lymphomas, or develop skin tumours, lung adenocarcinomas or teratomas[10,11]. *Pole*$^{exo/exo}$ mice die prematurely of intestinal adenomas and adenocarcinomas[12]. *Pole*$^{exo/exo}$;*Pold1*$^{exo/exo}$ double exonuclease-mutant mice die even more rapidly from thymic lymphomas than single-mutant mice[12]. Mice carrying a mutator allele of *Pold1* (which confers an increase in nucleotide misincorporation and genomic instability) are not viable in the homozygous state, emphasizing the importance of high fidelity DNA replication for the survival of an organism[13]. In view of these results in mouse models, it is intriguing that sporadic sequence changes have been found in *POLD1* in human colon cancer cell lines and patient tumour tissue samples[14]. Most of these changes seem to have no functional effect; however, an R689W mutation caused lethality when modelled as a homologous change in *pol3*, which encodes the catalytic subunit of Pol δ in *S. cerevisiae*[15]. The expression of low levels of normal Pol δ rescued this lethality, but was associated with an increased mutation rate. It is consequently possible that some mutations in *POLD1* or *POLE* might contribute to tumorigenesis or tumour progression in humans by increasing mutation rates.

Template switching
An error-avoiding strategy for DNA damage tolerance that uses the newly synthesized, undamaged strand of a sister chromatid for bypass replication.

Checkpoint
A control mechanism to verify whether each phase of the cell cycle has been completed accurately. If DNA damage is present, some checkpoint controls prevent or delay progression through the cell cycle, for example from G1 to S phase or from G2 phase to mitosis.

Leading and lagging strands
DNA synthesis can only add nucleotides to the terminal 3′-OH group of a growing polymer. The strand synthesized continuously during DNA replication is the leading strand. The strand of DNA that is synthesized in discontinuous segments is the lagging strand.

TLS

Despite the existence of DNA repair mechanisms, some DNA damage escapes repair and can stall the replication machinery. Stalled DNA replication forks seem to be fragile, and if not resolved they can collapse into structures that cause a DSB to be formed, thereby increasing genomic instability (FIG. 1).

The main strategy by which cells are able to tolerate DNA damage during replication is by synthesizing DNA past the damaged bases (FIG. 2). The replicative DNA polymerases are particularly specific for normal DNA base pairs, but they have very little capacity for replication opposite damaged bases[8]. Mammalian cells have at least seven enzymes with substantial TLS activity. These include four Y-family polymerases (Pol η (also known as POLH), Pol ι (also known as POLI), Pol κ (also known as POLK) and REV1), one B-family polymerase (Pol ζ, the catalytic subunit of which is REV3L) and two A-family polymerases (Pol θ (also known as POLQ) and Pol ν (also known as POLN)). None of the TLS DNA polymerases has proofreading exonuclease activity, and they possess unique DNA damage bypass and fidelity profiles. In the context of TLS, these DNA polymerases are not DNA repair enzymes, but are DNA damage tolerance factors. As described below, physiological roles in lesion bypass are established for only some of these enzymes[16,17].

Bypass of DNA damage caused by UV radiation. The most notable TLS polymerase for the bypass of UV radiation-induced DNA damage is Pol η. Currently, Pol η is the only polymerase for which a deficiency is known to predispose humans to cancer[18]. The inherited disorder xeroderma pigmentosum is associated with a greatly increased risk of sunlight-induced carcinomas of the skin, and individuals with the variant type of the condition, XP-V, have disabling mutations in Pol η[18,19]. Pol η can bypass a TT-cyclobutane pyrimidine dimer (CPD) — the major form of DNA damage that is induced by UV radiation — with high efficiency and fidelity. Purified human Pol η usually correctly inserts A deoxynucleotides opposite the linked bases of a TT-CPD[20]. Co-crystal structures show that Pol η is particularly suited for bypassing TT-CPDs as it makes many specific contacts with the lesion within the spacious active site[21,22]. Mutations in *POLH* that occur in individuals with XP-V disable specific interactions of the enzyme with the TT-CPD[21,22].

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Box 1 | DNA repair mechanisms

DNA repair mechanisms generally involve the removal of damaged or incorrect bases, and require a DNA polymerase to resynthesize DNA, using the undamaged strand as a template.

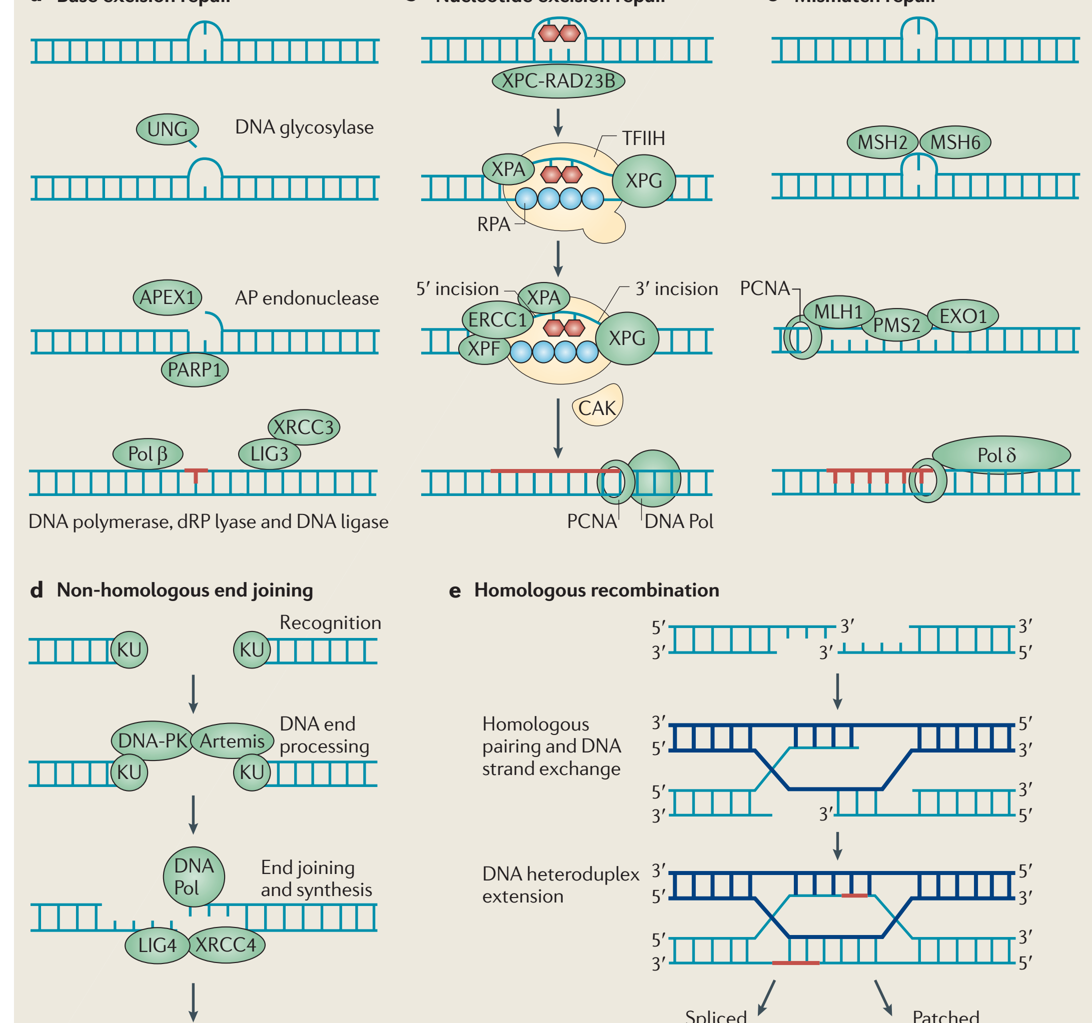
Base excision repair (BER) typically mediates the removal and replacement of a single base residue (see part **a** of the figure). Substrates include uracil residues in DNA and damaged bases caused by reactive oxygen species, hydrolytic reactions and methylation. A damaged base is removed by a specific DNA glycosylase (UNG for uracil is shown in the figure). The resulting abasic site (apurinic or apyrimidinic (AP) site) is incised by an AP endonuclease (APEX1). The 5′-deoxyribose-phosphate (dRP) residue is removed by a dRP lyase, leaving a one nucleotide gap that is filled in by DNA polymerase β (Pol β).

Nucleotide excision repair (NER) can remove various helix-distorting adducts, including those caused by ultraviolet (UV) radiation, cisplatin and polycyclic aromatic hydrocarbons (part **b** of the figure). A distorted region is recognized, and two incisions are made on either side of the adduct to excise the damaged DNA. The resulting 27–29 nucleotide gap is filled by Pol δ or Pol ε and under some circumstances Pol κ.

Mismatch repair (MMR) is an excision repair process that removes mismatched bases (part **c** of the figure). It is initiated by mismatch recognition proteins, and a segment of DNA is excised between the mismatch and a nearby nick. The gap that is left in the DNA is filled by Pol δ.

DNA double-strand breaks (DSBs) can be repaired by non-homologous end joining (NHEJ) (part **d** of the figure). Strand breaks caused by ionizing radiation or by enzymes that cleave DNA usually do not yield DNA ends that can be ligated directly. End-trimming and resynthesis of bases is therefore required to join breaks.

An alternative strategy for DSB repair is homologous recombination (HR) (part **e** of the figure). HR only operates when a double-stranded copy of the sequence is available; for example, as a sister chromatid in late S or G2 phase of the cell cycle.



Exonuclease
An exonuclease cleaves DNA phosphodiester bonds to release nucleotides from one end of a polynucleotide chain. DNA polymerases synthesize DNA in a 5′–3′ direction and some DNA polymerases have an intrinsic 3′– 5′ exonuclease activity that enables proofreading of their own mistakes.

Y-family
The prototypes for Y-family polymerases are the *Escherichia coli* TLS DNA polymerases DinB (Pol IV) and UmuC (Pol V).

B-family
B-family DNA polymerases show similarity to *E. coli* Pol II.

A-family
The A-family DNA polymerase domain is similar to *E. coli* DNA polymerase I (Pol I), encoded by the bacterial *PolA* gene.

Xeroderma pigmentosum
An inherited human syndrome characterized by severe photosensitivity, a high incidence of skin cancer and neurological abnormalities. The disorder is caused by a deficiency in NER genes (*XPA-XPG*), or in TLS past UV radiation-induced DNA damage (the XP-V type is caused by mutations in *POLH*).

Cyclobutane pyrimidine dimer
(CPD). The most frequent UV radiation-induced DNA lesion, formed by the covalent linkage of the C5 and C6 bonds of adjacent pyrimidines to form a cyclobutane ring, without directly altering the base pairing faces of the dimerized bases. Such dimers are formed most commonly between adjacent thymines, but also between thymine and cytosine or two adjacent cytosines.

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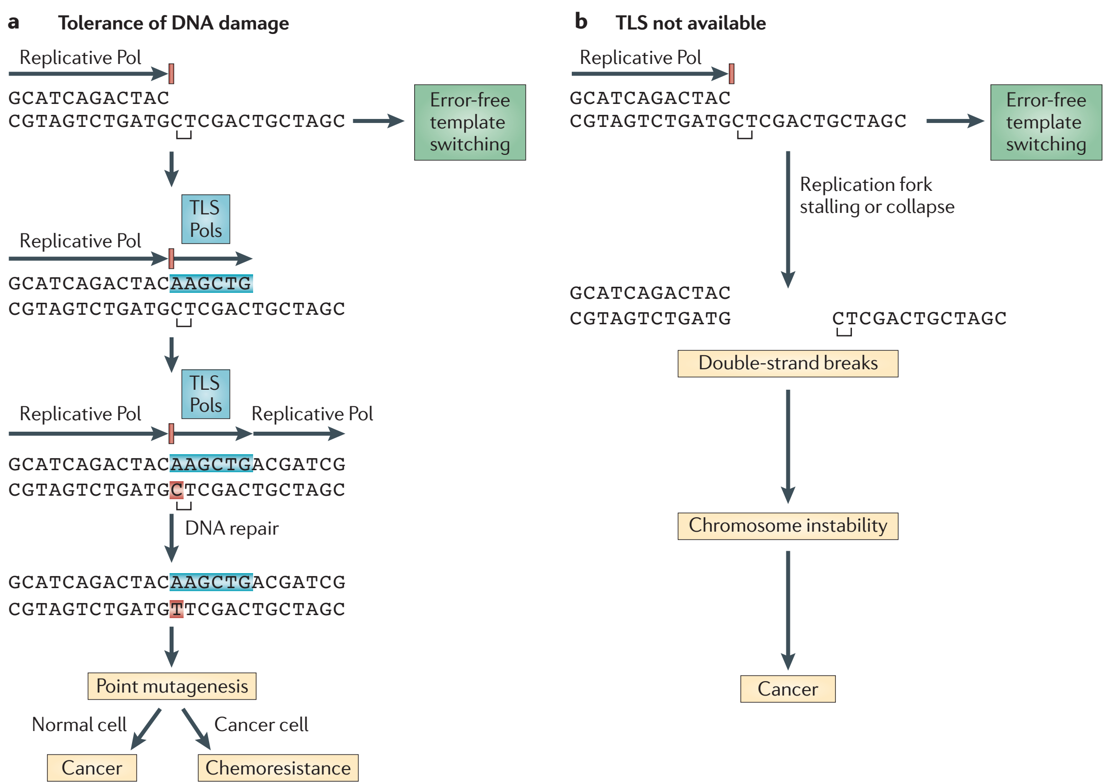

Figure 1 | **DNA damage tolerance and carcinogenesis. a** | Replication forks can be blocked by lesions in DNA. When a translesion DNA synthesis (TLS) polymerase (Pol; blue) is available, a lesion can be bypassed by TLS, potentially causing point mutagenesis, or template switching can mediate bypass in an error-free manner (FIG. 2). Point mutagenesis (highlighted in red) can lead to cancer formation in normal cells or to resistance to therapeutic agents in cancer cells. **b** | In the absence of a TLS polymerase, no translesion bypass occurs (although there will still be some template switching) and replication forks collapse, leading to double-strand breaks and chromosome instability, which increases the incidence of cancer.

Polh$^{-/-}$ mice mirror the XP-V phenotype as they rapidly develop UV radiation-induced tumours (TABLE 2). *Polh*$^{+/-}$ mice are also susceptible to UV radiation-induced skin carcinogenesis, although at a lesser rate[23]. UV irradiation of XP-V cells causes DNA DSBs to form because the absence of the TLS function of Pol η causes DNA replication forks to stall and collapse at sites of DNA damage on the template strand[24]. Prolonged replication delay in the absence of Pol η may also inhibit DNA repair of UV radiation-induced lesions[25]. Unirradiated *POLH*$^{-/-}$ cells have been reported to have more chromatid breaks than normal, including breaks at a common fragile site[26].

Patients with XP-V have an increased incidence of squamous cell carcinoma of the skin, and so experiments have been conducted to determine whether there are mutations in *POLH* that are associated with sporadic skin carcinomas[27] or other human cancers. No mutations affecting the function of Pol η have yet been identified.

Another frequently formed type of UV radiation-induced DNA damage is the (6-4) photoproduct[28,29]. This adduct is more distorting to the DNA double helix than CPDs and is not handled efficiently by Pol η[30]; the enzyme mostly misincorporates one G residue (instead of two As) and cannot continue replicating. Pol ι is more suited to inserting the correct bases opposite UV radiation-induced (6-4) photoproducts[29,31]. Pol ι can also insert bases opposite a TT-CPD, particularly when Pol η is absent, although this bypass is mutagenic[32]. When allowed to replicate undamaged DNA, Pol ι may be the most error-generating DNA polymerase, commonly misincorporating G opposite a template T before stalling[31].

Poli$^{-/-}$ cells are no more sensitive than wild-type cells to UV radiation[33,34]. However, *Poli*$^{-/-}$;*Polh*$^{-/-}$ double knockout mice have increased rates of UV radiation-induced skin carcinogenesis, compared with *Polh*$^{-/-}$ mice[33,34]. The increase in UV radiation-induced mutagenesis that is observed in *Polh*$^{-/-}$ mice and cells is suppressed when Pol ι function is ablated[33,35], suggesting that Pol ι is an error-generating backup polymerase for the bypass of UV radiation-induced DNA lesions, and may be responsible for skin carcinogenesis in patients with XP-V.

Bypass of bulky adducts in DNA. Bulky or helix-distorting DNA adducts are clinically relevant because they are caused by both carcinogenic compounds (such as benzo[*a*]pyrene diol epoxide (BPDE)) and chemotherapeutic agents (such as cisplatin and mitomycin C (MMC)). Several lines of evidence suggest specialized roles for Pol κ in bypassing bulky adducts in DNA. *In vitro*, Pol κ is adept at bypassing adducts formed with the N^2 of G in the minor groove of DNA,

Fragile site
Heritable regions on chromosomes that are associated with an increased frequency of chromosome breaks, gaps and other aberrations. Fragile sites, and the genes that they contain, are frequently rearranged or deleted in cancer cells.

(6-4) photoproduct
The second most common type of UV radiation-induced DNA damage, involving linkage of the C6 position of a 5′ pyrimidine base to the C4 position of a 3′ adjacent pyrimidine base. (6-4) photoproducts distort the DNA helix more than a CPD and form most often at 5′ thymine-cytosine-3′ sequences.

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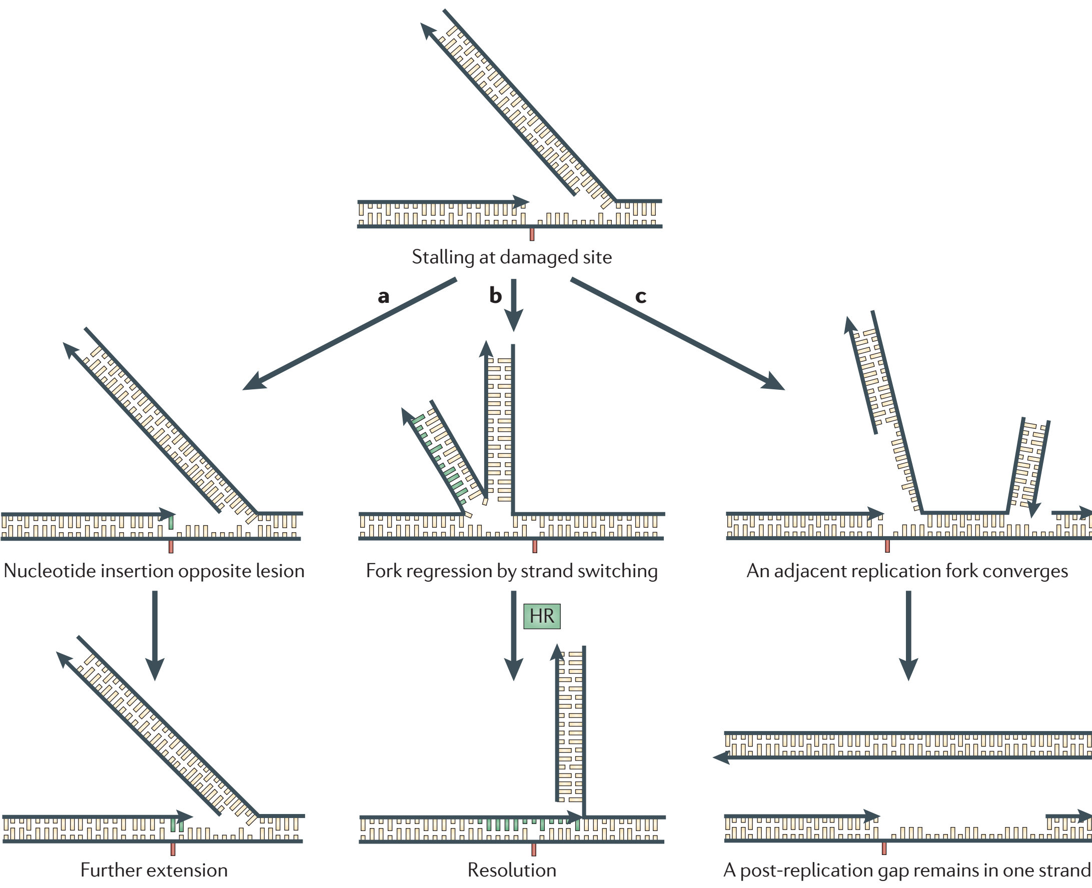

Figure 2 | **Strategies for translesion DNA synthesis.** Some types of DNA damage, if not repaired, will block the progression of a DNA replication fork. When a site of DNA damage on the leading strand is encountered by the DNA replication machinery and this prevents normal base pairing (red), replication is blocked. The lagging strand may continue replication, but the leading strand on which the replication machinery is blocked is fragile. Replication of the two strands can become uncoupled and dissociation of the DNA replication machinery causes collapse of the DNA replication fork, eventually leading to a DNA break (FIG. 1). Several possible strategies to overcome this block to replication may be activated. One strategy (part **a**) is to carry out translesion DNA synthesis (TLS) by successive steps. The replication machinery switches to a specialized DNA polymerase for the insertion of a base (green). This step is potentially mutagenic because the wrong base will sometimes be incorporated. A switch to a second specialized DNA polymerase may take place to extend the nonstandard terminus opposite the damage, and finally there is a switch to a replicative DNA polymerase (Pol ε or Pol δ). DNA polymerase switching is facilitated by post-translational modifications of DNA polymerases and their accessory factors, as summarized in the text and reviewed in depth elsewhere[1,3,99,100]. A second strategy (part **b**) is DNA replication fork regression. Here, the blocked leading strand switches templates and begins to copy the already-replicated lagging strand. The newly replicated bases are shown in green. The regressed fork resembles a four-way junction that can be processed by homologous recombination (HR) enzymes and resolved. This pathway avoids errors, as it makes use of genetic information from the undamaged strand. A third strategy is illustrated in part **c**. If the replication fork remains stalled for long enough, an adjacent replication fork will converge with it. This allows one strand to replicate fully, while one strand will contain a gap. This gap will then remain through to late S phase or G2 phase of the cell cycle. The gap is then filled by DNA synthesis. During gap filling, two different specialized DNA polymerases may also be needed to accomplish synthesis across a lesion, for insertion and extension, and this is potentially mutagenic. Gaps could also conceivably arise by re-initiation of DNA synthesis on the other side of a DNA adduct. Arrows indicate the direction of DNA replication, which is 5′ to 3′ with respect to the deoxyribose sugar-phosphate.

including BPDE adducts and crosslinked peptides[36–38]. The promoter region of *POLK* contains aryl-hydrocarbon receptor binding sites, and its expression can be increased by treatment with the carcinogenic polycyclic aromatic hydrocarbon 3-methylcholanthrene[39]. *Polk*$^{-/-}$ mouse embryonic fibroblasts are sensitive to BPDE[40] and depletion of Pol κ sensitizes cells to apoptosis and chromosome aberrations caused by MMC, which can crosslink N^2-Gs[38].

Polk$^{-/-}$ mice are viable, although survival is shorter than in *Polk*$^{+/-}$ and *Polk*$^{+/+}$ mice (TABLE 2). *Polk*$^{-/-}$ mice have a spontaneous mutator phenotype in tissues, including the kidney, liver and lung, and it has been suggested that Pol κ has a role in tolerating bulky DNA adducts generated endogenously, such as by cholesterol metabolism[41]. Despite its low fidelity when copying undamaged DNA, Pol κ seems to protect against spontaneous mutagenesis.

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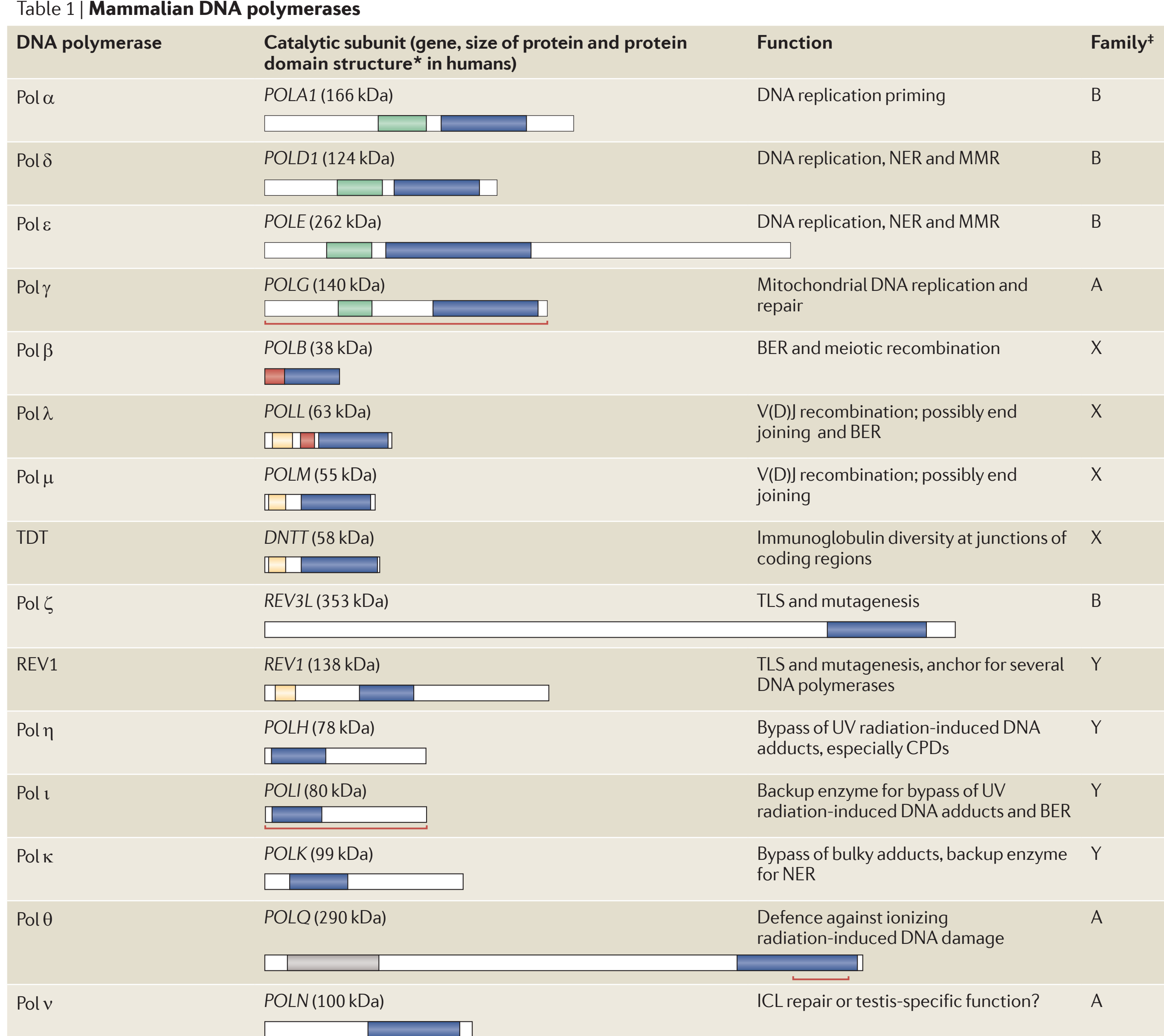
Table 1 | **Mammalian DNA polymerases**



DNA polymerase	Catalytic subunit (gene, size of protein and protein domain structure* in humans)	Function	Family‡
Pol α	*POLA1* (166 kDa)	DNA replication priming	B
Pol δ	*POLD1* (124 kDa)	DNA replication, NER and MMR	B
Pol ε	*POLE* (262 kDa)	DNA replication, NER and MMR	B
Pol γ	*POLG* (140 kDa)	Mitochondrial DNA replication and repair	A
Pol β	*POLB* (38 kDa)	BER and meiotic recombination	X
Pol λ	*POLL* (63 kDa)	V(D)J recombination; possibly end joining and BER	X
Pol μ	*POLM* (55 kDa)	V(D)J recombination; possibly end joining	X
TDT	*DNTT* (58 kDa)	Immunoglobulin diversity at junctions of coding regions	X
Pol ζ	*REV3L* (353 kDa)	TLS and mutagenesis	B
REV1	*REV1* (138 kDa)	TLS and mutagenesis, anchor for several DNA polymerases	Y
Pol η	*POLH* (78 kDa)	Bypass of UV radiation-induced DNA adducts, especially CPDs	Y
Pol ι	*POLI* (80 kDa)	Backup enzyme for bypass of UV radiation-induced DNA adducts and BER	Y
Pol κ	*POLK* (99 kDa)	Bypass of bulky adducts, backup enzyme for NER	Y
Pol θ	*POLQ* (290 kDa)	Defence against ionizing radiation-induced DNA damage	A
Pol ν	*POLN* (100 kDa)	ICL repair or testis-specific function?	A

BER, base excision repair; CPD, cyclobutane pyrimidine dimer; ICL, interstrand crosslink; MMR, mismatch repair; NER, nucleotide excision repair; Pol, polymerase; TDT, terminal deoxynucleotidyltransferase; TLS, translesion DNA synthesis; UV, ultraviolet. Blue, DNA polymerase domain; green, exonuclease domain; red, 5′-deoxyribose phosphate (dRP) lyase domain; yellow, BRCT domain; grey, helicase-like domain; red line, dRP lyase activity. *Most eukaryotic DNA polymerase proteins are named with Greek letters (for example, α, β and γ) and the genes are named with the corresponding roman letter. ‡In mammalian cells, these enzymes fall into four distinct families, designated A, B, X and Y, based on amino acid sequence relationships.

Abasic site
A site in a DNA chain that is missing a pyrimidine or purine base residue, but where the phosphodiester backbone remains intact. Such sites can arise when a base–sugar bond is cleaved by a DNA glycosylase during BER, or by a spontaneous hydrolytic reaction.

Pol η is another polymerase that, to some extent, can incorporate a base opposite bulky types of DNA damage that are produced by carcinogens and chemotherapeutic agents. In addition to its ability to bypass UV radiation-induced DNA damage, Pol η can bypass cisplatin-GG adducts[20], acetylaminofluorene-G[42], lesions induced by ionizing radiation, including 8-oxoG[43,44], and a major G adduct, γ-hydroxypropanodeoxyguanosine, which is formed with the mutagen acrolein[45]. In experiments in which DNA containing a single site of damage is transfected into mammalian cells, Pol η participates in the bypass of BPDE-G and cisplatin-GG adducts[46], as well as CPDs[18,20–22]. Furthermore, XP-V cells are more sensitive than normal cells to cisplatin[47]. Pol η also contributes to replication through naturally occurring alternative DNA structures, such as G-quartets[48].

Bypass of AP sites and thymine glycol. Abasic sites (apurinic or apyrimidinic (AP) sites) are the most frequent spontaneous lesions in DNA. They are formed by the release of bases from the sugar–phosphate backbone and as intermediates in DNA repair. Many DNA polymerases can insert a base opposite an AP site[49]: for example, Pol η and Pol ι can do so[31,42], but their activities are not needed for AP site bypass in human cells[50].

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Table 2 | **DNA polymerase knockout mouse phenotypes***

DNA polymerase	Gene for catalytic subunit	Phenotype of knockout mouse
Pol α	*Pola1*	Embryonic lethality likely
Pol δ	*Pold1*	Embryonic lethality after E4.5. Tumorigenesis in *Pold1*$^{exo/exo}$ and *Pold1*$^{exo/-}$ mice[11,12]
Pol ε	*Pole*	Embryonic lethality likely. Tumorigenesis in *Pole*$^{exo/exo}$ mice[12]
Pol γ	*Polg*	Embryonic lethality after E7.5 (REF. 194)
Pol β	*Polb*	Exhibit apoptosis in post-mitotic neuronal cells and die at birth. Loss of p53 rescues lethality[195–197]
Pol λ	*Poll*	Viable, fertile and display reduced immunoglobulin heavy chain junction variability[144]
Pol μ	*Polm*	Viable, fertile and display reduced immunoglobulin light chain junction variability[147]
TDT	*Dntt*	Viable, fertile and display reduced immunoglobulin heavy chain junction variability[143]
Pol ζ	*Rev3l*	Embryonic lethality after E9.5, p53 deficiency cannot rescue the lethality[72–74]. Spontaneous tumour development in conditional knockout mice[85]
REV1	*Rev1*	Inviable on the C57BL/6 background, but viable on the 129/OLA background[93]. Loss of C:G transversions during somatic hypermutation
Pol η	*Polh*	Viable and fertile, susceptible to UV radiation-induced skin cancer. Generation of A:T mutations during the somatic hypermutation of immunoglobulin genes[198]
Pol ι	*Poli*	Viable and fertile. The 129/OLA strain of mice has a naturally occurring nonsense mutation in *Poli*. *Polh*$^{-/-}$; *Poli*$^{-/-}$ mice show slightly earlier onset of skin tumour formation[198,199]
Pol κ	*Polk*	Viable and fertile[40,200]. Increased mutation frequencies in tissues[41]
Pol θ	*Polq*	Viable and fertile, and high micronuclei frequency in reticulocytes. Very low viability of *Polq*$^{-/-}$; *Atm*$^{-/-}$ mice[149,201]
Pol ν	*Poln*	Unknown

Atm, ataxia-telangiectasia mutated; E, embryonic day; exo, exonuclease-deficient; Pol, polymerase; TDT, terminal deoxynucleotidyltransferase; UV, ultraviolet. *A database of knockout mouse strains related to DNA damage processing is available[202].

Interstrand crosslink
(ICL). Covalently links the two complementary strands of duplex DNA. Such crosslinks are formed by some carcinogenic and chemotherapeutic agents and they are especially toxic because they block the complementary DNA strand separation that is necessary for DNA replication and transcription.

Mammalian Pol θ is unique in its ability both to efficiently insert an A opposite an AP site, and to extend past it[51]. It is more proficient at this TLS reaction *in vitro* than any of the Y-family DNA polymerases. On undamaged template DNA, Pol θ has a much lower fidelity than prokaryotic A-family DNA polymerases and a tendency to delete or add single bases during DNA synthesis[51,52].

Thymine glycol residues are a major product of ROS-induced damage to DNA and are frequently caused by ionizing radiation. Several DNA polymerases can bypass thymine glycols, including Pol θ[51], Pol η[53], Pol κ[54] and Pol ν[56]. Pol ν is unusually proficient at accurate bypass of 5*S*-thymine glycol[56,57] and of bulky major groove DNA lesions such as *N*^{6}-A crosslinks[58]. Further distinctive properties of Pol ν include its strong strand displacement activity[56] and low fidelity[56,59]. Physiological roles have been suggested for Pol ν in TLS, past a short oligonucleotide crosslinked to DNA, representing an intermediate in interstrand crosslink (ICL) repair, as well as in HR[60,61]. However, *POLN*$^{-/-}$ chicken DT40 cells are not sensitive to the chemotherapeutic agents MMC, cisplatin[62] or camptothecin[63]. *POLN* is preferentially expressed in testis[55], and is expressed weakly in other tissues or in cultured mammalian cells; *POLN* is located near the telomere of human chromosome 4p, an area of frequent loss in some human cancers[64].

Pol ζ and REV1, master control proteins for TLS. In *S. cerevisiae*, most mutagenesis that is induced by DNA-damaging agents is dependent on the action of Pol ζ[65–67]. Biochemical assays show that yeast Pol ζ (comprising the catalytic subunit Rev3 and an accessory subunit, Rev7) can facilitate bypass of several types of DNA damage. The closest mammalian homologue of *S. cerevisiae* Rev3 is Rev3-like (REV3L; the catalytic subunit of Pol ζ), but with more than 3,000 amino acid residues, it is twice the size of the yeast protein[68]. Mammalian REV7 (also known as MAD2L2) is thought to be a binding partner of REV3L[69].

Knowledge of the *in vitro* biochemical activity of Pol ζ comes from studies carried out in *S. cerevisiae* because an active form of mammalian Pol ζ has not yet been purified. However, from work in knockout and knockdown cell lines, information has been gathered about the role of Pol ζ in mammalian and other higher eukaryotic cells. Experiments with cell lines in which REV3L function is disrupted indicate that Pol ζ is involved in the bypass of many types of clinically relevant DNA lesions, including cisplatin-GG[46], BPDE-GG[46], (6-4) photoproducts[30,46,70], AP sites[46] and thymine glycols[54]. The role of Pol ζ in the bypass of these lesions is proposed to be primarily as an extender, after another polymerase has inserted a nucleotide opposite a damaged site[71]. Mammalian Pol ζ may operate principally to fill single-stranded DNA gaps following DNA replication (FIG. 2), rather than operating directly at the replication fork[70].

Yeast *rev3* knockouts are viable, but *Rev3l*$^{-/-}$ mice die at embryonic days 9.5–15.5 (REFS 72–76) (TABLE 2), and *Rev3l*$^{-/-}$ blastocysts are not viable in cell culture. This role in

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Sliding clamp
A mobile platform for DNA replication and repair machinery. The eukaryotic sliding clamp PCNA binds to DNA polymerases and is crucial for the switching of polymerases during TLS and DNA repair.

X-family
X-family polymerases in mammalian cells are Pol β, Pol λ, Pol μ and TDT.

maintaining viability is unique among the DNA polymerases that are involved in TLS. *Rev3l*$^{-/-}$ cell lines can only proliferate if p53 function is compromised. This is a necessary but not a sufficient condition, as loss of p53 does not rescue the embryonic lethality of *Rev3l* deletion in mice[74–76]. The frequency of point mutations was decreased by up to 90% in mouse and human *Rev3l*-knockout and knockdown cells, and the knockout cells are sensitive to DNA-damaging agents[68,70,77–84]. *Rev3l*$^{-/-}$ cells also have a high frequency of spontaneous chromosome aberrations, particularly translocations[75,79,81–83].

The consequences of Pol ζ disruption for carcinogenesis have been explored in a viable mouse model in which one allele of *Rev3l* is stably deleted and the second allele contains loxP sites. The second allele can be deleted on expression of Cre recombinase from the mouse mammary tumour virus (*MMTV*) promoter, which is active in a substantial proportion of epithelial and haematopoietic cells[85]. On a *Trp53*$^{-/-}$ background, the mosaic mice in which *Rev3l* is conditionally deleted developed thymic lymphomas more rapidly than *Trp53*$^{-/-}$ mice with a wild-type copy of *Rev3l*. Importantly, most of the tumours arose from cells in which both copies of *Rev3l* were deleted, despite an initial growth disadvantage caused by *Rev3l* deletion[85]. Moreover, these tumours were frequently multiclonal, indicating that loss of REV3L confers a selective advantage for tumour formation. In *Trp53*$^{+/+}$ and *Trp53*$^{+/-}$ backgrounds, the induction of *Rev3l*$^{-/-}$ cells predisposed mosaic mice to the development of spontaneous mammary tumours. Most of these tumours had *Rev3l*$^{-/-}$ cells, were multifocal and were associated with mammary intraepithelial neoplasia[85]. Although there is no direct evidence that these tumours had increased chromosome aberrations, extensive data from REV3L-defective cell lines, as well as from another conditional *Rev3l*-deletion mouse model[75,79,81–83,86], strongly suggest the induction of genomic instability in *Rev3l*-deleted tumours. These observations indicate that if a preneoplastic cell can overcome checkpoint responses that are induced on deletion of *Rev3l*, an accelerated rate of gross genetic change can drive carcinogenesis (FIG. 1). It is interesting that the human *REV3L* gene is located at a fragile site (*FRA6F*) on chromosome 6q21 (REFS 82,87), which is a common region of deletion in human haematopoietic neoplasms[84].

In *S. cerevisiae*, Rev1 functions together with Pol ζ[88], and *rev1* mutants have dramatically reduced frequencies of mutagenesis induced by DNA-damaging agents[89]. Mouse and chicken cells with mutations causing REV1 deficiency have decreased damage-induced mutagenesis and are more sensitive to DNA-damaging agents, but they also have an increased incidence of chromosome aberrations[90–92]. Deletion of *Rev1* from mouse cells does not affect cellular viability[91,93–95]. Knock out of *REV1* induces cell cycle defects in chicken DT40 cells[90], but not in mouse embryonic fibroblasts[92].

REV1 is a DNA polymerase in only a very limited sense, as it cannot synthesize a DNA polymer, but can add a single C residue to a primer end in special cases. Human REV1 preferentially inserts C opposite a template G, U or an AP site[96]. Mammalian REV1 is involved in the bypass of UV radiation-induced damage[92] and crosslinks[97], but this role does not involve its deoxycytidine monophosphate (dCMP) transferase activity. The cellular sensitivity of *Rev1*-knockout cells to some DNA-damaging agents can be rescued with a catalytically inactive REV1 mutant protein[98]. The primary role of REV1 during TLS may be in DNA polymerase switching, as described below.

DNA polymerase choice and switching during TLS. The structure of Y-family DNA polymerases such as Pol η is specially adapted for DNA damage bypass (BOX 2). However, the capacious active site of Y-family DNA polymerases is not suitable for accurate DNA replication of undamaged templates (the base substitution error rates of Y-family enzymes are more than 10^{-3}, which is about 10–100-fold higher than those of replicative DNA polymerases[7]). Therefore, the access of Y-family polymerases to DNA is tightly controlled by post-translational modifications of interacting proteins. Each Y-family polymerase has a ubiquitin-binding domain that interacts with ubiquitylated forms of the sliding clamp proliferating cell nuclear antigen (PCNA)[99]. Blocks to DNA replication trigger monoubiquitylation of PCNA by E3 ubiquitin ligases, including RAD18 (REFS 100,101). This creates a binding site for Y-family polymerases and, because PCNA is in contact with DNA, this facilitates the interaction of these polymerases with DNA. There is also evidence that Pol η is ubiquitylated in response to DNA damage[102]. Although not so intensively studied, access of A-family, B-family and X-family polymerases to DNA is also likely to be tightly regulated.

Monoubiquitylated PCNA is not the only factor responsible for regulating TLS[103]. The extreme carboxyl terminus of REV1 interacts with Y-family polymerases: Pol η, Pol ι and Pol κ[104–107]. Through distinct ubiquitin-binding motifs, REV1 also interacts with ubiquitylated PCNA[98,108]. These interactions may allow REV1 to help regulate DNA polymerase switching and this may be the primary role of REV1 in the cell[99,109]. It will be interesting to learn whether the DNA damage-sensing clamp RAD9–HUS1–RAD1 (also known as the 9-1-1 complex) is also involved in TLS polymerase loading and switching.

The mechanism of selection of a particular TLS polymerase is under investigation. Perhaps the polymerase that catalyses the most thermodynamically favourable reaction at a particular lesion is most likely to mediate bypass of that lesion[110]. The abundance of a polymerase may also have a role[111]. In addition, in yeast, TLS polymerases may be used in lesion bypass when cells are in the G2 phase of the cell cycle, when replication is complete[112,113]. A gap can arise in DNA when an adjacent replication origin converges on a stalled replication fork (FIG. 2), or if the replication apparatus can reprime on the DNA template downstream of a DNA lesion[112]. There is evidence for post-replication gaps in mouse cells in which *Rev1* and *Rev3l* are deleted, suggesting a role for these polymerases in bypassing DNA lesions during G2 (REFS 70,92). The relative contribution of different TLS polymerases during replication and gap filling is actively under discussion[70,114,115], and many of the same proteins that enable the polymerase switch are also required for gap filling[116,117].

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Hydrolytic reactions
Decomposition of a chemical compound or a molecular bond by reaction with water.

Alkylating agent
An electrophilic compound that can covalently add an alkyl group to a DNA base, or to other biological macromolecules. These compounds act as both carcinogens (for example, methyl chloride) and as chemotherapeutic agents (for example, mechloroethamine).

Nonsense mutation
A change in the codon for an amino acid to a stop codon. Nonsense mutations cause protein truncation and often nonsense-mediated decay of the encoding mRNA.

BER

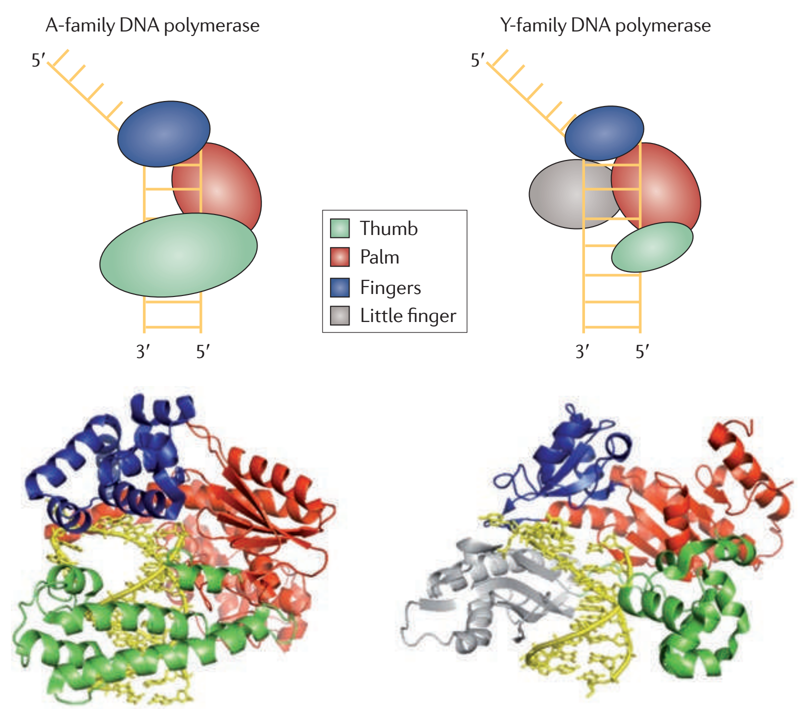
BER (BOX 1) is responsible for repairing many DNA adducts that are generated by ROS, hydrolytic reactions and alkylating agents. Pol β (also known as POLB) is the primary enzyme used for gap filling DNA synthesis during BER in the nucleus[118]. Pol β also has a 5′-deoxyribose phosphate (dRP) lyase activity that is normally required to remove the sugar–phosphate residue that is produced by the action of an AP endonuclease during BER. In mitochondria, the replicative polymerase Pol γ is responsible for gap filling in BER[119].

In some human tumours, sporadic sequence changes have been reported in *POLB* cDNA. In only a few cases have these changes been confirmed in genomic DNA[120]. These modifications usually encode single amino acid substitutions, and the expression of some of them can transform cultured cells, allowing growth on soft agar[121]. It remains to be seen whether *POLB* mutations will be found by cancer genome sequencing projects or whether such mutations contribute to a mutator phenotype. Mice that are *Polb*$^{+/-}$ develop normally, but have an increased incidence of lymphomas[122] (TABLE 2).

Other DNA polymerases have been proposed as backup enzymes for BER. A dRP lyase activity is present in Pol ι[123], Pol λ (also known as POLL)[124] and Pol θ[125]. It has been reported that BER is defective in cells in which *POLI* is knocked down[126], and *POLI* can compensate for the loss of *POLB* in the removal of uracil by BER in cell extracts[127]. However, the mouse 129/OLA strain carries a homozygous nonsense mutation in *Poli*, and has no obvious indications of a BER defect. Pol λ-deficient cells are sensitive to H_2O_2 (but not to alkylating agents)[124], so Pol λ might function as a backup for the repair of a subset of lesions in BER[128]. Knock out of *POLQ* (which encodes Pol θ) in the chicken DT40 B cell line causes increased sensitivity to H_2O_2, and it has been suggested that Pol θ might function in BER[62]. Cells from *Polq*$^{-/-}$ mice, however, do not have higher than normal sensitivity to H_2O_2 (REF. 129). Mammalian Pol θ may instead help cells tolerate DNA damage, perhaps by TLS of lesions such as thymine glycol or by participating in DNA end joining, as described below.

Box 2 | **DNA polymerase structure**

DNA polymerases have structural domains regularly likened to a 'right hand', in which DNA lies in the 'palm' and is surrounded by 'finger' and 'thumb' domains. Y-family DNA polymerases have substantially smaller finger and thumb domains than those of replicative DNA polymerases, as well as spacious active sites that enable them to bypass bulky DNA adducts[187]. The 'little-finger' domain that is uniquely encoded in Y-family enzymes not only helps the short finger and thumb domains to stably bind template DNA, but also influences fidelity and bypass activity[188]. The figure shows a structural comparison of the large fragment of *Thermus aquaticus* DNA polymerase I, a member of the A-family (protein data bank (PDB) ID: 3M8R[189]) and Pol η (a member of the Y-family; PDB ID: 3MR3 (REF. 22)).



NER

NER is a pathway responsible for the repair of many types of DNA damage caused by carcinogenic and chemotherapeutic agents, such as BPDE, UV radiation and cisplatin. In mammalian cell extracts *in vitro*, either Pol ε or Pol δ can synthesize DNA to fill the gap of about 27 nucleotides that is produced during NER. This reaction is dependent on PCNA and the single-stranded DNA binding protein replication protein A (RPA). Pol ε seems to be well-suited to filling gaps that can be sealed by DNA ligase I (LIG1)[130]. A second mode of NER gap filling uses a combination of Pol δ and Pol κ, with LIG3 to seal the nick. This mode may be used preferentially when deoxynucleotide concentrations are low, as is the case in non-cycling cells[131]. Inherited defects in components of the NER pathway that recognize and incise damaged DNA are the main cause of xeroderma pigmentosum[132]. However, defects in Pol ε, Pol δ or Pol κ have not been associated with this skin cancer-prone syndrome, or with other cancer predispositions. As mentioned above, mutations in Pol η give rise to the XP-V subtype of xeroderma pigmentosum and cells from afflicted individuals are proficient in NER but defective in TLS.

Repair of DNA breaks

Breaks in the DNA can be caused by many agents, including ionizing radiation, antibiotics such as bleomycin and chemotherapeutic agents such as etoposide. The DNA polymerases Pol β, Pol ζ, Pol η, Pol θ, Pol λ, Pol μ and terminal deoxynucleotidyltransferase (TDT) have been implicated in different aspects of DNA DSB repair by HR or NHEJ. Pol β is necessary for normal meiotic synapsis and functions in an early stage in the processing of DSBs that initiate HR during meiosis[133]. Pol η has been suggested to be involved in some aspects of DSB repair as *POLH*-deficient chicken DT40 cells have been reported to have a reduced frequency of the DSB-initiated HR-mediated gene conversion events that create immunoglobulin diversity[134]. Furthermore, Pol η

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Terminal deoxynucleotidyl-transferase
(TDT). A template-independent DNA synthesis activity that catalyses the addition of nucleotides to the 3′ terminus end of DNA. The TDT enzyme in human cells contributes to immune diversity by adding nucleotides of varying lengths between gene segments during V(D)J recombination.

V(D)J recombination
Assembles immunoglobulin and T cell receptor genes from different segments. The RAG1–RAG2 nuclease introduces DNA DSBs to produce segments that are joined by NHEJ.

Micronuclei
Pieces of DNA that reside outside of the nucleus, caused by chromosome breakage leading to accentric chromosome fragments that lack spindle attachments, or by chromosome mis-segregation during mitosis. Micronuclei are most easily detected in mature erythrocytes that lack nuclear DNA.

can mediate extension of a D-loop (an HR intermediate) and second strand capture *in vitro*, reactions that involve RAD51 and RAD52, both of which are major components of the HR pathway[135]. The multiple roles of Pol η in DNA repair and TLS might mean that several factors could contribute to the phenotype of cells from patients with XP-V, including a defect in TLS that causes replication fork collapse and consequently the formation of DSBs, a deficiency in DSB repair and a replacement of the TLS function of Pol η with an error-generating polymerase that increases point mutagenesis. There is also evidence that Pol ζ may be involved in some aspects of DSB repair[65,67,79–81,136] and ICL repair[137,138].

DNA end joining. NHEJ is a major mode of DSB repair in mammalian somatic cells and it is also a central process for generating diversity in the immune system through V(D)J recombination (BOX 3). The X-family enzymes Pol λ, Pol μ (also known as POLM) and TDT have all been implicated in immunoglobulin V(D)J gene recombination. These DNA polymerases contain a BRCT domain (TABLE 1) that binds to NHEJ factors such as KU80–KU70 and the XRCC4–LIG4 complex[139,140]. Pol μ and TDT are closely related and contain a protein domain called loop1 that is proposed to preclude binding to a template DNA strand. Both enzymes can add bases to the end of a DNA strand in the absence of a template[141,142]. TDT is the key enzyme for generating diversity during V(D)J recombination by adding random nucleotides at the junctions between the V, D and J elements[143]. Pol λ and Pol μ are also implicated in gap filling during V(D)J recombination[144]. There is evidence for increased sensitivity to ionizing radiation and reduced DSB repair in cells from *Polm*$^{-/-}$ mice[145,146] (TABLE 2). This suggests a function for Pol μ in NHEJ, in addition to a specialized function in V(D)J recombination. Hypersensitivity to ionizing radiation or bleomycin has not been detected in other studies of *Polm*$^{-/-}$ or *Poll*$^{-/-}$ mice[144,147].

Pol θ and defence against breaks caused by ionizing radiation. Evidence is emerging for the importance of Pol θ in the defence against DNA damage caused by ionizing radiation. Mouse bone marrow cell lines in which *Polq* is deleted are more sensitive than normal cells to ionizing radiation and bleomycin, but not to the ROS-generating agents H_2O_2 and paraquat[129]. This suggests that cells might rely on Pol θ for the repair of some DNA DSBs[129]. Knock down of *POLQ* by small interfering RNA (siRNA) in human tumour cell lines also increases the sensitivity to ionizing radiation and causes persistent phosphorylation of histone H2AX[148]. *Polq*$^{-/-}$ mice develop normally, but have increased frequencies of spontaneous and radiation-induced micronuclei in erythrocytes, which can arise from chromosome breakage[149] (TABLE 2). The viability of *Polq*$^{-/-}$ mice is severely compromised by an additional mutation in ataxia-telangiectasia mutated (*Atm*), suggesting that Pol θ is particularly important in the absence of the checkpoint and repair functions of ATM[149]. These results indicate that Pol θ is involved in a DSB repair process, or that it prevents the generation of DSBs at stalled DNA replication forks by mediating TLS opposite some lesions that are generated by ionizing radiation. A homologue of Pol θ in *Drosophila melanogaster*, mutagen-sensitive 308 (MUS308), is involved in a microhomology-mediated pathway of DNA end joining[150,151]. It is possible that Pol θ is involved in a similar pathway in mammalian cells, but it is not yet certain whether Pol θ and *D. melanogaster* MUS308 are functionally orthologous. Although MUS308 and mammalian Pol θ share an unusual helicase-polymerase domain organization, they differ substantially in their polymerase motifs[51].

DNA polymerase gene expression in cancer

Many studies have been conducted to determine changes in gene expression of DNA polymerases in human cancers. A pattern of expression in tumours has been difficult to discern. For example, the expression levels of the human *REV3L*, *POLK* and *POLI* genes have been explored in various types of cancer, but no consistent picture has emerged[69,152–157]. *Poli* was proposed as a candidate for the pulmonary adenoma resistance locus, designated *Par2* in mice, but further analysis excluded *Poli* and identified *4930503L19Rik* (also known as *Las2*), which is adjacent to *Poli*, as the tumour susceptibility gene that is mutated in *Par2* mice[158].

Some polymerases do show a trend in gene expression in tumours. The expression of *POLH* is reduced in lung and stomach cancers in comparison to paired normal tissues[153]. In non-small-cell lung cancer, higher *POLH* expression correlated with poorer outcome[159]. Increased expression of *POLB* has been reported in gastric[155,160], uterine[155], prostate[155], ovarian[155] and thyroid[161] carcinomas, and forced overexpression of *POLB* in cells can interfere with normal replication, causing mutagenesis[162,163]. Transgenic mice overexpressing *Polb* have several pathologies, including increased development of osteosarcoma[161]. This suggests that proper regulation of *POLB* expression is important for preventing carcinogenesis.

In addition, *POLQ* expression may be higher in tumour cells than in normal cells. *POLQ* expression is higher in samples of colorectal cancer than in matched surrounding normal tissues, independently of the proliferation state of the tumours[164,165]. When the expression profiles for all of the human nuclear DNA polymerase genes were

Box 3 | **Somatic hypermutation of antibody genes**

Following V(D)J recombination, antibody variable genes undergo a process of mutagenesis in B cells, initiated when activation-induced cytidine deaminase (AID; also known as AICDA) mediates cytosine deamination. The uracil-DNA glycosylase removes the resulting uracil to form an apurinic or apyrimidinic (AP) site and this is followed by repair of the AP site by base excision repair (BER) and an error-generating DNA polymerase for DNA repair synthesis. Alternatively, translesion DNA synthesis (TLS) might be used to bypass the AP site. This process of somatic hypermutation involves DNA polymerase η (Pol η)[190,191], as well as REV1 and Rev3-like (REV3L)[86,90–94]. The AP site bypass activity of Pol θ has encouraged the suggestion that it might be involved in somatic hypermutation[63], but it seems to have only a minor role in this process in mammalian cells[192]. In the chicken DT40 cell line, both immunoglobulin gene diversification by recombination and somatic hypermutation are reported to depend on Pol η, Pol θ, Pol ν and REV1 (REFS 63,193).

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examined in patients with previously untreated primary breast cancers, *POLQ* was the only one significantly higher in breast cancer tumour samples compared with normal breast tissues, and higher expression correlated with poor clinical outcome[154,166]. Ectopic overexpression of *POLQ* in human cell lines impairs DNA replication fork progression and causes chromosome damage[154]. Error-generating, low processivity DNA polymerases are normally expressed at low levels in cells, and overexpression might lead to a more frequent imposition of these enzymes at replication forks (temporarily replacing higher processivity, error-free replicative polymerases), leading to mutagenesis and replication fork stalling.

DNA polymerases as therapeutic targets

Drugs that inhibit DNA replication, such as folate inhibitors, pyrimidine analogues and hydroxyurea, are in standard use for chemotherapy, but some of these therapies are highly toxic as they do not selectively target cancer cells. This is promoting searches for other drugs to target DNA polymerases for chemotherapeutic benefit[203]. For example, derivatives of the antibiotic dehydroaltenusin inhibit Pol α but not Pol β, Pol δ, Pol ε or Pol γ[167,168]. The drug suppresses HeLa cell xenograft tumour growth in mice[169]. Eicosapentaenoic acid radiosensitizes cells, and is also an inhibitor of Pol β, Pol δ and Pol ε[170].

Pol β, the principal DNA polymerase for gap filling DNA synthesis in BER, is being explored as a therapeutic target. Several small-molecule inhibitors of Pol β have been found[171], including some that can potentiate the toxic effects of the chemotherapeutic agents bleomycin[172] and temozolomide[173,174]. Reduction of *POLB* expression also increases sensitivity to the chemotherapeutic agent oxaliplatin[175]. The mechanism for some of these effects is unclear, as the specific function of BER in response to the DNA damage caused by oxaliplatin or bleomycin is not yet known.

TLS DNA polymerases are also possible targets for enhancing DNA-damaging therapies, and inhibitors of some of these are beginning to emerge[176,177]. Studies on DNA polymerase inhibitors are intriguing but are still in their early stages. As strategies for small-molecule screens are developed[177], they will have to be coupled with screens for the most pharmacologically effective compounds. For example, the most potent DNA polymerase inhibitors might not be transported efficiently into cells[167]. As drugs are found that inhibit specific polymerases and that are preferentially toxic to tumour cells, it will be important to determine whether the inhibition of DNA polymerase activity is mechanistically responsible for mediating any antitumour effects.

Additionally, suppression of *Rev1* and *Rev3l* expression is being explored in mouse cancer models. A *Rev1*-specific ribozyme was delivered to the lungs of BPDE-treated mice, and it decreased BPDE-induced lung tumours by 50%[178], presumably by decreasing point mutagenesis. Recent work also demonstrates that lymphomas with decreased expression of *Rev1* or *Rev3l* are more sensitive to cisplatin and cyclophosphamide in a xenograft model, and tumours in which *Rev1* expression is knocked down do not develop resistance to cyclophosphamide[179]. *Rev3l* downregulation increased the sensitivity to cisplatin in a xenograft model of non-small-cell lung cancer[180]. Vigilance will be needed in the development of drugs targeting REV1 or REV3L, because the loss of *Rev1* or *Rev3l* in cells and the conditional deletion of *Rev3l* in mice results in chromosome instability, as described above. Even though *Rev3l* is essential for base change mutagenesis, conditional loss of *Rev3l* in mice accelerated spontaneous tumour formation[85]. Furthermore, mice carrying disruptions of the BRCT domain of REV1 in *Xpc*$^{-/-}$ mice have accelerated UV radiation-induced skin carcinogenesis[95]. This disruption of the BRCT domain of REV1 does not confer a marked defect in UV radiation-induced mutagenesis in mouse skin, but it substantially enhances the toxicity of UV radiation, resulting in greatly increasing inflammation that contributes to accelerated formation of skin cancer. Consequently, REV3L and REV1 may be problematic targets for cancer therapeutics, because their suppression may lead to additional genomic instability and inflammatory responses in surviving cells.

Ribozyme
A catalytic enzyme made entirely of RNA. Some ribozymes are nucleases and can include base-pairing regions that enable specific binding and cleavage of a target RNA molecule.

Conclusions and perspectives

The 15 DNA polymerases encoded by mammalian genomes are specialized for different functions, including DNA replication, DNA repair, recombination and TLS. Genetic and biochemical experiments have shown that different DNA polymerases are suited for TLS of particular types of DNA damage. For some DNA polymerases that can carry out TLS, firm identification of the most biologically relevant substrates remains a challenge. Newly obtained structures of DNA polymerases in complexes with lesions show how the architecture of an active site can accommodate relatively bulky adducts, and different enzymes seem to solve this problem in different ways[21,22,181,182]. Nevertheless, evidence is accumulating that even those DNA polymerases that are especially adept at TLS have additional and unexpected roles in human cells.

It seems that some DNA polymerases are more important in particular tissues than others, and more analysis is needed on tissue-specific and developmental expression patterns. The range of available DNA polymerases in stem cells is worthy of attention, as it is imperative that these cells maintain stable genomes. One specialized DNA polymerase, Pol ζ, has now been shown to function effectively as a suppressor of spontaneous tumorigenesis in mice[85]. However, other than *POLH* in XP-V cells, no definitive evidence has yet emerged in human cancer cells for frequent homozygous loss or inactivating mutation of any gene encoding a DNA polymerase. As whole-genome cancer analyses continue it will be interesting to monitor the loss, rearrangement or mutation of genes encoding specialized DNA polymerases. Because tumours are genetically heterogeneous, sequencing of individual cell genomes might be required to achieve this. New evidence indicates that *POLQ* is the DNA polymerase gene with the greatest difference in expression between breast tumours and non-tumour cells[183]. Further analysis will be needed to determine whether this is also the case for other types of cancer, and whether DNA polymerase expression is regulated or sometimes silenced by DNA methylation,

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microRNAs or other mechanisms. Investigation of DNA polymerase protein levels in tumours is also needed, as nearly all work in this area has only explored variations in gene expression. Numerous single nucleotide polymorphisms exist in DNA polymerases, reflecting the differences between individuals, and it is of interest to determine whether any of them have a meaningful functional effect on activity and predisposition to developing cancer.

Because many non-surgical cancer therapies work by damaging DNA, consideration of DNA repair capacity is an ongoing concern in improving responses to treatments. In a few cases, knowledge of the DNA repair status of a tumour allows tailoring of therapy. For example, patients with glioblastoma benefit from treatment with temozolomide when the promoter for O^6-methylguanine-DNA methyltransferase (*MGMT*) is methylated in their tumours[184].

The more that is known about the associations between the expression of genes encoding DNA repair proteins and others involved in maintaining genomic stability and prognosis, the more progress can be made in this area. This requires highly specific reagents and careful quality control[185]. Like other gene products involved in processing DNA damage, DNA polymerases are potential targets. Specific inhibitors of a DNA polymerase that is important for tolerating particular types of damage could be useful as an adjuvant for DNA-damaging radiation or chemotherapy. As more is known about the active sites of specialized DNA polymerases, more specific small-molecule inhibitors may be selected or designed. Many surveys of small-molecule libraries for DNA polymerase inhibitors are underway, but no studies have yet screened all 15 mammalian polymerases simultaneously to determine specificity. Alternatively, the forced overexpression of a DNA polymerase in a tumour might also cause desired toxic effects by interfering with replication. Increasing the expression of a DNA polymerase to intentionally cause an increase in mutagenesis and the potential loss of essential genes is another suggested strategy[16]. Toxicity is apparent when Pol β, Pol κ or Pol θ are overexpressed at levels much higher than the variations in expression seen in human tumour samples[162]; cells that are engineered to overexpress Pol η do not show obvious toxicity[186].

It is possible that further enzymes with DNA polymerase activity may be discovered in mammalian genomes, and it seems certain that additional accessory proteins will be found that modulate the activity of the known DNA polymerases. Improved knowledge of these factors may also have implications for carcinogenesis and for designing anticancer strategies.

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Competing interests statement
The authors declare no competing financial interests.

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