COMPREHENSIVE INVITED REVIEW

Biological and Therapeutic Relevance of Nonreplicative DNA Polymerases to Cancer

Jason L. Parsons,¹ Nils H. Nicolay,^{1,2} and Ricky A. Sharma¹

Abstract

Apart from surgical approaches, the treatment of cancer remains largely underpinned by radiotherapy and pharmacological agents that cause damage to cellular DNA, which ultimately causes cancer cell death. DNA polymerases, which are involved in the repair of cellular DNA damage, are therefore potential targets for inhibitors for improving the efficacy of cancer therapy. They can be divided, according to their main function, into two groups, namely replicative and nonreplicative enzymes. At least 15 different DNA polymerases, including their homologs, have been discovered to date, which vary considerably in processivity and fidelity. Many of the nonreplicative (specialized) DNA polymerases replicate DNA in an error-prone fashion, and they have been shown to participate in multiple DNA damage repair and tolerance pathways, which are often aberrant in cancer cells. Alterations in DNA repair pathways involving DNA polymerases have been linked with cancer survival and with treatment response to radiotherapy or to classes of cytotoxic drugs routinely used for cancer treatment, particularly cisplatin, oxaliplatin, etoposide, and bleomycin. Indeed, there are extensive pre-clinical data to suggest that DNA polymerase inhibition may prove to be a useful approach for increasing the effectiveness of therapies in patients with cancer. Furthermore, specialized DNA polymerases warrant examination of their potential use as clinical biomarkers to select for particular cancer therapies, to individualize treatment for patients. *Antioxid. Redox Signal.* 18, 851–873.

I. Introduction	852
II. Biochemistry and Molecular Biology of Nonreplicative DNA Polymerases	853
A. X family polymerases	853
1. DNA polymerase beta	853
2. DNA polymerase lambda	854
3. DNA polymerase mu	856
B. Y family polymerases	856
1. TLS pathways	856
2. DNA polymerase eta	857
3. DNA polymerase iota	858
4. DNA polymerase kappa	858
C. A family polymerases	859
1. DNA polymerase theta	859
D. B family polymerases	859
1. DNA polymerase zeta	859
III. Biological Relevance of Nonreplicative DNA Polymerases to Carcinogenesis	859
A. X family polymerases	859
B. Y family polymerases	860
C. A and B family polymerases	860

Reviewing Editors: Emma Bolderson, Ida Casorelli, Sylvie Doublié, Ahmad R. Heydari, David Li, Sudha Sharma, Michael P. Stone, and Conchita Vens

¹Cancer Research UK-Medical Research Council, Oncology Department, Gray Institute for Radiation Oncology and Biology, University of Oxford, Oxford, United Kingdom.

²Department of Radiation Oncology, Heidelberg University Hospital, Heidelberg, Germany.

IV. The Potential Role of Nonreplicative DNA Polymerases in Cancer Treatment	861
A. Involvement of DNA polymerases in cellular responses to chemotherapy	861
B. The role of nonreplicative polymerases in cellular responses to radiotherapy	862
C. Targeting specialized DNA polymerases to improve cancer treatment	863
V. Conclusions	864

I. Introduction

CELLS ARE CONTINUOUSLY EXPOSED to DNA damage from exogenous (e.g., ionizing radiation) and endogenous (e.g., cellular reactive oxygen species) sources, and it is estimated that each individual cell incurs a DNA-damaging event about 50,000 times per day (149) (Table 1). To cope with these potentially lethal lesions, cells employ an elaborate system of different DNA repair pathways to prevent genomic instability and carcinogenesis, and ultimately maintain the integrity of their genetic information. Cellular repair pathways are quite diverse and enable cells to directly reverse damage, to repair single-strand breaks (SSBs) and double-strand breaks (DSBs), or to excise base lesions or base mismatches (102).

The majority of DNA lesions are amenable to repair by excision of the damaged bases, or nucleotide sequences, and subsequent gap filling against the undamaged template strand, which is carried out by a DNA polymerase (pol). Pols can be divided according to their main function into two groups, namely replicative and nonreplicative enzymes. The doubling of genomic information during the S phase of the cell cycle is carried out by an elaborate replication machinery involving pols that can synthesize the nascent DNA strands with high processivity and fidelity, and enable cells to efficiently replicate their ~3 billion base pairs of genomic and 17,000 base pairs of mitochondrial DNA (33, 114, 115). Pols α , δ , and ϵ have been identified as the major replicative polymerases in eukaryotic cells, and pols δ and ϵ possess a 3'-5' exonucleolytic activity that allows the immediate removal of mispaired nucleotides during strand elongation, thus

increasing replication fidelity considerably (135, 136) (Fig. 1). Most other pols that have been discovered so far do not assist replication, but carry out a variety of other cellular functions, often associated with the repair of DNA damage. To date, at least 15 different DNA polymerases, and their homologs, have been discovered and characterized, which are grouped into classes according to sequence homology (Table 2). The A family contains pols γ , θ , and ν ; the B family comprises pols α , δ , ϵ , and ζ ; the X family includes pols β , λ and μ , and the Y polymerase family incorporates pols η , ι , and κ . Additionally, two DNA polymerase homologs have been described, the deoxycytidyl-transferase Rev1, belonging to the Y family, and the terminal deoxyribonucleotidyltransferase (TdT), as a part of the X family (40). These pols vary considerably in processivity and fidelity, and many nonreplicative pols lack proofreading activity, therefore replicating DNA in an error-prone fashion.

Apart from their involvement in DNA replication and repair, certain pols have been linked to the cellular ability to perform strand synthesis past unrepaired DNA lesion sites, thereby temporarily tolerating DNA damage (81, 256) (Table 2). Since cells cannot repair all lesions immediately, some forms of DNA damage may persist into the S phase of the cell cycle, in which they have the potential to inhibit replicative pols and block the replication machinery, resulting in stalling, and ultimately the collapse of replication forks. A disruption of replication threatens cell viability, and therefore cells have developed ways to prevent replication fork stalling (10, 173). Human cells employ a set of specialized pols that are able to perform DNA replication past damage sites, a mechanism

TABLE 1. TYPES OF SPONTANEOUS CELLULAR DNA DAMAGE AND THEIR RELATIVE FORMATION IN HUMAN CELLS

Type of damage	Cellular events per day
Oxidation	
8-oxoG	500–1000
Ring-saturated pyrimidines	1000
Lipid peroxidation products	1000
	~3000
Methylation	
7-MeG	3000–4000
3-MeA	600
O ⁶ -MeG	10–50
	~4000
Hydrolysis	
Depurination	9000–10,000
Depyrimidation	300–500
C-deamination	50–500
5-MeC deamination	5–50
	~20,000
Polymerase errors	30,000–1,000, 000
Total	> 50,000

Adapted from (208).

FIG. 1. **Determinants of replication fidelity.** The major determinants of the fidelity of DNA replication by eukaryotic polymerases are shown in boxes. Polymerase-inherent replication fidelity and the enzymatic 3'-5' exonuclease activity are the main determinants of replication fidelity. Additional exogenous determinants of replication fidelity are the cellular DNA damage bypass ability and the efficient repair of newly created mismatches. A further factor is the composition of the cellular deoxynucleotide pool.

TABLE 2. LIST OF HUMAN DNA POLYMERASES AND THEIR INVOLVEMENT IN SPECIFIC DNA REPAIR PATHWAYS

<i>Polymerase family</i>	<i>Enzyme</i>	<i>Gene</i>	<i>Chromosomal location</i>	<i>Repair pathway</i>
A family	Pol γ	<i>POLG</i>	15q25	BER, TLS
	Pol θ	<i>POLQ</i>	3q13.33	
	Pol ν	<i>POLN</i>	4p16.3	
B family	Pol α	<i>POLA1</i>	Xp22.1-p21.3	TLS, DSBR
	Pol δ	<i>POLD1</i>	19q13.3	
	Pol ϵ	<i>POLE</i>	12q24.3	
	Pol ζ	<i>REV3L</i>	6q21	
	Pol β	<i>POLB</i>	8p11.2	
X family	Pol λ	<i>POLL</i>	10q23	BER, DSBR
	Pol μ	<i>POLM</i>	7p13	DSBR, TLS
	TdT (homolog)	<i>DNTT</i>	10q23-q24	
Y family	Pol η	<i>POLH</i>	6p21.1	DSBR, TLS
	Pol ι	<i>POLI</i>	18q21.1	BER, TLS
	Pol κ	<i>POLK</i>	5q13	TLS, NER
	Rev1 (homolog)	<i>REV1</i>	2q11.1-q11.2	TLS

BER, base excision repair; DSBR, double-strand break repair; NER, nucleotide excision repair; Pol, DNA polymerase; SSBR, single-strand break repair; TLS, translesion synthesis.

called translesion synthesis (TLS) (91, 92, 95, 256). The Y family pols η , ι , κ , and Rev1 have well-characterized TLS activity, but pols of other families, such as pols ζ and β , have also been shown to be able to perform lesion bypass. The TLS pol η is to date the only pol that is causally linked to the development of cancer (35), but deregulations of other pols have been reported in various tumor tissues and have been linked to mutagenesis and drug resistance (33, 134, 135, 140).

In this review article, the links between nonreplicative (specialized) pols and cancer will be discussed in detail. We will focus on the unique properties of nonreplicative pols and discuss the involvement of these enzymes in DNA repair and TLS. The benefits and possibilities of targeting nonreplicative pols will also be reviewed, with a view to improving current treatments for cancer.

II. Biochemistry and Molecular Biology of Nonreplicative DNA Polymerases

A. X family polymerases

The X family of pols contains pols β , λ , μ , and TdT, which share structural similarities and limited sequence homology (264). X family pols have been found across species, and vertebrates express all four pols (242). All X family pols contain an 8-kDa domain, although it only has functional relevance for pols β and λ , where it exhibits 5'-deoxyribose phosphate (dRP) lyase activity. The polymerase domain of all X family pols contains thumb, palm, and finger domains that are required for nucleotide insertions, as has been reported for most pols characterized to date (70, 232, 264). X family pols have been found to participate in the repair of base damage, SSBs, and DSBs, as well as variable, diversity and joining gene segments (VDJ) recombination during antibody diversification (177). Pol β was the first X family pol identified, and it has been extensively studied both mechanistically and in the context of human cancers (231).

1. DNA polymerase beta. Pol β is present in all vertebrates and exhibits a high sequence homology between all mammalian species (20). In yeast, a pol β homologous gene was found encoding a protein with pol β -like enzymatic

function, and was named pol IV (207). In humans, pol β is encoded by the *POLB* gene on chromosome 8 comprising 33 kb and 14 exons, and the protein has a calculated molecular weight of 39 kDa (163). Several different tightly folded protein domains within the pol β protein have been identified that have been attributed to its different activities, namely DNA strand and nucleotide binding, nucleotidyltransferase, and dRP lyase activities (19). The N-terminal region of pol β , with a molecular weight of 8 kDa, comprises the lyase domain, which by itself has a high binding affinity to single-stranded DNA, but only weak affinity to DNA double strands (205). The 31-kDa C-terminal region of pol β holds the pol activity and can be divided into three subdomains: a catalytic, a DNA double-strand binding domain, and a domain binding the nascent base pairs (18). As for most pols, pol β requires divalent cations, Mg^{2+} or Mn^{2+} , as cofactors for the pol reaction (20). During the insertion of new nucleotides, the pol interacts with the DNA backbone, therefore probing the newly formed base pair for adequate Watson-Crick base pairing and proper formation of hydrogen bonds in the minor groove of DNA. This interaction therefore helps to increase the fidelity of the enzymatic activity of pol β (20, 197).

On a functional level, pol β has been shown to participate in different DNA repair and damage tolerance pathways, of which its involvement in base-excision repair (BER) is the most well understood. BER enables cells to remove damaged DNA bases and to repair DNA SSBs that are created both endogenously and exogenously by DNA-damaging therapies, such as ionizing radiation or various anticancer drugs (66, 78, 221, 257). The initial step of the BER pathway (Fig. 2) is the removal of a damaged base that is recognized and cut from the sugar-phosphate backbone by a damage-specific DNA glycosylase (82, 168). The removal of the damaged base results in the formation of an abasic site (AP site) that is recognized and cleaved by AP endonuclease-1 (APE1), creating an SSB with 5'-dRP and 3'-hydroxyl ends. In the short-patch BER pathway (Fig. 2, left scheme), this 5'-dRP residue is further processed by the dRP lyase activity of pol β , and a 5'-phosphate residue is formed that can be used by the pol activity of pol β , which subsequently adds one nucleotide to the

FIG. 2. Schematic depiction of the short- and long-patch base excision repair (BER) pathways. The excision of a damaged base from a DNA strand is initiated by a damage-specific glycosylase (DSG), and then the remaining abasic site (AP) is incised by AP endonuclease 1 (APE1), creating a 5'-deoxyribose phosphate (dRP) group. This group is removed by pol β , which subsequently fills the one nucleotide gap created by APE1, and the strand is ligated by DNA ligase 3-x-ray repair cross-complementing protein-1 (XRCC1) complex (short-patch BER). Alternatively, following pol β one-nucleotide addition, strand extension from that nucleotide is performed by pol δ or ϵ , thereby creating an oligonucleotide overhang. The overhang is then excised by flap endonuclease 1 (FEN1), and the remaining nick is sealed by DNA ligase 1 (long-patch BER).

3'-OH group of the SSB. The DNA ends are then sealed by the DNA ligase 3 α -X-ray repair cross-complementing protein 1 (XRCC1) heterodimer (67, 195, 226, 258). As the key pol during short-patch BER, the lyase and pol activities of pol β are both absolutely required, and these enzymatic components are tightly coordinated. Indeed, pol β -deficient cell extracts supplemented with either dRP lyase or pol-deficient pol β mutants could not support short-patch BER (7).

If the 5'-dRP residue is oxidized or otherwise modified, BER requires additional proteins to repair the lesion, since the pol β lyase activity is not able to perform its function on modified 5'-residues (131, 164). During this so-called long-patch BER pathway (Fig. 2, right scheme), the gap-filling step is initiated by pol β , which adds one nucleotide, and pols δ and ϵ then extend from the inserted nucleotide creating a single-stranded flap overhang (13). This overhang is excised by flap endonuclease 1 (FEN1) and the remaining nick is sealed by DNA ligase 1 (long-patch BER) (196, 225). Although long-patch BER pathway is able to replace two to five nucleotides, it likely only contributes to a minority of the total cellular BER capacity (221).

During BER, gap filling by pol β is carried out with relatively high fidelity compared to other nonreplicative pols. The most common errors of pol β are single-nucleotide deletions, and the overall calculated error frequency is about $5\text{--}10 \times 10^{-4}$ (23, 215). Despite the fact that pol β itself does not have any proofreading activity, the hydrophobic hinge region positions the DNA within the active site of pol β , and has been shown to be critical for the selection of the correct nucleotide (159, 263). There are also suggestions that APE1, as well as WRN and TREX1 or TREX2, may function as an external proofreading enzyme during BER (72, 222, 233).

Apart from its involvement in BER, the activity of pol β has been linked to TLS. Although the majority of TLS is carried

out by the low-fidelity pols of the Y family, X family pols, such as pol β , can also replicate past different DNA lesions, particularly AP sites (29, 73). AP sites cannot be bypassed by replicative pols and are therefore usually repaired by BER. However, a minority of these lesions persist to the S phase of the cell cycle, and therefore have the potential to collapse replication forks. Pol β can skip over AP sites by incorporating a nucleotide that is complementary to a template base adjacent to the lesion site, therefore leading to base deletions and substitutions (29). Pol β has also been shown to follow the A-rule when the AP site is opposite a single nucleotide gap (17, 269).

Other DNA lesions may also be amenable to TLS by pol β . Indeed, pol β can incorporate deoxyribonucleotide triphosphates (dNTPs) opposite thymine glycol lesions, and UV-induced cyclobutane dimers and pyrimidine-pyrimidone (6,4) photoproducts (22, 24, 220). Additionally, 1,2-GG and 1,2-AG DNA adducts created by platinum-containing anticancer drugs can be bypassed by pol β in a mutagenic fashion (15). The efficiency of pol β to replicate past these lesions is considerably lower compared to other TLS pols, and the relevance of pol β as a TLS polymerase *in vivo* remains poorly understood. This is particularly evident considering that pol β wild-type and pol β -deficient cells are equally sensitive to UV irradiation and cisplatin treatment (53, 186, 227). *In vitro* experiments have shown that fork-like DNA flaps or other higher-order DNA structures can increase the TLS capacity of pol β , but the regulation of this activity *in vivo* is still largely unclear (63).

2. DNA polymerase lambda. The X family enzyme, pol λ , is encoded by the *POLL* gene on chromosome 10, and the pol has a molecular weight of 68 kDa (264). It shares 54% sequence homology with pol β and is 34% identical, and pol λ is highly conserved throughout all mammalian species (11). Pol λ

comprises domains for DNA binding, nucleotide selection, and 5'-dRP lyase activity, suggesting an involvement in BER (85, 109). While pol λ has been shown to be able to remove 5'-dRP groups from strand-break ends, cell-based studies did not reveal an increased sensitivity of pol λ -deficient cells to alkylating agents (240). Pol λ is thought to act as a backup enzyme in the repair of base damage, since cells depleted of both pol β and λ are incapable of carrying out successful BER (31). Pol λ , like pol β , can carry out gap filling during short-patch and long-patch BER, and while the fidelity of the enzyme is comparable during single-nucleotide insertions, this fidelity decreases significantly with increasing length of the gap. It has therefore been suggested that pol λ may be more suited to short-patch BER (36). Indeed, *in vitro* assays have confirmed a potential role for pol λ in short-patch BER, and the enzyme has been shown to be able to interact with upstream BER glycosylases (31). Interestingly, pol λ exhibits a BRCA-1 C-terminal (BRCT) domain at its N-terminus, which is known to mediate interactions with other proteins required for the recognition and binding to DNA ends, possibly sug-

gesting a role beyond BER. Indeed, the BRCT domain of pol λ has been shown to enable the protein to interact with non-homologous end-joining (NHEJ) proteins (Fig. 3) (86, 178).

Further evidence for the involvement of the enzyme in NHEJ comes from observations that pol λ was able to perform error-free gap filling that was dependent on XRCC4 and DNA ligase 4, as well as other proteins that are exclusively involved in NHEJ (143, 154). Furthermore, pol λ -deficient mouse embryonic fibroblasts were found to be sensitive to camptothecin and etoposide, which create SSBs and DSBs (48, 250). Recent data also suggest the involvement of pol λ in homologous recombination (HR) (Fig. 4), and a polymorphic splice variant

FIG. 3. Schematic depiction of the nonhomologous end-joining (NHEJ) pathway. NHEJ is initiated by the Ku70-Ku80 complex (Ku) that recognizes DNA double-strand breaks (DSBs) and recruits DNA-PK. If the DNA ends cannot be ligated directly due to complex DNA damage, end resection is carried out by the Artemis endonuclease. The removed strand segments are resynthesized by a DNA polymerase (pol), before ligation is carried out by the DNA ligase 4-XRCC4 complex.

FIG. 4. Schematic overview of the homologous recombination (HR) pathway. The HR pathway requires a double-stranded homolog to serve as a template for the damaged strand, and therefore only occurs during the late S and G2 phases of the cell cycle. The 3'-end of the damaged strand, following end-processing by the MRE11-Rad50-NBS1 complex, invades and pairs with the homologous sequence of the sister chromatid. Strand invasion leads to the formation of a D-loop structure, from which the lesion-containing strand is resynthesized by a DNA polymerase (pol) and finally a resolvase restores the two sister chromatids.

was found to affect cellular HR activity (47). As with other X family pols, pol λ lacks a 3'-5' exonuclease proofreading ability, and *in vitro* experiments have shown a low fidelity of pol λ that is comparable to that of pol β (37). Furthermore, it has been shown that a loop structure upstream of the pol domain regulates the fidelity of pol λ by controlling the movements of the template strand induced by the incoming dNTPs, as the enzyme switches from an inactive to an active conformation (21). Phosphorylation of threonine residues on pol λ seems to stabilize the enzyme during cell cycle progression and prevent proteasomal degradation (259).

3. DNA polymerase μ . Pol μ is encoded by the *POLM* gene on chromosome 7, and pol μ is a 55-kDa single-subunit enzyme (264). Pol μ is primarily found in lymphoid tissue, and mice lacking the pol show defects in B-cell differentiation and impaired rearrangements of IgG light chains (25, 26). Steady-state kinetic analyses have identified pol μ as a lower-fidelity pol with a misinsertion frequency of 10^{-2} – 10^{-4} (211), and one-base deletion frameshifts were reported to be the major polymerization error caused by pol μ (271). The pol μ 8-kDa subunit lacks the 5'-dRP lyase activity reported in pols β and λ , but could interact with the downstream DNA required for the enzyme's end-bridging activity (264). Interestingly, pol μ has been reported to exhibit RNA polymerase activity and could insert ribonucleotides instead of deoxynucleotides on oligonucleotide-based constructs with a similar fidelity (184, 211, 212).

Pol μ has been demonstrated to exhibit TLS activity *in vitro*, and the pol was found to insert nucleotides opposite various DNA lesion sites with a relatively high efficiency, including AP sites, 8-oxoguanine and 1,N(6)-ethenoadenine lesions, thymidine dimers, and adducts caused by platinum-based chemotherapeutic drugs (62, 101, 270). As described for pol λ , pol μ exhibits an N-terminal BRCT domain that can interact with XRCC4 and DNA ligase 4, which suggests a role in NHEJ during VDJ recombination (64, 153). However, further structural comparisons of the BRCT domains from both pol λ and pol μ are required to provide an insight into their functions. While a lack of pol μ leads to a delay in DSB repair kinetics and an increased amount of unrepaired damage (54), it has yet to be shown if pol μ has a general role in DNA DSB repair outside of VDJ rearrangements. Pol μ has the ability to form foci upon radiation-induced DNA damage that have been described to co-localize with γ H2AX foci supporting a possible recruitment of the pol to sites of DNA DSBs (157). However, conflicting data have been reported examining expression patterns of pol μ in the context of radiation-induced DNA damage. Upregulation of pol μ on the protein level has been reported after γ -irradiation, suggesting that pol μ may be involved in the repair of ionizing radiation-induced damage (157). In contrast, *POLM* mRNA was reportedly downregulated after cellular treatment with various DNA-damaging agents, including alkylating agents, UV light, or γ -irradiation, and it has been suggested that the expression of the pol is regulated on a post-transcriptional level after DNA damage that may include post-transcriptional modifications or stabilization on chromatin containing DNA damage (11).

B. Y family polymerases

The Y family of pols comprises the enzymes, pols η , κ , ι , and Rev1, and all family members have been found to exhibit TLS

activity. Generally speaking, the Y family pols are highly conserved throughout species and share structural homology among each other, but also with TLS pols of other pol families (95, 256).

1. TLS pathways. Two models have been proposed for the mechanism of TLS, the polymerase-switch model (144, 165) and the gap-filling model (167, 256). In the polymerase-switch model (Fig. 5, left scheme), TLS activity is required at replication forks when the replication machinery is blocked, and the lesion bypass is crucial to prevent fork collapse and to continue DNA synthesis. During this process, the E3 ubiquitin ligase Rad18 and the E2-conjugating enzyme Rad6 are recruited to the DNA lesion site (83, 255). The Rad18-Rad6 complex then monoubiquitinates the DNA-sliding clamp, proliferating cell nuclear antigen (PCNA), which allows recruitment of TLS pols (9, 103). This is achieved through the binding of monoubiquitinated PCNA to the ubiquitin-binding zinc finger or ubiquitin-binding motif present within most TLS pols (27), although there is evidence that TLS pols can get recruited to sites of DNA damage independent of the presence of Rad18 or PCNA ubiquitination (3, 216). Interestingly, polyubiquitination of PCNA, which also occurs on the same lysine residue (K164) as monoubiquitination, by the MMS2-UBC13 complex and RAD5 has differential effects by recruiting alternative factors required for postreplicative repair (32, 100). Nevertheless, the relevance of PCNA monoubiquitination for the TLS pathway has not yet been completely unraveled, although it has been suggested that the modification may contribute to strengthening the bond between the pol and PCNA, or may facilitate the switch at a stalled replication fork between the blocked replicative pol and the incoming TLS enzyme (55). This pol switch thereby allows the TLS pol to access the DNA lesion site and carry out translesional bypass (144).

It has been suggested that depending on the structure and extent of the DNA damage, TLS may require sequential activity of more than one pol to extend from the lesion site. Insertion of nucleotides opposite certain lesion sites is carried out by specific TLS pols, whereas strand extension from an inserted nucleotide seems to be mainly performed by pol ζ and also by pol κ (99, 118, 254). Upon completion of lesion bypass, a second pol switch may lead to the removal of the TLS pol from the strand and the reinstatement of a replicative pol that can continue high-fidelity nucleotide insertion. Although the exact regulatory mechanism for this second switch is still largely unknown, it has been suggested that deubiquitination of PCNA may be involved in this process (38). Beyond the Rad18-Rad6-mediated regulatory mechanism, additional pathways have also been proposed to lead to the pol switch during TLS. Indeed, the alternative processivity complex 9-1-1 has been shown to be loaded onto damaged DNA strands and interact with the TLS enzymes, pol ζ and pol κ , in yeast models (120, 214).

The second model proposed involves gap filling by the TLS pathway postreplication and is independent of replication fork stalling. TLS-mediated gap filling (Fig. 5, right scheme) may contribute to the sealing of single-stranded gaps that have been left unresolved by the replication machinery during the S phase of the cell cycle (167, 256). The gap-filling model was established in yeast models and has not yet been sufficiently studied in human cells. The recruitment of TLS pols to DNA lesion sites

FIG. 5. Schematic depiction of the translesion synthesis (TLS) polymerase switch and gap-filling models. In the TLS polymerase model, the replication machinery cannot bypass distorting or bulky DNA lesions and therefore stalls. Replication fork stalling leads to the monoubiquitination of proliferating cell nuclear antigen (PCNA) by the Rad18-Rad6 heterodimer, resulting in the removal of the replicative DNA polymerase from the strand and the recruitment of a TLS polymerase that has the ability to carry out translesional bypass. After insertion of nucleotides opposite the lesion, a second polymerase switch takes place, replacing the TLS polymerase with a replicative enzyme. In TLS gap filling, this mechanism occurs independently of replication and deals with single-strand gaps that may have been left behind by the replication machinery. The TLS polymerase is recruited to the gap in the DNA and is able to insert nucleotides opposite the DNA lesion, before a polymerase switch that may lead to a replicative polymerase taking over the strand elongation before the remaining nick is sealed by a DNA ligase.

outside of the S phase may involve the same mechanisms as reported for the pol switch model, but since the replication machinery has already passed the damage site, a pol switch is not required for the TLS pol to access the lesion-containing strand. However, after sealing of the remaining nick by a DNA ligase, a pol switch to a replicative pol may still occur.

2. DNA polymerase η . Pol η was the first TLS pol to be discovered and characterized, and still to date, pol η is the most well-studied TLS enzyme, and is the only pol causally linked to the development of cancer (35). Pol η was first described as the enzyme lacking in the variant form of xeroderma pigmentosum (XPV), a rare autosomal-recessive disorder that leads to a massive increase in UV sensitivity in patients (117, 125, 162). The symptoms of XPV patients resemble those suffering from other forms of xeroderma pigmentosum, despite a functional nucleotide excision repair (NER) pathway (Fig. 6) (147).

Pol η is encoded by the *POLH* gene on chromosome 6, and the protein contains 713 amino acids (272). The active site is located in the N-terminal region of the enzyme and is highly conserved throughout the Y pol family. It exhibits a more open configuration to enable the enzyme to accommodate even bulky DNA adducts, such as thymidine dimers caused

by UV irradiation (8, 203). The C-terminus of pol η contains sequences that allow the enzyme to be recruited to DNA lesion sites (35, 123). Pol η has been cocrystallized with DNA, and it has been found that it only shares minimal sequence homology with other pols outside the Y family (8, 28, 224). However, it shares basic structural similarities, such as a palm domain containing the glutamate and aspartate residues to coordinate the divalent cations that facilitate the nucleophilic attack on the alpha-phosphate and stabilize the pyrophosphate-leaving group on the incoming dNTP (14). Pol η also contains thumb and finger domains that hold the DNA and make connections to the primer and template strands, respectively (203). Furthermore, the pol contains a polymerase-associated domain, which is a characteristic of all Y family pol members, which makes additional contacts with the DNA (150). Moreover, as with all pols of the Y family, pol η lacks intrinsic 3'-5'-exonuclease proofreading activity, and despite this, after insertion of an incorrect nucleotide, pol η was found not to be able to continue strand elongation and dissociates from the DNA lesion site (253). It is believed that the misinsertion rate of pol η is determined by hydrogen bonds formed between the catalytic domain of the enzyme, the incoming dNTPs, and the nascent DNA strand (8). Therefore, the fidelity of the TLS pol depends on its ability to insert the

FIG. 6. Schematic depiction of the nucleotide excision repair (NER) pathway. The NER pathway can carry out repair of most nucleotidic damage and lesions that cause distortion of the DNA double helix. The DNA damage is recognized by the XPC-Rad23B complex, and the helicase activity of the transcription factor IIH (TFIIH), consisting of XPB and XPD, carries out strand unwinding at the damage site. The first incision is carried out by the endonuclease XPG 3'—to the lesion, and the second incision, 5'—to the damage—is performed by the XPF/ERCC1 complex. After removal of the damaged nucleotide sequence, the remaining gap is filled by a DNA polymerase, and the nick is sealed by a DNA ligase.

correct nucleotide and to only continue strand elongation from the correctly paired primer termini.

Pol η can bypass thymidine–thymidine dimers (cyclobutane pyrimidine dimers [CPDs]) and pyrimidine–6/4-pyrimidone photoadducts caused by UV radiation in an almost error-free manner (253). Apart from UV-induced damage, pol η has been reported to be able to bypass a variety of other DNA lesions, including 8-oxoguanine, O^6 -methylguanine, benzo[a]pyrene-N2-deoxyguanine DNA adducts, as well as DNA damage created by treatment with the anticancer drugs, cisplatin, gemcitabine, and oxaliplatin (57, 58, 156, 210, 244). Processivity of pol η largely depends on the DNA structure, and while it is low on undamaged DNA, pol η was reported to insert nucleotides opposite CPDs with significantly higher processivity (166). Since overexpression of pol η in human cells does not increase cellular mutagenesis, and vector-based multicopy expression proved nonviable in cell lines, it has been concluded that pol η activity is highly regulated, and the enzyme is prevented from accessing undamaged DNA *in vivo* (130, 239).

Pol η requires Rad18 to monoubiquitinate PCNA to be loaded onto damaged DNA (255), although it has been suggested that Rad18-dependent PCNA ubiquitination may not be essential for Pol η recruitment (3, 216). Pol η itself then gets monoubiquitinated, although the relevance of this modification for its TLS activity has not been revealed (27). Translesional bypass by pol η only spans the lesion site, and a few adjacent bases before the binding of the pol to the primer terminus get destabilized, and a pol switch removes the enzyme, allowing replicative pols α or δ to continue strand elongation (137, 161). Beyond its involvement in TLS, pol η has been suggested to be involved in the repair of DSBs by HR, and *in vitro* experiments have shown that the pol is able to perform the D-loop extension step during HR (126, 170).

3. DNA polymerase iota. Despite its sequence homology with pol η , pol ι has been suggested to play a distinct role in TLS. *Saccharomyces cerevisiae*, often used as a model organism for the study of the translesional pathways, lacks the enzyme, and the function and involvement of pol ι in the TLS system are therefore relatively unexplored (169). Human pol ι is encoded by the *POLI* gene on chromosome 18, and it is believed that the pol ι protein is used as a backup pol employed for the bypass of UV-induced lesions in the absence of pol η , where it has been shown to contribute to a unique spectrum and high rate of mutations, as reported in XPV (94, 252). Lack of pol ι in turn increases mutation rates and has been shown to result in a higher incidence of different cancers in both humans and in mouse models (71, 142, 193). However, unlike in the absence of pol η , there is no distinct phenotype associated with pol ι deficiency, and no immune defects or deficiencies of UV lesion bypass have been reported in the absence of pol ι in mice (94, 223). Similarly, deficiency of pol ι did not increase cellular sensitivity to alkylating agents (201). A deficiency of pol ι in human cell lines has been demonstrated to lead to an increased sensitivity to oxidative damage, and due to the established interaction of pol ι with the BER enzyme XRCC1, this evidence suggests a role for pol ι in the cellular response to DNA damage induced by oxidative stress (198).

Pol ι has a considerably lower fidelity than pol η , but *in vitro* experiments have shown that the fidelity of pol ι could be increased by substituting the divalent cation, Mg^{2+} with Mn^{2+} (79). The fidelity of pol ι was found to vary between different template nucleotides, and the enzyme incorporated dNTPs opposite purines with higher fidelity than opposite pyrimidines, a finding that may be explained by the preference of pol ι for Hogsteen base pairing (59, 116, 181). The presence of the auxiliary proteins, PCNA, replication protein A (RPA), and replication factor C (RFC) has been shown to increase the enzymatic activity of the pol *in vitro*, and structural analysis of pol ι has revealed a binding motif for ubiquitinated PCNA and dependence on Rad18 activity (41, 98, 121, 156). Pol ι was also found to be able to physically interact with pol η , although the impact and mechanistic relevance of this interaction are not currently understood (124).

4. DNA polymerase kappa. Pol κ , encoded by the *POLK* gene on chromosome 5, is different from the other Y family TLS pols in several respects. Pol κ is the most highly expressed and most-conserved TLS pol characterized to date (256) and has only weak translesional activity and limited ability in DNA lesion bypass. Indeed, the highest TLS efficiency and

accuracy of pol κ have been demonstrated across N^2 guanine adducts or interstrand crosslinks (60, 174). Unlike most other Y family pols, pol κ is capable of replicating undamaged DNA with relatively high processivity. However, while the *Escherichia coli* pol κ homolog, pol IV, has been shown to perform high-fidelity replication on undamaged DNA, human pol κ has misinsertion frequencies comparable with other TLS pols (132, 190). Apart from its translesional activity, pol κ performs extension from nucleotides inserted opposite DNA lesions and acts as the extender pol during TLS, which makes it dependent on other inserter pols to bypass the site of DNA damage (152, 254). Beyond its involvement in TLS, a role for pol κ has been implied during the polymerization steps of NER (Fig. 6), most likely in cooperation with pol δ (189).

Pol κ deficiency leads to an increase in cellular sensitivity to UV radiation and alkylating agents, and the enzyme has been found to be involved in high-fidelity bypass past UV-induced CPDs, in collaboration with pol η in *Xenopus laevis* oocytes (188, 235, 262). Pol κ has been found to interact with PCNA and the 9-1-1 complex, but unlike pol η and ι , pol κ foci were found not to co-localize with PCNA upon DNA damage, suggesting that the pol may not be a vital part of the TLS machinery during the S phase of the cell cycle (187, 191). Overexpression of pol κ has been shown to result in genomic instability and an increase in DNA damage, and mice deficient in pol κ were found to exhibit a mutator phenotype (16, 230). Furthermore, pol κ overexpression resulted in slowing down of replication fork progression without activation of the replication checkpoint (199).

C. A family polymerases

1. DNA polymerase theta. The A family pol, pol θ , is encoded by the *POLQ* gene located on chromosome 3. The enzyme is composed of three domains, an N-terminal ATPase-helicase-like domain, a central spacer domain, and a pol domain located at the C-terminus (217). On undamaged DNA, pol θ was found to insert nucleotides with an ~ 100 -fold increased misinsertion rate (2.4×10^{-3} nucleotides) compared to other A family pols (12). On the other hand, pol θ was observed to be very efficient at bypassing AP sites (107, 218). Pol θ has also been shown to contain a 5'-dRP lyase domain, and therefore a dual role for pol θ as a pol involved in both TLS and short-patch BER of AP sites has been suggested, indicating an important role for pol θ in the repair of these DNA lesions (206). These findings are supported by data showing that pol β and pol θ have overlapping functions and cooperate during BER *in vitro* (268).

Studies have also suggested that pol θ acts as an extender pol that can extend from mismatches and base pairs containing CPDs or pyrimidine-6/4-pyrimidone photoproducts *in vitro* (219). Investigations of pol θ in *Drosophila melanogaster* have uncovered an important role for the enzyme in a DNA ligase 4-independent alternative end-joining pathway (52), and *C. elegans* pol θ has been implied in interstrand crosslink repair (179), although the relevance of these findings for higher eukaryotes remains unclear. The lack of pol θ in human cells was found to increase radiosensitivity in tumor and bone marrow cell lines (89, 105), and mice deficient in pol θ are viable, but cells from these mice produce an increased level of ionizing radiation-induced micronuclei (89). Furthermore, pol θ has previously been implicated in somatic hypermutation

and immunoglobulin diversification (133), although this is still under debate (160).

D. B family polymerases

1. DNA polymerase zeta. The B family pol, pol ζ , is comprised of two subunits, the catalytic subunit Rev3, encoded by the *REV3L* gene on chromosome 6, and the accessory subunit Rev7, which has been shown to considerably increase the catalytic activity of the pol (256). Mice lacking pol ζ are not viable, and furthermore, loss of the catalytic subunit of pol ζ leads to early embryonic lethality (75, 260). In contrast to the other pols of the B family, the replicative enzymes pol δ and ϵ , pol ζ is devoid of a 3'-5'-proofreading exonuclease activity, and it has been suggested that pol ζ contributes considerably to mutagenesis in the majority of eukaryotic organisms (1, 68). However, a deficiency of this pol has been found to result in increased chromosomal instability, and it has been suggested that pol ζ mediates cellular tolerance to spontaneously occurring DNA damage (261). Although pol ζ was shown to be able to insert nucleotides opposite certain DNA lesions, its main role is believed to be in extension from nucleotides inserted opposite DNA lesions, thereby cooperating with another specialized pol during TLS (2, 273). Indeed, several interactions between pol ζ and other TLS proteins have been described, and both the catalytic Rev3 subunit and the accessory Rev7 subunit can bind to the Y family pol-like protein Rev1 (97). Additionally, the enzymatic activity of pol ζ has been found to increase in the presence of the replication factors PCNA, RPA, and RFC, as well as the 9-1-1 complex (214, 273). Pol ζ protein expression levels are reportedly low in most cell types, and studies performed in *S. cerevisiae* showed increased mutagenesis and resistance upon UV irradiation when pol ζ was upregulated (209).

III. Biological Relevance of Nonreplicative DNA Polymerases to Carcinogenesis

A. X family polymerases

While no causal connection between X family pols and cancer has been established to date, various associations and deregulation patterns have been described for certain enzymes.

Pol β has been the most thoroughly studied X pol member in the context of cancer. Because of the significant amount of DNA damage occurring spontaneously under normal conditions, all cells depend on BER to repair the majority of DNA lesions (108). While the DNA glycosylases involved in this pathway have redundant functions, pol β has a unique function in gap filling, and has been shown to be essential for cellular viability. Mice deficient in pol β die very early during embryogenesis, and embryonic fibroblasts derived from these animals are hypersensitive to methylating agents that induce DNA damage repaired by BER (93, 202, 234). Due to its essential role during BER, pol β is constitutively expressed in all tissues, and pol β expression has been shown not to change during the cell cycle (274). Pol β levels have been reported to be low in most tissue types, with the exception of normal testicular tissue, and low pol expression may help to prevent pol β from interfering with replication by high-fidelity pols (106, 221). Based on data from artificial model systems, it has also been proposed that overexpressed pol β has the ability to replace the replicative pols, pol δ , and ϵ , during the gap-filling step of NER (46).

Cellular upregulation of pol β has been reported after treatment with cytotoxic agents or exposure to oxidative stress, and increased pol β levels correlate with resistance to oxidative agents, such as hydrogen peroxide and the alkylating agents, methyl methanesulfonate, melphalan, and cisplatin (44, 56). Upregulation of pol β was found to lead to an increase in frameshift mutations during BER (51); conversely, downregulated levels of pol β have also been linked to an increased rate of spontaneous chromosomal aberrations and increased cancer risk, as observed in pol β haploinsufficient mice (42, 43). Interestingly, a reduction of pol β levels in some tumor tissues was reported to correlate with a downregulation of other pols, suggesting a connection between BER and other pol-dependent DNA repair pathways (6).

Pol β levels have been studied in clinical samples of various tumor types. Analysis of mRNA expression arrays has shown a reduction in pol β levels in ~20% of all screened cancer samples, and downregulation of pol β has been found mainly in breast and colorectal cancers (6). In general, pol β overexpression seems to be considerably more prevalent than underexpression in tumor samples, and it has also been shown for various cancer cell lines (45, 228). It has been reported that increased pol β mRNA and protein levels are observed in ~35% of tumor tissues in comparison to the corresponding normal tissues, and overexpression was most marked in samples from ovarian, uterine, prostate, and gastric cancers (6).

The short arm of chromosome 8 that contains the *POLB* gene coding for pol β has been found to be lost in various tumor types, including hepatocellular, colorectal, prostate, and lung cancers (74, 155, 247). In a meta-analysis, pol β mutants or splice variants have been reported in almost half of all cancers, and about 10% of the analyzed cancer samples express a truncated pol β protein (231). Several pol β variants that are expressed in tumor cells have been found to impede BER activity or pol fidelity (138, 139), and one deletion variant has been reported to act in a dominant-negative fashion by impeding gap filling during BER (61, 182, 183, 249).

Pol λ has also been found to be overexpressed in different tumor tissues, and more than a twofold upregulation was reported for almost 25% of examined samples (6). In a study examining pol λ expression in bronchial tissue from smokers, expression correlated with the amount of smoking in normal tissues, but not cancer tissues, and lack of pol λ expression in heavy smokers was linked to an advanced tumor stage (192). Interestingly, a naturally occurring pol λ splice variant has been characterized that results in a mutator phenotype by impeding the NHEJ pathway (238).

The third X family pol, pol μ , has been shown to be preferentially expressed in secondary lymphoid tissue where it seems to contribute to somatic hypermutation (213). However, little is known about expression of pol μ in cancer tissues, and potential deregulations in expression have not yet been characterized.

B. Y family polymerases

Y family pols have been implicated in mutagenesis and tumorigenesis, and to date, pol η is the only pol that appears to exhibit a causal connection with the development of skin cancers (35). Various mutations in the *POLH* gene leading to nonfunctional pol η protein have been described in XPV

patients from Europe, Asia, and North America (110, 236). Additional variants of the *POLH* gene have been suggested as low-penetrance alleles predisposing for malignant melanoma (65). In contrast, no mutations or variations in the *POLH* gene were found in a panel of basal and squamous skin cancers (77, 87).

Expression patterns of the Y family TLS pols have been studied in a range of tumor samples, although with conflicting results. An upregulation of pol η and ι in a panel of tumor samples, whereas pol κ generally showed lower levels, compared to corresponding normal tissues has been reported (6). In this study, pol ι was found to be increased more than twofold in almost 30% of all analyzed samples, whereas a downregulation was observed in about one-quarter of the samples. In a panel of breast cancer biopsies, pol ι was generally found overexpressed, and it has been suggested that deregulation of the pol leads to a decrease in fidelity, thereby contributing to increased genomic instability in these tumors (265). In glioma samples, pol ι upregulation was observed in 27% of all examined cases, and detection of increased expression by immunohistochemistry correlated with shorter patient survival (251). It has also been suggested that hypoxia is able to upregulate pol ι expression in human tumor cell lines (112).

In contrast, a general downregulation of the TLS pols, pol ι , η , κ , and ζ , in tumor specimens derived from lung, gastric, and colorectal cancers has been reported (194). Conversely, two further studies reported no alterations in pol η expression in lung and gastric cancer compared to normal tissues, but a significant inverse correlation between expression levels of pol η and patient survival was found (50, 237). Another study assessed gene expression profiles in nonsmall-cell lung cancer biopsies and reported an upregulation of pol η (246). Furthermore, in a small cohort of skin cancer patients, pol η was found downregulated in 4 of the 10 examined basal-cell carcinoma biopsies and upregulated in 3 of 10 specimens, whereas only 1 out of 7 squamous cell cancer samples exhibited a significant increase in pol η (77).

In a panel of lung cancer samples, increased pol κ levels were found in 21 of 29 examined samples (185), and pol κ upregulation has been linked to genetic alterations and tumorigenesis in a mouse model (16). In a glioma study, pol κ was reported to be upregulated in 72 out of 104 tumor samples, and immunohistochemical detection of pol κ in these samples correlated with a poor prognosis (251).

C. A and B family polymerases

Only a few studies to date have been published on the expression of A and B family DNA pols in cancer tissues. Expression of the Rev3 subunit of the B family pol, pol ζ , has been found to be significantly reduced in a panel of 74 colorectal cancer samples when compared to normal colon tissue (34). Several single-nucleotide polymorphisms in the *POLZ* gene have also been found to correlate with either breast cancer development or prognosis (248).

The A family pol, pol θ , is generally observed at very low levels in most tissues, although significant normal tissue expression has been reported for the lymphatic system, and has also been observed in certain tumors (127). Two studies found a correlation between overexpression of pol θ in tissues of breast cancer patients and an adverse prognosis for these

patients (104, 145). Furthermore, overexpression of the pol in colorectal cancer samples has been linked with poor patient survival (200). In cell lines, pol θ overexpression led to disturbances in replication, and eventually genomic instability, which may contribute to carcinogenesis (145).

IV. The Potential Role of Nonreplicative DNA Polymerases in Cancer Treatment

An understanding of the complexity of the involvement of multiple nonreplicative pols in DNA damage repair pathways (summarized in Table 2), and the implication that nonreplicative pols are involved in carcinogenesis (Section III above) has led to studies on the role of these enzymes in determining cellular responses to cancer treatment and the search for potential correlations with patient response and survival.

In addition to surgical approaches to cancer, chemotherapeutic treatment with cytotoxic drugs and radiotherapy using ionizing radiation remain the mainstays of current cancer therapy. Both of these nonsurgical approaches can use the DNA of cancer cells as a target, thereby inhibiting further proliferation and eventually leading to cell death. However, cancer cells have evolved pathways that can repair treatment-induced DNA lesions and prevent the accumulation of lethal damage. Several of these pathways require specialized pols for intermediate steps, for example, for gap filling or lesion bypass. Based on this knowledge, certain specialized pols may prove to be valuable targets to improve tumor therapy, or to inhibit the development of resistance in various cancers.

A. Involvement of DNA polymerases in cellular responses to chemotherapy

A significant proportion of solid tumors are either inherently resistant to different chemotherapeutic drugs or develop resistance during the course of treatment. Several mechanisms have been proposed in the development of resistance to cytotoxic treatment, among them decreased cellular uptake (96, 151) or cytosolic inactivation (171, 172) (Fig. 7). Alterations in several DNA repair pathways have also been linked to the development of drug resistance in cellular assays and have been shown to correlate with treatment response or outcome in patient-based studies. As specialized pols are integral parts of many DNA repair pathways, their role in the development of resistance to chemotherapy has been widely examined.

In vitro assays have shown that the X family pol, pol β , can efficiently bypass or repair DNA adducts created by cisplatin and oxaliplatin, and it has therefore been hypothesized that pol β may mediate tolerance of cancer cells to platinum-based treatments (15, 53, 243, 245) (Fig. 8). Furthermore, an increased resistance of cultured cells overexpressing pol β to cisplatin, melphalan, and mechlorethamine has been observed (44), and cellular depletion of pol β by RNA interference has revealed an increased sensitivity to monofunctional alkylating agents, such as methyl methanesulfonate (186). Cells deficient in pol β were also found to be hypersensitive to oxaliplatin (266). Similarly, inhibition of pol β , but not pol λ , sensitized cancer cells to the alkylating agent, temozolomide, an effect that was augmented in cells with mutations in the *BRCA2* gene (229, 240, 241). The cellular response to the topoisomerase II inhibitor, etoposide, was also reported to be

FIG. 7. Mechanisms for the resistance of cancer cells to cytotoxic drugs. Cancer cells employ several mechanisms that lead to the formation of resistance to chemotherapeutic drugs, such as cisplatin or etoposide. Decreased uptake by copper transporters or increased removal by the multidrug-resistance receptor proteins may lead to a diminished concentration of the drug at the target structure. Binding or inactivation in the cytosol, for example, through glutathione metabolism, has also been shown to contribute to increased resistance. Alterations and increases in several different DNA repair pathways lead to an increased removal of drug-induced DNA lesions and have the potential to reduce the efficacy of cancer treatment.

affected by pol β status, since inhibition of the pol resulted in an increased amount of DNA damage and an increased sensitivity to the chemotherapeutic drug (141). In addition, *in vitro* data have shown that pol β was able to incorporate antimetabolite drugs, gemcitabine and cytarabine, into DNA, which would suggest that these drugs may be more beneficial in tumors overexpressing pol β (204).

Similar to pol β , the Y family pol, pol η , has also been shown to be able to bypass DNA adducts created by platinum-based cytotoxic drugs *in vitro*, and the structural basis for this bypass mechanism has been studied in detail (8, 15, 244). Moreover, a deficiency of pol η has been shown to increase sensitivity to cisplatin and oxaliplatin, as well as a cisplatin/gemcitabine combination treatment in cell lines (5, 57). It has been suggested that expression levels of POLH mRNA may predict response to treatment with cisplatin in nonsmall-cell lung cancer patients and to treatment with oxaliplatin in gastric cancer patients (50, 237). Pol η was furthermore found to be capable of bypassing DNA adducts induced by the cancer drug tamoxifen *in vitro* (267). In contrast, the enzymatic activity of the Y family pols, pol ι or pol κ , has not been linked to the response of cells to chemotherapeutic agents, and in particular, a deficiency in pol ι was shown in several studies not to increase cellular sensitivity to alkylating agents (198, 201, 240). Conversely, *in vitro* studies in *S. cerevisiae* showed that both pol ι and pol κ could bypass DNA damage caused by the alkylating agent methyl methanesulfonate, suggesting that they may have a nonexclusive role in the cellular response to

FIG. 8. Chemical structures of commonly used cytotoxic chemotherapy drugs used to treat cancer.

alkylating agents (119). Similar results have been published for pol θ , which did not mediate a cellular response to the alkylating and methylating agent, temozolomide (105). However, cells derived from pol θ -deficient mice have been demonstrated to be hypersensitive to the chemotherapeutic drug bleomycin (89).

The B family pol, pol ζ , has been suggested to play a role in the cellular bypass of platinum-induced DNA adducts, since RNA interference-based depletion of pol ζ in head-and-neck squamous cell carcinoma cell lines resulted in an increased cellular response to cisplatin treatment (4). Similar results have been reported for tumors with an acquired resistance to platinum-based chemotherapy, and furthermore, a knock-down of pol ζ in colorectal cancer cell lines deficient in mismatch repair was reported to resensitize these cells to cisplatin (148). In a mouse model of cisplatin-resistant lung cancer, suppression of the catalytic subunit of pol ζ , Rev3, led to resensitization of these tumors to cisplatin treatment, and resulted in an increased survival of the treated mice (69).

B. The role of nonreplicative polymerases in cellular responses to radiotherapy

Similar to cancer treatment with cytotoxic drugs, radiotherapy targets cellular DNA causing DNA damage that ultimately leads to cell death. Unlike most chemotherapeutic agents that create specific DNA lesions, ionizing radiation

FIG. 9. Types of DNA damage caused by ionizing radiation. Ionizing radiation causes DNA damage directly, either by depositing its energy onto the DNA strands or by ionizing cellular water, creating reactive oxygen species that lead to secondary DNA lesions. Common types of ionizing radiation-induced DNA damage include base damage, single-strand breaks (SSBs), and DSBs. The repair pathways that deal with these types of DNA damage include the BER, NER, TLS, single-strand break repair (SSBR), nonhomologous end joining (NHEJ), and HR pathways.

results in a plethora of different types of DNA damage, including oxidative base damage, SSBs, and DSBs, as well as complex DNA damage containing two or more different lesions within one helical turn of the DNA (90, 158) (Fig. 9). Although DSBs are a minority of all DNA damage induced by ionising radiation, they are believed to be the major DNA lesions by which radiation causes cell death. Because of the complexity of radiation-induced DNA damage, several DNA repair pathways are involved in the repair of these lesions, and specialized pols have been implicated in the cellular repair of ionizing radiation-induced damage (Fig. 9).

Recently, a link between deficiency in the A family pol member, pol θ , and the cellular response to ionizing radiation has been found by two independent research teams. Pol θ has been identified as a mediator of the cellular radiation response in a siRNA-based screen, and subsequently, a knockdown of pol θ increased radiosensitivity in tumor cell lines (105). Similarly, a deficiency in pol θ was found to radiosensitize bone marrow stromal cells, causing an increase in cellular micronuclei, a marker of DNA damage (89).

Likewise, the X family pol member, pol β , has been linked to the cellular response to radiation. While a deficiency in

pol β alone was reported not to result in an increase in radiosensitivity, a dominant-negative variant has been described that could radiosensitize cells by inhibiting the later stages of DNA repair (182, 183, 249). In addition, deregulation of pol β , at levels corresponding to those reported in some tumors, has been suggested to contribute to nucleotidic perturbances and chromosomal instabilities after ionizing radiation treatment (80). Similar observations have been reported for another X family pol member, pol μ . Pol μ is thought to be involved in DSB repair through the NHEJ pathway, and overexpression of pol μ has been shown to lead to an increase in cellular sensitivity to ionizing radiation (49, 157).

At present, the role of other specialized pols in the cellular response to ionizing radiation treatment remains unclear. There is certainly scope for further research in this area, since, using *in vitro* or cell-based assays, several other pols have been shown to contribute to the various DNA repair pathways employed during the repair of ionizing radiation-induced DNA damage (Fig. 9). This research is required to further establish whether nonreplicative pols may be viable targets for inhibitors to improve the efficacy of radiotherapy in cancer treatment.

C. Targeting specialized DNA polymerases to improve cancer treatment

Taking into account the effects of specialized pols in the cellular responses to cytotoxic cancer treatments and radiotherapy, there may be scope for targeting these enzymes with pharmacological agents (Fig. 10) as a means of improving cancer treatment. Indeed, inhibitors for several specialized pols have been identified and characterized already, and several studies have tested the potential benefits of pol inhibition in combination with cytotoxic drugs in cell culture.

Inhibition of the X family pol member, pol β , has been shown to sensitize cells to the alkylating agent, temozolomide, and the DNA cross-linker, cisplatin, highlighting the important involvement of pol β in repairing the types of DNA damage generated by these agents that are processed by BER and TLS pathways (30, 113). Therefore, the potential importance of targeting pol β in cancer treatment is evident (88). Furthermore, an additional benefit of BER inhibition by blocking pol β activity was reported in BRCA2-deficient tumors, which showed a further increase in the cellular response to temozolomide treatment (229). This concept of synthetic lethality previously observed by inhibition of poly(ADP-ribose) polymerase-1 (PARP-1) in BRCA2-deficient tumors (39, 76) suggests that targeting pol β activity, in combination with a deficiency in DSB repair through HR, may serve as an important approach to cancer treatment. Several molecules have also been described that have differential inhibitory effects on pol β , and while the triterpenoid inhibitors oleanolic acid and betulinic acid were shown to inhibit both the 5'-dRP lyase and pol activities of pol β , the plant sterol inhibitor stigmasterol was found to selectively block lyase activity (84) (Fig. 10). Stigmasterol-mediated inhibition of pol β has been shown to lead to a potentiation of the cytotoxic effect of the radiomimetic cancer agent, bleomycin, which induces DNA strand breaks that may require the SSB repair activity of pol β (84, 146).

Tocotrienol is an agent that can inhibit the activity of pol λ and may have additional effects as an angiogenesis inhibitor (Fig. 10), although no benefit as a combinatorial treatment

FIG. 10. Chemical structures of pharmacological inhibitors of DNA polymerases.

with any anticancer drug has yet been demonstrated (176). Several other inhibitors for X family pols have been identified and characterized *in vitro*, but have not yet been tested for potential increases in tumor cell sensitivity to chemotherapeutic treatment (111, 122, 128, 180). Furthermore, Penicillols A and B were the first published inhibitors shown to specifically inhibit Y family pols *in vitro* (129). Since then, the Y family pol inhibitor 3-O-methylfunicone has been shown to inhibit tumor cell growth and decrease clonogenic survival of cells treated with UV irradiation, linking pol inhibition with the cellular response to DNA damage (175).

The potential advantage of inhibiting nonreplicative pols has been shown *in vitro*, and agents that may be used for this purpose are currently at preclinical stages of development for cancer treatment. While the potential use of novel inhibitors targeting nonreplicative pols as potential cancer therapy targets is at an early stage, one caveat is the redundancy among the pols, and therefore inhibition of one particular pol may not have a significant effect on tumor cell killing due to its replacement with another pol that is able to undertake the same function. Therefore, multiple pols may need to be targeted, although the effect of inhibiting two or more of these enzymes in normal cells, in comparison to tumor cells, would need to be studied in detail to ensure preferential toxicity to the tumor cells. It should also be noted that due to the differential expression of nonreplicative pols in various normal as well as cancer tissues, but also due to the interindividual variation of expression of these enzymes, these considerations must be

taken into effect when using inhibitors against these pols as potential cancer therapy agents. Therefore, more, detailed studies on tissue-specific expression of nonreplicative pols are required as well as further information on the expression of these enzymes in various different tumor types. The molecular mechanisms that control the expression of nonreplicative pols also warrant future research, and both the regulation of expression at a transcriptional level, but also protein stability *via* post-translational modifications, should be investigated in more detail and subsequently analyzed in various tumor cells to establish whether these mechanisms are defective.

V. Conclusions

Cancer is routinely treated by agents that act *via* cellular DNA damage and ultimately cause tumor cell death. Non-replicative (specialized) pols have been shown to participate in DNA damage repair and tolerance pathways relevant to carcinogenesis and the treatment of cancer. An alteration in these DNA repair pathways, specifically in tumor cells, has been linked with resistance of these cells to certain classes of cytotoxic drugs used during cancer treatment. There is also evidence that specialized pols can contribute to cellular tolerance to certain types of DNA damage. *In vitro* inhibition of specialized pols by RNA interference or small-molecule inhibitors has been reported to increase sensitivity of tumor cells to cancer treatment, and pol inhibition may ultimately prove to be a useful approach for increasing the effectiveness of the current therapies for patients with cancer. However, more detailed information is required on the expression, as well as the mechanisms controlling this expression, of nonreplicative pols in various normal and tumor tissues to be able to predict the normal-*versus*-tumor-tissue response to pol inhibition in individual patients. Furthermore, as drugs that target non-replicative pols become available, full mechanistic details on their mode of action will be required to fully ensure that pol inhibition is responsible for mediating the toxic effects to tumor cells. Subsequently, when these drugs are available for clinical trials, clinical studies will be required to evaluate the potential of pol inhibition as a way of optimizing noninvasive cancer treatment and a means of targeting treatment-resistant tumors. In the meantime, specialized pols warrant examination of their potential use as clinical biomarkers to select for certain chemotherapies and radiotherapy, with the ultimate goal of individualizing cancer treatment.

Acknowledgments

R.A.S. is funded by the NIHR Biomedical Research Centre Oxford, the Experimental Cancer Medicine Centre, Cancer Research UK, the UK Medical Research Council, and the Higher Education Funding Council for England.

References

1. Abdulovic AL and Jinks-Robertson S. The *in vivo* characterization of translesion synthesis across UV-induced lesions in *Saccharomyces cerevisiae*: insights into Pol zeta- and Pol eta-dependent frameshift mutagenesis. *Genetics* 172: 1487–1498, 2006.
2. Acharya N, Johnson RE, Prakash S, and Prakash L. Complex formation with Rev1 enhances the proficiency of *Saccharomyces cerevisiae* DNA polymerase zeta for mismatch extension and for extension opposite from DNA lesions. *Mol Cell Biol* 26: 9555–9563, 2006.
3. Acharya N, Yoon JH, Gali H, Unk I, Haracska L, Johnson RE, Hurwitz J, Prakash L, and Prakash S. Roles of PCNA-binding and ubiquitin-binding domains in human DNA polymerase eta in translesion DNA synthesis. *Proc Natl Acad Sci U S A* 105: 17724–17729, 2008.
4. Adachi M, Ijichi K, Hasegawa Y, Ogawa T, Nakamura H, Yasui Y, Fukushima M, and Ishizaki K. Hypersensitivity to cisplatin after hRev3 mRNA knockdown in head and neck squamous cell carcinoma cells. *Mol Med Report* 1: 695–698, 2008.
5. Albertella MR, Green CM, Lehmann AR, and O'Connor MJ. A role for polymerase eta in the cellular tolerance to cisplatin-induced damage. *Cancer Res* 65: 9799–9806, 2005.
6. Albertella MR, Lau A, and O'Connor MJ. The over-expression of specialized DNA polymerases in cancer. *DNA Repair (Amst)* 4: 583–593, 2005.
7. Allinson SL, Dianova II, and Dianov GL. DNA polymerase beta is the major dRP lyase involved in repair of oxidative base lesions in DNA by mammalian cell extracts. *EMBO J* 20: 6919–6926, 2001.
8. Alt A, Lammens K, Chiocchini C, Lammens A, Pieck JC, Kuch D, Hopfner KP, and Carell T. Bypass of DNA lesions generated during anticancer treatment with cisplatin by DNA polymerase eta. *Science* 318: 967–970, 2007.
9. Andersen PL, Xu F, and Xiao W. Eukaryotic DNA damage tolerance and translesion synthesis through covalent modifications of PCNA. *Cell Res* 18: 162–173, 2008.
10. Andreassen PR, Ho GP, and D'Andrea AD. DNA damage responses and their many interactions with the replication fork. *Carcinogenesis* 27: 883–892, 2006.
11. Aoufouchi S, Flatter E, Dahan A, Faili A, Bertocci B, Storck S, Delbos F, Cocea L, Gupta N, Weill JC, and Reynaud CA. Two novel human and mouse DNA polymerases of the polX family. *Nucleic Acids Res* 28: 3684–3693, 2000.
12. Arana ME, Seki M, Wood RD, Rogozin IB, and Kunkel TA. Low-fidelity DNA synthesis by human DNA polymerase theta. *Nucleic Acids Res* 36: 3847–3856, 2008.
13. Asagoshi K, Liu Y, Masaoka A, Lan L, Prasad R, Horton JK, Brown AR, Wang XH, Bdour HM, Sobol RW, Taylor JS, Yasui A, and Wilson SH. DNA polymerase beta-dependent long patch base excision repair in living cells. *DNA Repair (Amst)* 9: 109–119, 2010.
14. Baker TA and Bell SP. Polymerases and the replisome: machines within machines. *Cell* 92: 295–305, 1998.
15. Bassett E, Vaisman A, Havener JM, Masutani C, Hanaoka F, and Chaney SG. Efficiency of extension of mismatched primer termini across from cisplatin and oxaliplatin adducts by human DNA polymerases beta and eta *in vitro*. *Biochemistry* 42: 14197–14206, 2003.
16. Bavoux C, Leopoldino AM, Bergoglio V, J OW, Ogi T, Bieth A, Judde JG, Pena SD, Poupon MF, Helleday T, Tagawa M, Machado C, Hoffmann JS, and Cazaux C. Up-regulation of the error-prone DNA polymerase [kappa] promotes pleiotropic genetic alterations and tumorigenesis. *Cancer Res* 65: 325–330, 2005.
17. Beard WA, Shock DD, Batra VK, Pedersen LC, and Wilson SH. DNA polymerase beta substrate specificity: side chain modulation of the “A-rule”. *J Biol Chem* 284: 31680–31689, 2009.
18. Beard WA, Shock DD, Yang XP, DeLauder SF, and Wilson SH. Loss of DNA polymerase beta stacking interactions with templating purines, but not pyrimidines, alters cata-

- lytic efficiency and fidelity. *J Biol Chem* 277: 8235–8242, 2002.
19. Beard WA and Wilson SH. Purification and domain-mapping of mammalian DNA polymerase beta. *Methods Enzymol* 262: 98–107, 1995.
20. Beard WA and Wilson SH. Structure and mechanism of DNA polymerase beta. *Chem Rev* 106: 361–382, 2006.
21. Bebenek K, Garcia-Diaz M, Zhou RZ, Povirk LF, and Kunkel TA. Loop 1 modulates the fidelity of DNA polymerase lambda. *Nucleic Acids Res* 38: 5419–5431, 2010.
22. Belousova EA, Maga G, Fan Y, Kubareva EA, Romanova EA, Lebedeva NA, Oretskaya TS, and Lavrik OI. DNA polymerases beta and lambda bypass thymine glycol in gapped DNA structures. *Biochemistry* 49: 4695–4704, 2010.
23. Bennett SE, Sung JS, and Mosbaugh DW. Fidelity of uracil-initiated base excision DNA repair in DNA polymerase beta-proficient and -deficient mouse embryonic fibroblast cell extracts. *J Biol Chem* 276: 42588–42600, 2001.
24. Bergoglio V, Ferrari E, Hubscher U, Cazaux C, and Hoffmann JS. DNA polymerase beta can incorporate ribonucleotides during DNA synthesis of undamaged and CPD-damaged DNA. *J Mol Biol* 331: 1017–1023, 2003.
25. Bertocci B, De Smet A, Berek C, Weill JC, and Reynaud CA. Immunoglobulin kappa light chain gene rearrangement is impaired in mice deficient for DNA polymerase mu. *Immunity* 19: 203–211, 2003.
26. Bertocci B, De Smet A, Weill JC, and Reynaud CA. Nonoverlapping functions of DNA polymerases mu, lambda, and terminal deoxynucleotidyltransferase during immunoglobulin V(D)J recombination *in vivo*. *Immunity* 25: 31–41, 2006.
27. Bienko M, Green CM, Sabbioneda S, Crosetto N, Matic I, Hibbert RG, Begovic T, Niimi A, Mann M, Lehmann AR, and Dikic I. Regulation of translesion synthesis DNA polymerase eta by monoubiquitination. *Mol Cell* 37: 396–407, 2010.
28. Biertumpfel C, Zhao Y, Kondo Y, Ramon-Maiques S, Gregory M, Lee JY, Masutani C, Lehmann AR, Hanaoka F, and Yang W. Structure and mechanism of human DNA polymerase eta. *Nature* 465: 1044–1048, 2010.
29. Blanca G, Villani G, Shevelev I, Ramadan K, Spadari S, Hubscher U, and Maga G. Human DNA polymerases lambda and beta show different efficiencies of translesion DNA synthesis past abasic sites and alternative mechanisms for frameshift generation. *Biochemistry* 43: 11605–11615, 2004.
30. Boudsocq F, Benaïm P, Canitrot Y, Knibiehler M, Ausseil F, Capp JP, Bieth A, Long C, David B, Shevelev I, Frierich-Heineken E, Hubscher U, Amalric F, Massiot G, Hoffmann JS, and Cazaux C. Modulation of cellular response to cisplatin by a novel inhibitor of DNA polymerase beta. *Mol Pharmacol* 67: 1485–1492, 2005.
31. Braithwaite EK, Kedar PS, Stumpo DJ, Bertocci B, Freedman JH, Samson LD, and Wilson SH. DNA polymerases beta and lambda mediate overlapping and independent roles in base excision repair in mouse embryonic fibroblasts. *PLoS One* 5: e12229, 2010.
32. Branzei D, Seki M, and Enomoto T. Rad18/Rad5/Mms2-mediated polyubiquitination of PCNA is implicated in replication completion during replication stress. *Genes Cells* 9: 1031–1042, 2004.
33. Brieba LG. Template dependent human DNA polymerases. *Curr Top Med Chem* 8: 1312–1326, 2008.
34. Brondello JM, Pillaire MJ, Rodriguez C, Gourraud PA, Selves J, Cazaux C, and Piette J. Novel evidences for a tumor suppressor role of Rev3, the catalytic subunit of Pol zeta. *Oncogene* 27: 6093–6101, 2008.
35. Broughton BC, Cordonnier A, Kleijer WJ, Jaspers NG, Fawcett H, Raams A, Garritsen VH, Stary A, Avril MF, Boudsocq F, Masutani C, Hanaoka F, Fuchs RP, Sarasin A, and Lehmann AR. Molecular analysis of mutations in DNA polymerase eta in xeroderma pigmentosum-variant patients. *Proc Natl Acad Sci U S A* 99: 815–820, 2002.
36. Brown JA, Pack LR, Sanman LE, and Suo Z. Efficiency and fidelity of human DNA polymerases lambda and beta during gap-filling DNA synthesis. *DNA Repair (Amst)* 10: 24–33, 2011.
37. Brown JA, Pack LR, Sherrer SM, Kshetry AK, Newmister SA, Fowler JD, Taylor JS, and Suo Z. Identification of critical residues for the tight binding of both correct and incorrect nucleotides to human DNA polymerase lambda. *J Mol Biol* 403: 505–515, 2010.
38. Brown S, Niimi A, and Lehmann AR. Ubiquitination and deubiquitination of PCNA in response to stalling of the replication fork. *Cell Cycle* 8: 689–692, 2009.
39. Bryant HE, Schultz N, Thomas HD, Parker KM, Flower D, Lopez E, Kyle S, Meuth M, Curtin NJ, and Helleday T. Specific killing of BRCA2-deficient tumours with inhibitors of poly(ADP-ribose) polymerase. *Nature* 434: 913–917, 2005.
40. Burgers PM, Koonin EV, Bruford E, Blanco L, Burtis KC, Christman MF, Copeland WC, Friedberg EC, Hanaoka F, Hinkle DC, Lawrence CW, Nakanishi M, Ohmori H, Prakash L, Prakash S, Reynaud CA, Sugino A, Todo T, Wang Z, Weill JC, and Woodgate R. Eukaryotic DNA polymerases: proposal for a revised nomenclature. *J Biol Chem* 276: 43487–43490, 2001.
41. Burschowsky D, Rudolf F, Rabut G, Herrmann T, Peter M, and Wider G. Structural analysis of the conserved ubiquitin-binding motifs (UBMs) of the translesion polymerase iota in complex with ubiquitin. *J Biol Chem* 286: 1364–1373, 2011.
42. Cabelof DC, Guo Z, Raffoul JJ, Sobol RW, Wilson SH, Richardson A, and Heydari AR. Base excision repair deficiency caused by polymerase beta haploinsufficiency: accelerated DNA damage and increased mutational response to carcinogens. *Cancer Res* 63: 5799–5807, 2003.
43. Cabelof DC, Ikeno Y, Nyska A, Busuttill RA, Anyangwe N, Vijg J, Matherly LH, Tucker JD, Wilson SH, Richardson A, and Heydari AR. Haploinsufficiency in DNA polymerase beta increases cancer risk with age and alters mortality rate. *Cancer Res* 66: 7460–7465, 2006.
44. Canitrot Y, Cazaux C, Frechet M, Bouayadi K, Lesca C, Salles B, and Hoffmann JS. Overexpression of DNA polymerase beta in cell results in a mutator phenotype and a decreased sensitivity to anticancer drugs. *Proc Natl Acad Sci U S A* 95: 12586–12590, 1998.
45. Canitrot Y, Frechet M, Servant L, Cazaux C, and Hoffmann JS. Overexpression of DNA polymerase beta: a genomic instability enhancer process. *FASEB J* 13: 1107–1111, 1999.
46. Canitrot Y, Hoffmann JS, Calsou P, Hayakawa H, Salles B, and Cazaux C. Nucleotide excision repair DNA synthesis by excess DNA polymerase beta: a potential source of genetic instability in cancer cells. *FASEB J* 14: 1765–1774, 2000.
47. Capp JP, Boudsocq F, Bergoglio V, Trouche D, Cazaux C, Blanco L, Hoffmann JS, and Canitrot Y. The R438W polymorphism of human DNA polymerase lambda triggers cellular sensitivity to camptothecin by compromising the

- homologous recombination repair pathway. *Carcinogenesis* 31: 1742–1747, 2010.
48. Capp JP, Boudsocq F, Bertrand P, Laroche-Clary A, Pourquier P, Lopez BS, Cazaux C, Hoffmann JS, and Canitrot Y. The DNA polymerase lambda is required for the repair of non-compatible DNA double strand breaks by NHEJ in mammalian cells. *Nucleic Acids Res* 34: 2998–3007, 2006.
 49. Capp JP, Boudsocq F, Besnard AG, Lopez BS, Cazaux C, Hoffmann JS, and Canitrot Y. Involvement of DNA polymerase mu in the repair of a specific subset of DNA double-strand breaks in mammalian cells. *Nucleic Acids Res* 35: 3551–3560, 2007.
 50. Ceppi P, Novello S, Cambieri A, Longo M, Monica V, Lo Iacono M, Gaj-Lavra M, Saviozzi S, Volante M, Papotti M, and Scagliotti G. Polymerase eta mRNA expression predicts survival of non-small cell lung cancer patients treated with platinum-based chemotherapy. *Clin Cancer Res* 15: 1039–1045, 2009.
 51. Chan K, Houlbrook S, Zhang QM, Harrison M, Hickson ID, and Dianov GL. Overexpression of DNA polymerase beta results in an increased rate of frameshift mutations during base excision repair. *Mutagenesis* 22: 183–188, 2007.
 52. Chan SH, Yu AM, and McVey M. Dual roles for DNA polymerase theta in alternative end-joining repair of double-strand breaks in *Drosophila*. *PLoS Genet* 6: e1001005, 2010.
 53. Chaney SG, Campbell SL, Bassett E, and Wu Y. Recognition and processing of cisplatin- and oxaliplatin-DNA adducts. *Crit Rev Oncol Hematol* 53: 3–11, 2005.
 54. Chayot R, Danckaert A, Montagne B, and Ricchetti M. Lack of DNA polymerase mu affects the kinetics of DNA double-strand break repair and impacts on cellular senescence. *DNA Repair (Amst)* 9: 1187–1199, 2010.
 55. Chen J, Bozza W, and Zhuang Z. Ubiquitination of PCNA and its essential role in eukaryotic translesion synthesis. *Cell Biochem Biophys* 60: 47–60, 2011.
 56. Chen KH, Yakes FM, Srivastava DK, Singhal RK, Sobol RW, Horton JK, Van Houten B, and Wilson SH. Up-regulation of base excision repair correlates with enhanced protection against a DNA damaging agent in mouse cell lines. *Nucleic Acids Res* 26: 2001–2007, 1998.
 57. Chen YW, Cleaver JE, Hanaoka F, Chang CF, and Chou KM. A novel role of DNA polymerase eta in modulating cellular sensitivity to chemotherapeutic agents. *Mol Cancer Res* 4: 257–265, 2006.
 58. Choi JY, Chowdhury G, Zang H, Angel KC, Vu CC, Peterson LA, and Guengerich FP. Translesion synthesis across O6-alkylguanine DNA adducts by recombinant human DNA polymerases. *J Biol Chem* 281: 38244–38256, 2006.
 59. Choi JY, Lim S, Eoff RL, and Guengerich FP. Kinetic analysis of base-pairing preference for nucleotide incorporation opposite template pyrimidines by human DNA polymerase iota. *J Mol Biol* 389: 264–274, 2009.
 60. Choi JY, Zang H, Angel KC, Kozekov ID, Goodenough AK, Rizzo CJ, and Guengerich FP. Translesion synthesis across 1,N2-ethenoguanine by human DNA polymerases. *Chem Res Toxicol* 19: 879–886, 2006.
 61. Clairmont CA and Sweasy JB. The Pol beta-14 dominant negative rat DNA polymerase beta mutator mutant commits errors during the gap-filling step of base excision repair in *Saccharomyces cerevisiae*. *J Bacteriol* 180: 2292–2297, 1998.
 62. Covo S, Blanco L, and Livneh Z. Lesion bypass by human DNA polymerase mu reveals a template-dependent, sequence-independent nucleotidyl transferase activity. *J Biol Chem* 279: 859–865, 2004.
 63. Daube SS, Arad G, and Livneh Z. Translesion replication by DNA polymerase beta is modulated by sequence context and stimulated by fork-like flap structures in DNA. *Biochemistry* 39: 397–405, 2000.
 64. DeRose EF, Clarkson MW, Gilmore SA, Galban CJ, Tripathy A, Havener JM, Mueller GA, Ramsden DA, London RE, and Lee AL. Solution structure of polymerase mu's BRCT Domain reveals an element essential for its role in nonhomologous end joining. *Biochemistry* 46: 12100–12110, 2007.
 65. Di Lucca J, Guedj M, Lacapere JJ, Fagnoli MC, Bourillon A, Dieude P, Dupin N, Wolkenstein P, Aegerter P, Saiag P, Descamps V, Lebbe C, Basset-Seguin N, Peris K, Grandchamp B, and Soufir N. Variants of the xeroderma pigmentosum variant gene (POLH) are associated with melanoma risk. *Eur J Cancer* 45: 3228–3236, 2009.
 66. Dianov GL and Parsons JL. Co-ordination of DNA single strand break repair. *DNA Repair (Amst)* 6: 454–460, 2007.
 67. Dianova II, Sleeth KM, Allinson SL, Parsons JL, Breslin C, Caldecott KW, and Dianov GL. XRCC1-DNA polymerase beta interaction is required for efficient base excision repair. *Nucleic Acids Res* 32: 2550–2555, 2004.
 68. Diaz M, Watson NB, Turkington G, Verkoczy LK, Klinman NR, and McGregor WG. Decreased frequency and highly aberrant spectrum of ultraviolet-induced mutations in the hprt gene of mouse fibroblasts expressing antisense RNA to DNA polymerase zeta. *Mol Cancer Res* 1: 836–847, 2003.
 69. Doles J, Oliver TG, Cameron ER, Hsu G, Jacks T, Walker GC, and Hemann MT. Suppression of Rev3, the catalytic subunit of Pol{zeta}, sensitizes drug-resistant lung tumors to chemotherapy. *Proc Natl Acad Sci U S A* 107: 20786–20791, 2010.
 70. Double S, Sawaya MR, and Ellenberger T. An open and closed case for all polymerases. *Structure* 7: R31–R35, 1999.
 71. Dumstorf CA, Clark AB, Lin Q, Kissling GE, Yuan T, Kucherlapati R, McGregor WG, and Kunkel TA. Participation of mouse DNA polymerase iota in strand-biased mutagenic bypass of UV photoproducts and suppression of skin cancer. *Proc Natl Acad Sci U S A* 103: 18083–18088, 2006.
 72. Dyrkheeva NS, Lomzov AA, Pyshnyi DV, Khodyreva SN, and Lavrik OI. Efficiency of exonucleolytic action of apurinic/apyrimidinic endonuclease 1 towards matched and mismatched dNMP at the 3' terminus of different oligomeric DNA structures correlates with thermal stability of DNA duplexes. *Biochim Biophys Acta* 1764: 699–706, 2006.
 73. Efrati E, Tocco G, Eritja R, Wilson SH, and Goodman MF. Abasic translesion synthesis by DNA polymerase beta violates the "A-rule". Novel types of nucleotide incorporation by human DNA polymerase beta at an abasic lesion in different sequence contexts. *J Biol Chem* 272: 2559–2569, 1997.
 74. Emi M, Fujiwara Y, Nakajima T, Tsuchiya E, Tsuda H, Hirohashi S, Maeda Y, Tsuruta K, Miyaki M, and Nakamura Y. Frequent loss of heterozygosity for loci on chromosome 8p in hepatocellular carcinoma, colorectal cancer, and lung cancer. *Cancer Res* 52: 5368–5372, 1992.
 75. Esposito G, Godindagger I, Klein U, Yaspo ML, Cumano A, and Rajewsky K. Disruption of the Rev3l-encoded catalytic subunit of polymerase zeta in mice results in early embryonic lethality. *Curr Biol* 10: 1221–1224, 2000.
 76. Farmer H, McCabe N, Lord CJ, Tutt AN, Johnson DA, Richardson TB, Santarosa M, Dillon KJ, Hickson I, Knights C,

- Martin NM, Jackson SP, Smith GC, and Ashworth A. Targeting the DNA repair defect in BRCA mutant cells as a therapeutic strategy. *Nature* 434: 917–921, 2005.
77. Flanagan AM, Rafferty G, O'Neill A, Rynne L, Kelly J, McCann J, and Carty MP. The human POLH gene is not mutated, and is expressed in a cohort of patients with basal or squamous cell carcinoma of the skin. *Int J Mol Med* 19: 589–596, 2007.
 78. Fortini P and Dogliotti E. Base damage and single-strand break repair: mechanisms and functional significance of short- and long-patch repair subpathways. *DNA Repair (Amst)* 6: 398–409, 2007.
 79. Frank EG and Woodgate R. Increased catalytic activity and altered fidelity of human DNA polymerase iota in the presence of manganese. *J Biol Chem* 282: 24689–24696, 2007.
 80. Frechet M, Canitrot Y, Bieth A, Dogliotti E, Cazaux C, and Hoffmann JS. Deregulated DNA polymerase beta strengthens ionizing radiation-induced nucleotidic and chromosomal instabilities. *Oncogene* 21: 2320–2327, 2002.
 81. Friedberg EC. Suffering in silence: the tolerance of DNA damage. *Nat Rev Mol Cell Biol* 6: 943–953, 2005.
 82. Fromme JC, Banerjee A, and Verdine GL. DNA glycosylase recognition and catalysis. *Curr Opin Struct Biol* 14: 43–49, 2004.
 83. Fu Y, Zhu Y, Zhang K, Yeung M, Durocher D, and Xiao W. Rad6-Rad18 mediates a eukaryotic SOS response by ubiquitinating the 9-1-1 checkpoint clamp. *Cell* 133: 601–611, 2008.
 84. Gao Z, Maloney DJ, Dedkova LM, and Hecht SM. Inhibitors of DNA polymerase beta: activity and mechanism. *Bioorg Med Chem* 16: 4331–4340, 2008.
 85. Garcia-Diaz M, Bebenek K, Krahn JM, Kunkel TA, and Pedersen LC. A closed conformation for the Pol lambda catalytic cycle. *Nat Struct Mol Biol* 12: 97–98, 2005.
 86. Garcia-Diaz M, Dominguez O, Lopez-Fernandez LA, de Lera LT, Saniger ML, Ruiz JF, Parraga M, Garcia-Ortiz MJ, Kirchhoff T, del Mazo J, Bernad A, and Blanco L. DNA polymerase lambda (Pol lambda), a novel eukaryotic DNA polymerase with a potential role in meiosis. *J Mol Biol* 301: 851–867, 2000.
 87. Glick E, White LM, Elliott NA, Berg D, Kiviat NB, and Loeb LA. Mutations in DNA polymerase eta are not detected in squamous cell carcinoma of the skin. *Int J Cancer* 119: 2225–2227, 2006.
 88. Goellner EM, Svilar D, Almeida KH, and Sobol RW. Targeting DNA polymerase ss for therapeutic intervention. *Curr Mol Pharmacol* 5: 68–87, 2012.
 89. Goff JP, Shields DS, Seki M, Choi S, Epperly MW, Dixon T, Wang H, Bakkenist CJ, Dertinger SD, Torous DK, Wittschieben J, Wood RD, and Greenberger JS. Lack of DNA polymerase theta (POLQ) radiosensitizes bone marrow stromal cells *in vitro* and increases reticulocyte micronuclei after total-body irradiation. *Radiat Res* 172: 165–174, 2009.
 90. Goodhead DT. Initial events in the cellular effects of ionizing radiations: clustered damage in DNA. *Int J Radiat Biol* 65: 7–17, 1994.
 91. Goodman MF. Error-prone repair DNA polymerases in prokaryotes and eukaryotes. *Annu Rev Biochem* 71: 17–50, 2002.
 92. Goodman MF and Tiffin B. The expanding polymerase universe. *Nat Rev Mol Cell Biol* 1: 101–109, 2000.
 93. Gu H, Marth JD, Orban PC, Mossmann H, and Rajewsky K. Deletion of a DNA polymerase beta gene segment in T cells using cell type-specific gene targeting. *Science* 265: 103–106, 1994.
 94. Gueranger Q, Stary A, Aoufouchi S, Faili A, Sarasin A, Reynaud CA, and Weill JC. Role of DNA polymerases eta, iota and zeta in UV resistance and UV-induced mutagenesis in a human cell line. *DNA Repair (Amst)* 7: 1551–1562, 2008.
 95. Guo C, Kosarek-Stancel JN, Tang TS, and Friedberg EC. Y-family DNA polymerases in mammalian cells. *Cell Mol Life Sci* 66: 2363–2381, 2009.
 96. Hall MD, Okabe M, Shen DW, Liang XJ, and Gottesman MM. The role of cellular accumulation in determining sensitivity to platinum-based chemotherapy. *Annu Rev Pharmacol Toxicol* 48: 495–535, 2008.
 97. Hara K, Hashimoto H, Murakumo Y, Kobayashi S, Kogame T, Unzai S, Akashi S, Takeda S, Shimizu T, and Sato M. Crystal structure of human REV7 in complex with a human REV3 fragment and structural implication of the interaction between DNA polymerase zeta and REV1. *J Biol Chem* 285: 12299–12307, 2010.
 98. Haracska L, Acharya N, Unk I, Johnson RE, Hurwitz J, Prakash L, and Prakash S. A single domain in human DNA polymerase iota mediates interaction with PCNA: implications for translesion DNA synthesis. *Mol Cell Biol* 25: 1183–1190, 2005.
 99. Haracska L, Prakash L, and Prakash S. Role of human DNA polymerase kappa as an extender in translesion synthesis. *Proc Natl Acad Sci U S A* 99: 16000–16005, 2002.
 100. Haracska L, Torres-Ramos CA, Johnson RE, Prakash S, and Prakash L. Opposing effects of ubiquitin conjugation and SUMO modification of PCNA on replicational bypass of DNA lesions in *Saccharomyces cerevisiae*. *Mol Cell Biol* 24: 4267–4274, 2004.
 101. Havener JM, Nick McElhinny SA, Bassett E, Gauger M, Ramsden DA, and Chaney SG. Translesion synthesis past platinum DNA adducts by human DNA polymerase mu. *Biochemistry* 42: 1777–1788, 2003.
 102. Helleday T, Petermann E, Lundin C, Hodgson B, and Sharma RA. DNA repair pathways as targets for cancer therapy. *Nat Rev Cancer* 8: 193–204, 2008.
 103. Hibbert RG, Huang A, Boelens R, and Sixma TK. E3 ligase Rad18 promotes monoubiquitination rather than ubiquitin chain formation by E2 enzyme Rad6. *Proc Natl Acad Sci U S A* 108: 5590–5595, 2011.
 104. Higgins GS, Harris AL, Prevo R, Helleday T, McKenna WG, and Buffa FM. Overexpression of POLQ confers a poor prognosis in early breast cancer patients. *Oncotarget* 1: 175–184, 2010.
 105. Higgins GS, Prevo R, Lee YF, Helleday T, Muschel RJ, Taylor S, Yoshimura M, Hickson ID, Bernhard EJ, and McKenna WG. A small interfering RNA screen of genes involved in DNA repair identifies tumor-specific radiosensitization by POLQ knockdown. *Cancer Res* 70: 2984–2993, 2010.
 106. Hirose F, Hotta Y, Yamaguchi M, and Matsukage A. Difference in the expression level of DNA polymerase beta among mouse tissues: high expression in the pachytene spermatocyte. *Exp Cell Res* 181: 169–180, 1989.
 107. Hogg M, Seki M, Wood RD, Double S, and Wallace SS. Lesion bypass activity of DNA polymerase theta (POLQ) is an intrinsic property of the pol domain and depends on unique sequence inserts. *J Mol Biol* 405: 642–652, 2011.
 108. Horton JK, Baker A, Berg BJ, Sobol RW, and Wilson SH. Involvement of DNA polymerase beta in protection against

- the cytotoxicity of oxidative DNA damage. *DNA Repair (Amst)* 1: 317–333, 2002.
109. Hubscher U, Maga G, and Spadari S. Eukaryotic DNA polymerases. *Annu Rev Biochem* 71: 133–163, 2002.
 110. Inui H, Oh KS, Nadeem C, Ueda T, Khan SG, Metin A, Gozukara E, Emmert S, Slor H, Busch DB, Baker CC, Di-Giovanna JJ, Tamura D, Seitz CS, Gratchev A, Wu WH, Chung KY, Chung HJ, Azizi E, Woodgate R, Schneider TD, and Kraemer KH. Xeroderma pigmentosum-variant patients from America, Europe, and Asia. *J Invest Dermatol* 128: 2055–2068, 2008.
 111. Ishimaru C, Kuriyama I, Shimazaki N, Koiwai O, Sakaguchi K, Kato I, Yoshida H, and Mizushima Y. Cholesterol hemisuccinate: a selective inhibitor of family X DNA polymerases. *Biochem Biophys Res Commun* 354: 619–625, 2007.
 112. Ito A, Koshikawa N, Mochizuki S, Omura K, and Takenaga K. Hypoxia-inducible factor-1 mediates the expression of DNA polymerase iota in human tumor cells. *Biochem Biophys Res Commun* 351: 306–311, 2006.
 113. Jaiswal AS, Banerjee S, Aneja R, Sarkar FH, Ostrov DA, and Narayan S. DNA polymerase beta as a novel target for chemotherapeutic intervention of colorectal cancer. *PLoS One* 6: e16691, 2011.
 114. Johansson E and Macneill SA. The eukaryotic replicative DNA polymerases take shape. *Trends Biochem Sci* 35: 339–347, 2010.
 115. Johnson KA. The kinetic and chemical mechanism of high-fidelity DNA polymerases. *Biochim Biophys Acta* 1804: 1041–1048, 2010.
 116. Johnson RE, Haracska L, Prakash L, and Prakash S. Role of Hoogsteen edge hydrogen bonding at template purines in nucleotide incorporation by human DNA polymerase iota. *Mol Cell Biol* 26: 6435–6441, 2006.
 117. Johnson RE, Kondratyck CM, Prakash S, and Prakash L. hRAD30 mutations in the variant form of xeroderma pigmentosum. *Science* 285: 263–265, 1999.
 118. Johnson RE, Washington MT, Haracska L, Prakash S, and Prakash L. Eukaryotic polymerases iota and zeta act sequentially to bypass DNA lesions. *Nature* 406: 1015–1019, 2000.
 119. Johnson RE, Yu SL, Prakash S, and Prakash L. A role for yeast and human translesion synthesis DNA polymerases in promoting replication through 3-methyl adenine. *Mol Cell Biol* 27: 7198–7205, 2007.
 120. Kai M and Wang TS. Checkpoint responses to replication stalling: inducing tolerance and preventing mutagenesis. *Mutat Res* 532: 59–73, 2003.
 121. Kakar S, Watson NB, and McGregor WG. RAD18 signals DNA polymerase IOTA to stalled replication forks in cells entering S-phase with DNA damage. *Adv Exp Med Biol* 614: 137–143, 2008.
 122. Kamisuki S, Ishimaru C, Onoda K, Kuriyama I, Ida N, Sugawara F, Yoshida H, and Mizushima Y. Nodulisporol and Nodulisporone, novel specific inhibitors of human DNA polymerase lambda from a fungus, *Nodulisporium* sp. *Bioorg Med Chem* 15: 3109–3114, 2007.
 123. Kannouche P, Broughton BC, Volker M, Hanaoka F, Mulenders LH, and Lehmann AR. Domain structure, localization, and function of DNA polymerase eta, defective in xeroderma pigmentosum variant cells. *Genes Dev* 15: 158–172, 2001.
 124. Kannouche P, Fernandez de Henestrosa AR, Coull B, Vidal AE, Gray C, Zicha D, Woodgate R, and Lehmann AR. Localization of DNA polymerases eta and iota to the replication machinery is tightly co-ordinated in human cells. *EMBO J* 22: 1223–1233, 2003.
 125. Kannouche P and Sary A. Xeroderma pigmentosum variant and error-prone DNA polymerases. *Biochimie* 85: 1123–1132, 2003.
 126. Kawamoto T, Araki K, Sonoda E, Yamashita YM, Harada K, Kikuchi K, Masutani C, Hanaoka F, Nozaki K, Hashimoto N, and Takeda S. Dual roles for DNA polymerase eta in homologous DNA recombination and translesion DNA synthesis. *Mol Cell* 20: 793–799, 2005.
 127. Kawamura K, Bahar R, Seimiya M, Chiyo M, Wada A, Okada S, Hatano M, Tokuhisa T, Kimura H, Watanabe S, Honda I, Sakiyama S, Tagawa M, and O-Wang J. DNA polymerase theta is preferentially expressed in lymphoid tissues and upregulated in human cancers. *Int J Cancer* 109: 9–16, 2004.
 128. Kimura T, Nishida M, Kuramochi K, Sugawara F, Yoshida H, and Mizushima Y. Novel azaphilones, kasanosins A and B, which are specific inhibitors of eukaryotic DNA polymerases beta and lambda from *Talaromyces* sp. *Bioorg Med Chem* 16: 4594–4599, 2008.
 129. Kimura T, Takeuchi T, Kumamoto-Yonezawa Y, Ohashi E, Ohmori H, Masutani C, Hanaoka F, Sugawara F, Yoshida H, and Mizushima Y. Penicillins A and B, novel inhibitors specific to mammalian Y-family DNA polymerases. *Bioorg Med Chem* 17: 1811–1816, 2009.
 130. King NM, Nikolaishvili-Feinberg N, Bryant MF, Luche DD, Heffernan TP, Simpson DA, Hanaoka F, Kaufmann WK, and Cordeiro-Stone M. Overproduction of DNA polymerase eta does not raise the spontaneous mutation rate in diploid human fibroblasts. *DNA Repair (Amst)* 4: 714–724, 2005.
 131. Klungland A and Lindahl T. Second pathway for completion of human DNA base excision-repair: reconstitution with purified proteins and requirement for DNase IV (FEN1). *EMBO J* 16: 3341–3348, 1997.
 132. Kobayashi S, Valentine MR, Pham P, O'Donnell M, and Goodman MF. Fidelity of *Escherichia coli* DNA polymerase IV. Preferential generation of small deletion mutations by dNTP-stabilized misalignment. *J Biol Chem* 277: 34198–34207, 2002.
 133. Kohzaki M, Nishihara K, Hirota K, Sonoda E, Yoshimura M, Ekino S, Butler JE, Watanabe M, Halazonetis TD, and Takeda S. DNA polymerases nu and theta are required for efficient immunoglobulin V gene diversification in chicken. *J Cell Biol* 189: 1117–1127, 2010.
 134. Kunkel TA. Considering the cancer consequences of altered DNA polymerase function. *Cancer Cell* 3: 105–110, 2003.
 135. Kunkel TA and Bebenek K. DNA replication fidelity. *Annu Rev Biochem* 69: 497–529, 2000.
 136. Kunkel TA and Burgers PM. Dividing the workload at a eukaryotic replication fork. *Trends Cell Biol* 18: 521–527, 2008.
 137. Kusumoto R, Masutani C, Shimmyo S, Iwai S, and Hanaoka F. DNA binding properties of human DNA polymerase eta: implications for fidelity and polymerase switching of translesion synthesis. *Genes Cells* 9: 1139–1150, 2004.
 138. Lang T, Dalal S, Chikova A, DiMaio D, and Sweasy JB. The E295K DNA polymerase beta gastric cancer-associated variant interferes with base excision repair and induces cellular transformation. *Mol Cell Biol* 27: 5587–5596, 2007.

139. Lang T, Maitra M, Starcevic D, Li SX, and Sweasy JB. A DNA polymerase beta mutant from colon cancer cells induces mutations. *Proc Natl Acad Sci U S A* 101: 6074–6079, 2004.
140. Lange SS, Takata K, and Wood RD. DNA polymerases and cancer. *Nat Rev Cancer* 11: 96–110, 2011.
141. Lawson MH, Cummings NM, Rassl DM, Russell R, Brenton JD, Rintoul RC, and Murphy G. Two novel determinants of etoposide resistance in small cell lung cancer. *Cancer Res* 71: 4877–4887, 2011.
142. Lee GH and Matsushita H. Genetic linkage between Pol iota deficiency and increased susceptibility to lung tumors in mice. *Cancer Sci* 96: 256–259, 2005.
143. Lee JW, Blanco L, Zhou T, Garcia-Diaz M, Bebenek K, Kunkel TA, Wang Z, and Povirk LF. Implication of DNA polymerase lambda in alignment-based gap filling for nonhomologous DNA end joining in human nuclear extracts. *J Biol Chem* 279: 805–811, 2004.
144. Lehmann AR, Niimi A, Ogi T, Brown S, Sabbioneda S, Wing JF, Kannouche PL, and Green CM. Translesion synthesis: Y-family polymerases and the polymerase switch. *DNA Repair (Amst)* 6: 891–899, 2007.
145. Lemee F, Bergoglio V, Fernandez-Vidal A, Machado-Silva A, Pillaire MJ, Bieth A, Gentil C, Baker L, Martin AL, Leduc C, Lam E, Magdeleine E, Filleron T, Oumouhou N, Kaina B, Seki M, Grimal F, Lacroix-Triki M, Thompson A, Roche H, Bourdon JC, Wood RD, Hoffmann JS, and Cazaux C. DNA polymerase theta up-regulation is associated with poor survival in breast cancer, perturbs DNA replication, and promotes genetic instability. *Proc Natl Acad Sci U S A* 107: 13390–13395, 2010.
146. Li SS, Gao Z, Feng X, Jones SH, and Hecht SM. Plant sterols as selective DNA polymerase beta lyase inhibitors and potentiators of bleomycin cytotoxicity. *Bioorg Med Chem* 12: 4253–4258, 2004.
147. Lichon V and Khachemoune A. Xeroderma pigmentosum: beyond skin cancer. *J Drugs Dermatol* 6: 281–288, 2007.
148. Lin X, Trang J, Okuda T, and Howell SB. DNA polymerase zeta accounts for the reduced cytotoxicity and enhanced mutagenicity of cisplatin in human colon carcinoma cells that have lost DNA mismatch repair. *Clin Cancer Res* 12: 563–568, 2006.
149. Lindahl T and Wood RD. Quality control by DNA repair. *Science* 286: 1897–1905, 1999.
150. Ling H, Boudsocq F, Woodgate R, and Yang W. Crystal structure of a Y-family DNA polymerase in action: a mechanism for error-prone and lesion-bypass replication. *Cell* 107: 91–102, 2001.
151. Loh SY, Mistry P, Kelland LR, Abel G, and Harrap KR. Reduced drug accumulation as a major mechanism of acquired resistance to cisplatin in a human ovarian carcinoma cell line: circumvention studies using novel platinum (II) and (IV) ammine/amine complexes. *Br J Cancer* 66: 1109–1115, 1992.
152. Lone S, Townson SA, Uljon SN, Johnson RE, Brahma A, Nair DT, Prakash S, Prakash L, and Aggarwal AK. Human DNA polymerase kappa encircles DNA: implications for mismatch extension and lesion bypass. *Mol Cell* 25: 601–614, 2007.
153. Lucas D, Escudero B, Ligos JM, Segovia JC, Estrada JC, Terrados G, Blanco L, Samper E, and Bernad A. Altered hematopoiesis in mice lacking DNA polymerase mu is due to inefficient double-strand break repair. *PLoS Genet* 5: e1000389, 2009.
154. Ma Y, Lu H, Tippin B, Goodman MF, Shimazaki N, Koiwai O, Hsieh CL, Schwarz K, and Lieber MR. A biochemically defined system for mammalian nonhomologous DNA end joining. *Mol Cell* 16: 701–713, 2004.
155. Macoska JA, Trybus TM, Sakr WA, Wolf MC, Benson PD, Powell IJ, and Pontes JE. Fluorescence *in situ* hybridization analysis of 8p allelic loss and chromosome 8 instability in human prostate cancer. *Cancer Res* 54: 3824–3830, 1994.
156. Maga G, Villani G, Crespan E, Wimmer U, Ferrari E, Bertocchi B, and Hubscher U. 8-oxo-guanine bypass by human DNA polymerases in the presence of auxiliary proteins. *Nature* 447: 606–608, 2007.
157. Mahajan KN, Nick McElhinny SA, Mitchell BS, and Ramsden DA. Association of DNA polymerase mu (pol mu) with Ku and ligase IV: role for pol mu in end-joining double-strand break repair. *Mol Cell Biol* 22: 5194–5202, 2002.
158. Mahaney BL, Meek K, and Lees-Miller SP. Repair of ionizing radiation-induced DNA double-strand breaks by non-homologous end-joining. *Biochem J* 417: 639–650, 2009.
159. Maitra M, Gudzelak A, Jr., Li SX, Matsumoto Y, Eckert KA, Jager J, and Sweasy JB. Threonine 79 is a hinge residue that governs the fidelity of DNA polymerase beta by helping to position the DNA within the active site. *J Biol Chem* 277: 35550–35560, 2002.
160. Martomo SA, Saribasak H, Yokoi M, Hanaoka F, and Gearhart PJ. Reevaluation of the role of DNA polymerase theta in somatic hypermutation of immunoglobulin genes. *DNA Repair (Amst)* 7: 1603–1608, 2008.
161. Masutani C, Kusumoto R, Iwai S, and Hanaoka F. Mechanisms of accurate translesion synthesis by human DNA polymerase eta. *EMBO J* 19: 3100–3109, 2000.
162. Masutani C, Kusumoto R, Yamada A, Dohmae N, Yokoi M, Yuasa M, Araki M, Iwai S, Takio K, and Hanaoka F. The XPV (xeroderma pigmentosum variant) gene encodes human DNA polymerase eta. *Nature* 399: 700–704, 1999.
163. Matsukage A, Yamaguchi M, Utsumi KR, Hayashi Y, Ueda R, and Yoshida MC. Assignment of the gene for human DNA polymerase beta (POLB) to chromosome 8. *Jpn J Cancer Res* 77: 330–333, 1986.
164. Matsumoto Y and Kim K. Excision of deoxyribose phosphate residues by DNA polymerase beta during DNA repair. *Science* 269: 699–702, 1995.
165. McCulloch SD, Kokoska RJ, Chilkova O, Welch CM, Johansson E, Burgers PM, and Kunkel TA. Enzymatic switching for efficient and accurate translesion DNA replication. *Nucleic Acids Res* 32: 4665–4675, 2004.
166. McCulloch SD, Kokoska RJ, Masutani C, Iwai S, Hanaoka F, and Kunkel TA. Preferential cis-syn thymine dimer bypass by DNA polymerase eta occurs with biased fidelity. *Nature* 428: 97–100, 2004.
167. McCulloch SD and Kunkel TA. The fidelity of DNA synthesis by eukaryotic replicative and translesion synthesis polymerases. *Cell Res* 18: 148–161, 2008.
168. McCullough AK, Dodson ML, and Lloyd RS. Initiation of base excision repair: glycosylase mechanisms and structures. *Annu Rev Biochem* 68: 255–285, 1999.
169. McDonald JP, Tissier A, Frank EG, Iwai S, Hanaoka F, and Woodgate R. DNA polymerase iota and related rad30-like enzymes. *Philos Trans R Soc Lond B Biol Sci* 356: 53–60, 2001.
170. McIlwraith MJ, Vaisman A, Liu Y, Fanning E, Woodgate R, and West SC. Human DNA polymerase eta promotes DNA synthesis from strand invasion intermediates of homologous recombination. *Mol Cell* 20: 783–792, 2005.

171. Meijer C, Mulder NH, Timmer-Bosscha H, Sluiter WJ, Meersma GJ, and de Vries EG. Relationship of cellular glutathione to the cytotoxicity and resistance of seven platinum compounds. *Cancer Res* 52: 6885–6889, 1992.
172. Mellish KJ, Kelland LR, and Harrap KR. *In vitro* platinum drug chemosensitivity of human cervical squamous cell carcinoma cell lines with intrinsic and acquired resistance to cisplatin. *Br J Cancer* 68: 240–250, 1993.
173. Michel B, Grompone G, Flores MJ, and Bidnenko V. Multiple pathways process stalled replication forks. *Proc Natl Acad Sci U S A* 101: 12783–12788, 2004.
174. Minko IG, Harbut MB, Kozekov ID, Kozekova A, Jakobs PM, Olson SB, Moses RE, Harris TM, Rizzo CJ, and Lloyd RS. Role for DNA polymerase kappa in the processing of N2-N2-guanine interstrand cross-links. *J Biol Chem* 283: 17075–17082, 2008.
175. Mizushima Y, Motoshima H, Yamaguchi Y, Takeuchi T, Hirano K, Sugawara F, and Yoshida H. 3-O-methylfunicone, a selective inhibitor of mammalian Y-family DNA polymerases from an Australian sea salt fungal strain. *Mar Drugs* 7: 624–639, 2009.
176. Mizushima Y, Nakagawa K, Shibata A, Awata Y, Kuriyama I, Shimazaki N, Koiwai O, Uchiyama Y, Sakaguchi K, Miyazawa T, and Yoshida H. Inhibitory effect of tocotrienol on eukaryotic DNA polymerase lambda and angiogenesis. *Biochem Biophys Res Commun* 339: 949–955, 2006.
177. Moon AF, Garcia-Diaz M, Batra VK, Beard WA, Bebenek K, Kunkel TA, Wilson SH, and Pedersen LC. The X family portrait: structural insights into biological functions of X family polymerases. *DNA Repair (Amst)* 6: 1709–1725, 2007.
178. Mueller GA, Moon AF, Deroose EF, Havener JM, Ramsden DA, Pedersen LC, and London RE. A comparison of BRCT domains involved in nonhomologous end-joining: introducing the solution structure of the BRCT domain of polymerase lambda. *DNA Repair (Amst)* 7: 1340–1351, 2008.
179. Muzzini DM, Plevani P, Boulton SJ, Cassata G, and Marini F. *Caenorhabditis elegans* POLQ-1 and HEL-308 function in two distinct DNA interstrand cross-link repair pathways. *DNA Repair (Amst)* 7: 941–950, 2008.
180. Naganuma M, Nishida M, Kuramochi K, Sugawara F, Yoshida H, and Mizushima Y. 1-deoxyruberactone, a novel specific inhibitor of families X and Y of eukaryotic DNA polymerases from a fungal strain derived from sea algae. *Bioorg Med Chem* 16: 2939–2944, 2008.
181. Nair DT, Johnson RE, Prakash S, Prakash L, and Aggarwal AK. Replication by human DNA polymerase-iota occurs by Hoogsteen base-pairing. *Nature* 430: 377–380, 2004.
182. Neijenhuis S, Begg AC, and Vens C. Radiosensitization by a dominant negative to DNA polymerase beta is DNA polymerase beta-independent and XRCC1-dependent. *Radiother Oncol* 76: 123–128, 2005.
183. Neijenhuis S, Verwijs-Janssen M, Kasten-Pisula U, Rumping G, Borgmann K, Dikomey E, Begg AC, and Vens C. Mechanism of cell killing after ionizing radiation by a dominant negative DNA polymerase beta. *DNA Repair (Amst)* 8: 336–346, 2009.
184. Nick McElhinny SA and Ramsden DA. Polymerase mu is a DNA-directed DNA/RNA polymerase. *Mol Cell Biol* 23: 2309–2315, 2003.
185. O-Wang J, Kawamura K, Tada Y, Ohmori H, Kimura H, Sakiyama S, and Tagawa M. DNA polymerase kappa, implicated in spontaneous and DNA damage-induced mutagenesis, is overexpressed in lung cancer. *Cancer Res* 61: 5366–5369, 2001.
186. Ochs K, Sobol RW, Wilson SH, and Kaina B. Cells deficient in DNA polymerase beta are hypersensitive to alkylating agent-induced apoptosis and chromosomal breakage. *Cancer Res* 59: 1544–1551, 1999.
187. Ogi T, Kannouche P, and Lehmann AR. Localisation of human Y-family DNA polymerase kappa: relationship to PCNA foci. *J Cell Sci* 118: 129–136, 2005.
188. Ogi T and Lehmann AR. The Y-family DNA polymerase kappa (pol kappa) functions in mammalian nucleotide-excision repair. *Nat Cell Biol* 8: 640–642, 2006.
189. Ogi T, Limsirichaikul S, Overmeer RM, Volker M, Takenaka K, Cloney R, Nakazawa Y, Niimi A, Miki Y, Jaspers NG, Mullenders LH, Yamashita S, Foustier MI, and Lehmann AR. Three DNA polymerases, recruited by different mechanisms, carry out NER repair synthesis in human cells. *Mol Cell* 37: 714–727, 2010.
190. Ohashi E, Bebenek K, Matsuda T, Feaver WJ, Gerlach VL, Friedberg EC, Ohmori H, and Kunkel TA. Fidelity and processivity of DNA synthesis by DNA polymerase kappa, the product of the human DINB1 gene. *J Biol Chem* 275: 39678–39684, 2000.
191. Ohashi E, Hanafusa T, Kamei K, Song I, Tomida J, Hashimoto H, Vaziri C, and Ohmori H. Identification of a novel REV1-interacting motif necessary for DNA polymerase kappa function. *Genes Cells* 14: 101–111, 2009.
192. Ohba T, Kometani T, Shoji F, Yano T, Yoshino I, Taguchi K, Kuraoka I, Oda S, and Maehara Y. Expression of an X-family DNA polymerase, pol lambda, in the respiratory epithelium of non-small cell lung cancer patients with habitual smoking. *Mutat Res* 677: 66–71, 2009.
193. Ohkumo T, Kondo Y, Yokoi M, Tsukamoto T, Yamada A, Sugimoto T, Kanao R, Higashi Y, Kondoh H, Tatematsu M, Masutani C, and Hanaoka F. UV-B radiation induces epithelial tumors in mice lacking DNA polymerase eta and mesenchymal tumors in mice deficient for DNA polymerase iota. *Mol Cell Biol* 26: 7696–7706, 2006.
194. Pan Q, Fang Y, Xu Y, Zhang K, and Hu X. Down-regulation of DNA polymerases kappa, eta, iota, and zeta in human lung, stomach, and colorectal cancers. *Cancer Lett* 217: 139–147, 2005.
195. Parsons JL, Dianova II, Allinson SL, and Dianov GL. DNA polymerase beta promotes recruitment of DNA ligase III alpha-XRCC1 to sites of base excision repair. *Biochemistry* 44: 10613–10619, 2005.
196. Pascucci B, Stucki M, Jonsson ZO, Dogliotti E, and Hubscher U. Long patch base excision repair with purified human proteins. DNA ligase I as patch size mediator for DNA polymerases delta and epsilon. *J Biol Chem* 274: 33696–33702, 1999.
197. Pelletier H, Sawaya MR, Kumar A, Wilson SH, and Kraut J. Structures of ternary complexes of rat DNA polymerase beta, a DNA template-primer, and ddCTP. *Science* 264: 1891–1903, 1994.
198. Petta TB, Nakajima S, Zlatanou A, Despras E, Couve-Privat S, Ishchenko A, Sarasin A, Yasui A, and Kannouche P. Human DNA polymerase iota protects cells against oxidative stress. *EMBO J* 27: 2883–2895, 2008.
199. Pillaire MJ, Betous R, Conti C, Czaplicki J, Pasero P, Bensimon A, Cazaux C, and Hoffmann JS. Upregulation of error-prone DNA polymerases beta and kappa slows down fork progression without activating the replication checkpoint. *Cell Cycle* 6: 471–477, 2007.
200. Pillaire MJ, Selves J, Gordien K, Gourraud PA, Gentil C, Danjoux M, Do C, Negre V, Bieth A, Guimbaud R, Trouche

- D, Pasero P, Mechali M, Hoffmann JS, and Cazaux C. A 'DNA replication' signature of progression and negative outcome in colorectal cancer. *Oncogene* 29: 876–887, 2010.
201. Poltoratsky V, Horton JK, Prasad R, Beard WA, Woodgate R, and Wilson SH. Negligible impact of pol iota expression on the alkylation sensitivity of pol beta-deficient mouse fibroblast cells. *DNA Repair (Amst)* 7: 830–833, 2008.
 202. Poltoratsky V, Horton JK, Prasad R, and Wilson SH. REV1 mediated mutagenesis in base excision repair deficient mouse fibroblast. *DNA Repair (Amst)* 4: 1182–1188, 2005.
 203. Prakash S, Johnson RE, and Prakash L. Eukaryotic translesion synthesis DNA polymerases: specificity of structure and function. *Annu Rev Biochem* 74: 317–353, 2005.
 204. Prakasha Gowda AS, Polizzi JM, Eckert KA, and Spratt TE. Incorporation of gemcitabine and cytarabine into DNA by DNA polymerase beta and ligase III/XRCC1. *Biochemistry* 49: 4833–4840, 2010.
 205. Prasad R, Beard WA, Strauss PR, and Wilson SH. Human DNA polymerase beta deoxyribose phosphate lyase. Substrate specificity and catalytic mechanism. *J Biol Chem* 273: 15263–15270, 1998.
 206. Prasad R, Longley MJ, Sharief FS, Hou EW, Copeland WC, and Wilson SH. Human DNA polymerase theta possesses 5'-dRP lyase activity and functions in single-nucleotide base excision repair *in vitro*. *Nucleic Acids Res* 37: 1868–1877, 2009.
 207. Prasad R, Widen SG, Singhal RK, Watkins J, Prakash L, and Wilson SH. Yeast open reading frame YCR14C encodes a DNA beta-polymerase-like enzyme. *Nucleic Acids Res* 21: 5301–5307, 1993.
 208. Preston BD, Albertson TM, and Herr AJ. DNA replication fidelity and cancer. *Semin Cancer Biol* 20: 281–293, 2010.
 209. Rajpal DK, Wu X, and Wang Z. Alteration of ultraviolet-induced mutagenesis in yeast through molecular modulation of the REV3 and REV7 gene expression. *Mutat Res* 461: 133–143, 2000.
 210. Rechakoblit O, Zhang Y, Guo D, Wang Z, Amin S, Krzeminsky J, Louneva N, and Geacintov NE. trans-Lesion synthesis past bulky benzo[a]pyrene diol epoxide N2-dG and N6-dA lesions catalyzed by DNA bypass polymerases. *J Biol Chem* 277: 30488–30494, 2002.
 211. Roettger MP, Fiala KA, Sompalli S, Dong Y, and Suo Z. Pre-steady-state kinetic studies of the fidelity of human DNA polymerase mu. *Biochemistry* 43: 13827–13838, 2004.
 212. Ruiz JF, Juarez R, Garcia-Diaz M, Terrados G, Picher AJ, Gonzalez-Barrera S, Fernandez de Henestrosa AR, and Blanco L. Lack of sugar discrimination by human Pol mu requires a single glycine residue. *Nucleic Acids Res* 31: 4441–4449, 2003.
 213. Ruiz JF, Lucas D, Garcia-Palomero E, Saez AI, Gonzalez MA, Piris MA, Bernad A, and Blanco L. Overexpression of human DNA polymerase mu (Pol mu) in a Burkitt's lymphoma cell line affects the somatic hypermutation rate. *Nucleic Acids Res* 32: 5861–5873, 2004.
 214. Sabbioneda S, Minesinger BK, Giannattasio M, Plevani P, Muzi-Falconi M, and Jinks-Robertson S. The 9-1-1 checkpoint clamp physically interacts with polzeta and is partially required for spontaneous polzeta-dependent mutagenesis in *Saccharomyces cerevisiae*. *J Biol Chem* 280: 38657–38665, 2005.
 215. Sanderson RJ and Mosbaugh DW. Fidelity and mutational specificity of uracil-initiated base excision DNA repair synthesis in human glioblastoma cell extracts. *J Biol Chem* 273: 24822–24831, 1998.
 216. Schmutz V, Janel-Bintz R, Wagner J, Biard D, Shiomi N, Fuchs RP, and Cordonnier AM. Role of the ubiquitin-binding domain of Poleta in Rad18-independent translesion DNA synthesis in human cell extracts. *Nucleic Acids Res* 38: 6456–6465, 2010.
 217. Seki M, Marini F, and Wood RD. POLQ (Pol theta), a DNA polymerase and DNA-dependent ATPase in human cells. *Nucleic Acids Res* 31: 6117–6126, 2003.
 218. Seki M, Masutani C, Yang LW, Schuffert A, Iwai S, Bahar I, and Wood RD. High-efficiency bypass of DNA damage by human DNA polymerase Q. *EMBO J* 23: 4484–4494, 2004.
 219. Seki M and Wood RD. DNA polymerase theta (POLQ) can extend from mismatches and from bases opposite a (6–4) photoproduct. *DNA Repair (Amst)* 7: 119–127, 2008.
 220. Servant L, Cazaux C, Bieth A, Iwai S, Hanaoka F, and Hoffmann JS. A role for DNA polymerase beta in mutagenic UV lesion bypass. *J Biol Chem* 277: 50046–50053, 2002.
 221. Sharma RA and Dianov GL. Targeting base excision repair to improve cancer therapies. *Mol Aspects Med* 28: 345–374, 2007.
 222. Shevelev IV and Hubscher U. The 3' 5' exonucleases. *Nat Rev Mol Cell Biol* 3: 364–376, 2002.
 223. Shimizu T, Azuma T, Ishiguro M, Kanjo N, Yamada S, and Ohmori H. Normal immunoglobulin gene somatic hypermutation in Pol kappa-Pol iota double-deficient mice. *Immunol Lett* 98: 259–264, 2005.
 224. Silverstein TD, Johnson RE, Jain R, Prakash L, Prakash S, and Aggarwal AK. Structural basis for the suppression of skin cancers by DNA polymerase eta. *Nature* 465: 1039–1043, 2010.
 225. Sleeth KM, Robson RL, and Dianov GL. Exchangeability of mammalian DNA ligases between base excision repair pathways. *Biochemistry* 43: 12924–12930, 2004.
 226. Sobol RW, Horton JK, Kuhn R, Gu H, Singhal RK, Prasad R, Rajewsky K, and Wilson SH. Requirement of mammalian DNA polymerase-beta in base-excision repair. *Nature* 379: 183–186, 1996.
 227. Sobol RW, Watson DE, Nakamura J, Yakes FM, Hou E, Horton JK, Ladapo J, Van Houten B, Swenberg JA, Tindall KR, Samson LD, and Wilson SH. Mutations associated with base excision repair deficiency and methylation-induced genotoxic stress. *Proc Natl Acad Sci U S A* 99: 6860–6865, 2002.
 228. Srivastava DK, Husain I, Arteaga CL, and Wilson SH. DNA polymerase beta expression differences in selected human tumors and cell lines. *Carcinogenesis* 20: 1049–1054, 1999.
 229. Stachelek GC, Dalal S, Donigan KA, Campisi Hegan D, Sweasy JB, and Glazer PM. Potentiation of temozolomide cytotoxicity by inhibition of DNA polymerase beta is accentuated by BRCA2 mutation. *Cancer Res* 70: 409–417, 2010.
 230. Stancel JN, McDaniel LD, Velasco S, Richardson J, Guo C, and Friedberg EC. Polk mutant mice have a spontaneous mutator phenotype. *DNA Repair (Amst)* 8: 1355–1362, 2009.
 231. Starcevic D, Dalal S, and Sweasy JB. Is there a link between DNA polymerase beta and cancer? *Cell Cycle* 3: 998–1001, 2004.
 232. Steitz TA. DNA polymerases: structural diversity and common mechanisms. *J Biol Chem* 274: 17395–17398, 1999.
 233. Sukhanova MV, Khodyreva SN, Lebedeva NA, Prasad R, Wilson SH, and Lavrik OI. Human base excision repair enzymes apurinic/apyrimidinic endonuclease1 (APE1), DNA polymerase beta and poly(ADP-ribose) polymerase 1: interplay between strand-displacement DNA synthesis and

- proofreading exonuclease activity. *Nucleic Acids Res* 33: 1222–1229, 2005.
234. Sweasy JB, Lang T, Starcevic D, Sun KW, Lai CC, Dimaio D, and Dalal S. Expression of DNA polymerase {beta} cancer-associated variants in mouse cells results in cellular transformation. *Proc Natl Acad Sci U S A* 102: 14350–14355, 2005.
 235. Takenaka K, Ogi T, Okada T, Sonoda E, Guo C, Friedberg EC, and Takeda S. Involvement of vertebrate Polkappa in translesion DNA synthesis across DNA monoalkylation damage. *J Biol Chem* 281: 2000–2004, 2006.
 236. Tanioka M, Masaki T, Ono R, Nagano T, Otoshi-Honda E, Matsumura Y, Takigawa M, Inui H, Miyachi Y, Moriwaki S, and Nishigori C. Molecular analysis of DNA polymerase eta gene in Japanese patients diagnosed as xeroderma pigmentosum variant type. *J Invest Dermatol* 127: 1745–1751, 2007.
 237. Teng KY, Qiu MZ, Li ZH, Luo HY, Zeng ZL, Luo RZ, Zhang HZ, Wang ZQ, Li YH, and Xu RH. DNA polymerase eta protein expression predicts treatment response and survival of metastatic gastric adenocarcinoma patients treated with oxaliplatin-based chemotherapy. *J Transl Med* 8: 126, 2010.
 238. Terrados G, Capp JP, Canitrot Y, Garcia-Diaz M, Bebenek K, Kirchhoff T, Villanueva A, Boudsocq F, Bergoglio V, Cazaux C, Kunkel TA, Hoffmann JS, and Blanco L. Characterization of a natural mutator variant of human DNA polymerase lambda which promotes chromosomal instability by compromising NHEJ. *PLoS One* 4: e7290, 2009.
 239. Thakur M, Wernick M, Collins C, Limoli CL, Crowley E, and Cleaver JE. DNA polymerase eta undergoes alternative splicing, protects against UV sensitivity and apoptosis, and suppresses Mre11-dependent recombination. *Genes Chromosomes Cancer* 32: 222–235, 2001.
 240. Trivedi RN, Almeida KH, Fornasaglio JL, Schamus S, and Sobol RW. The role of base excision repair in the sensitivity and resistance to temozolomide-mediated cell death. *Cancer Res* 65: 6394–6400, 2005.
 241. Trivedi RN, Wang XH, Jelezcova E, Goellner EM, Tang JB, and Sobol RW. Human methyl purine DNA glycosylase and DNA polymerase beta expression collectively predict sensitivity to temozolomide. *Mol Pharmacol* 74: 505–516, 2008.
 242. Uchiyama Y, Takeuchi R, Kodera H, and Sakaguchi K. Distribution and roles of X-family DNA polymerases in eukaryotes. *Biochimie* 91: 165–170, 2009.
 243. Vaisman A and Chaney SG. The efficiency and fidelity of translesion synthesis past cisplatin and oxaliplatin GpG adducts by human DNA polymerase beta. *J Biol Chem* 275: 13017–13025, 2000.
 244. Vaisman A, Masutani C, Hanaoka F, and Chaney SG. Efficient translesion replication past oxaliplatin and cisplatin GpG adducts by human DNA polymerase eta. *Biochemistry* 39: 4575–4580, 2000.
 245. Vaisman A, Warren MW, and Chaney SG. The effect of DNA structure on the catalytic efficiency and fidelity of human DNA polymerase beta on templates with platinum-DNA adducts. *J Biol Chem* 276: 18999–19005, 2001.
 246. Valk K, Vooder T, Kolde R, Reintam MA, Petzold C, Vilo J, and Metspalu A. Gene expression profiles of non-small cell lung cancer: survival prediction and new biomarkers. *Oncology* 79: 283–292, 2010.
 247. van der Bosch K, Becker I, Savelyeva L, Bruderlein S, Schlag P, and Schwab M. Deletions in the short arm of chromosome 8 are present in up to 90% of human colorectal cancer cell lines. *Genes Chromosomes Cancer* 5: 91–95, 1992.
 248. Varadi V, Bevier M, Grzybowska E, Johansson R, Enquist K, Henriksson R, Butkiewicz D, Pamula-Pilat J, Tecza K, Hemminki K, Lenner P, and Forsti A. Genetic variation in genes encoding for polymerase zeta subunits associates with breast cancer risk, tumour characteristics and survival. *Breast Cancer Res Treat* 129: 235–245, 2011.
 249. Vens C, Dahmen-Mooren E, Verwijs-Janssen M, Blyweert W, Graversen L, Bartelink H, and Begg AC. The role of DNA polymerase beta in determining sensitivity to ionizing radiation in human tumor cells. *Nucleic Acids Res* 30: 2995–3004, 2002.
 250. Vermeulen C, Bertocci B, Begg AC, and Vens C. Ionizing radiation sensitivity of DNA polymerase lambda-deficient cells. *Radiat Res* 168: 683–688, 2007.
 251. Wang H, Wu W, Wang HW, Wang S, Chen Y, Zhang X, Yang J, Zhao S, Ding HF, and Lu D. Analysis of specialized DNA polymerases expression in human gliomas: association with prognostic significance. *Neuro Oncol* 12: 679–686, 2010.
 252. Wang Y, Woodgate R, McManus TP, Mead S, McCormick JJ, and Maher VM. Evidence that in xeroderma pigmentosum variant cells, which lack DNA polymerase eta, DNA polymerase iota causes the very high frequency and unique spectrum of UV-induced mutations. *Cancer Res* 67: 3018–3026, 2007.
 253. Washington MT, Johnson RE, Prakash L, and Prakash S. Accuracy of lesion bypass by yeast and human DNA polymerase eta. *Proc Natl Acad Sci U S A* 98: 8355–8360, 2001.
 254. Washington MT, Johnson RE, Prakash L, and Prakash S. Human DINB1-encoded DNA polymerase kappa is a promiscuous extender of mispaired primer termini. *Proc Natl Acad Sci U S A* 99: 1910–1914, 2002.
 255. Watanabe K, Tateishi S, Kawasuji M, Tsurimoto T, Inoue H, and Yamaizumi M. Rad18 guides poleta to replication stalling sites through physical interaction and PCNA monoubiquitination. *EMBO J* 23: 3886–3896, 2004.
 256. Waters LS, Minesinger BK, Wiltrout ME, D'Souza S, Woodruff RV, and Walker GC. Eukaryotic translesion polymerases and their roles and regulation in DNA damage tolerance. *Microbiol Mol Biol Rev* 73: 134–154, 2009.
 257. Weinfeld M. Complexities of BER. *Prog Nucleic Acid Res Mol Biol* 68: 125–127, 2001.
 258. Wiebauer K and Jiricny J. Mismatch-specific thymine DNA glycosylase and DNA polymerase beta mediate the correction of G.T mispairs in nuclear extracts from human cells. *Proc Natl Acad Sci U S A* 87: 5842–5845, 1990.
 259. Wimmer U, Ferrari E, Hunziker P, and Hubscher U. Control of DNA polymerase lambda stability by phosphorylation and ubiquitination during the cell cycle. *EMBO Rep* 9: 1027–1033, 2008.
 260. Wittschieben J, Shivji MK, Lalani E, Jacobs MA, Marini F, Gearhart PJ, Rosewell I, Stamp G, and Wood RD. Disruption of the developmentally regulated Rev3l gene causes embryonic lethality. *Curr Biol* 10: 1217–1220, 2000.
 261. Wittschieben JP, Reshmi SC, Gollin SM, and Wood RD. Loss of DNA polymerase zeta causes chromosomal instability in mammalian cells. *Cancer Res* 66: 134–142, 2006.
 262. Yagi Y, Ogawara D, Iwai S, Hanaoka F, Akiyama M, and Maki H. DNA polymerases eta and kappa are responsible for error-free translesion DNA synthesis activity over a cis-syn thymine dimer in *Xenopus laevis* oocyte extracts. *DNA Repair (Amst)* 4: 1252–1269, 2005.

263. Yamtich J, Starcevic D, Lauper J, Smith E, Shi I, Rangarajan S, Jaeger J, and Sweasy JB. Hinge residue I174 is critical for proper dNTP selection by DNA polymerase beta. *Biochemistry* 49: 2326–2334, 2010.
264. Yamtich J and Sweasy JB. DNA polymerase family X: function, structure, and cellular roles. *Biochim Biophys Acta* 1804: 1136–1150, 2010.
265. Yang J, Chen Z, Liu Y, Hickey RJ, and Malkas LH. Altered DNA polymerase iota expression in breast cancer cells leads to a reduction in DNA replication fidelity and a higher rate of mutagenesis. *Cancer Res* 64: 5597–5607, 2004.
266. Yang J, Parsons J, Nicolay NH, Caporali S, Harrington CF, Singh R, Finch D, D'Atri S, Farmer PB, Johnston PG, McKenna WG, Dianov G, and Sharma RA. Cells deficient in the base excision repair protein, DNA polymerase beta, are hypersensitive to oxaliplatin chemotherapy. *Oncogene* 29: 463–468, 2010.
267. Yasui M, Suzuki N, Laxmi YR, and Shibutani S. Translesion synthesis past tamoxifen-derived DNA adducts by human DNA polymerases eta and kappa. *Biochemistry* 45: 12167–12174, 2006.
268. Yoshimura M, Kohzaki M, Nakamura J, Asagoshi K, Sonoda E, Hou E, Prasad R, Wilson SH, Tano K, Yasui A, Lan L, Seki M, Wood RD, Arakawa H, Buerstedde JM, Hochegger H, Okada T, Hiraoka M, and Takeda S. Vertebrate POLQ and POLbeta cooperate in base excision repair of oxidative DNA damage. *Mol Cell* 24: 115–125, 2006.
269. Zahn KE, Wallace SS, and Doublie S. DNA polymerases provide a canon of strategies for translesion synthesis past oxidatively generated lesions. *Curr Opin Struct Biol* 21: 358–369, 2011.
270. Zhang Y, Wu X, Guo D, Rechkoblit O, Taylor JS, Geacintov NE, and Wang Z. Lesion bypass activities of human DNA polymerase mu. *J Biol Chem* 277: 44582–44587, 2002.
271. Zhang Y, Wu X, Yuan F, Xie Z, and Wang Z. Highly frequent frameshift DNA synthesis by human DNA polymerase mu. *Mol Cell Biol* 21: 7995–8006, 2001.
272. Zhang Y, Yuan F, Wu X, Rechkoblit O, Taylor JS, Geacintov NE, and Wang Z. Error-prone lesion bypass by human DNA polymerase eta. *Nucleic Acids Res* 28: 4717–4724, 2000.
273. Zhong X, Garg P, Stith CM, Nick McElhinny SA, Kissling GE, Burgers PM, and Kunkel TA. The fidelity of DNA synthesis by yeast DNA polymerase zeta alone and with accessory proteins. *Nucleic Acids Res* 34: 4731–4742, 2006.
274. Zmudzka BZ, Fornace A, Collins J, and Wilson SH. Characterization of DNA polymerase beta mRNA: cell-cycle and growth response in cultured human cells. *Nucleic Acids Res* 16: 9587–9596, 1988.

Address correspondence to:

Dr. Ricky A. Sharma
Cancer Research UK-Medical Research Council
Oncology Department
Gray Institute for Radiation Oncology and Biology
Old Road Campus Research Building
University of Oxford
Oxford OX3 7DQ
United Kingdom

E-mail: ricky.sharma@oncology.ox.ac.uk

Date of first submission to ARS Central, August 3, 2011; date of final revised submission, July 6, 2012; date of acceptance, July 15, 2012.

Abbreviations Used

AP = abasic site
 APE1 = AP endonuclease-1
 BER = base excision repair
 BRCA = breast cancer gene
 BRCT = BRCA-1 C-terminal
 CPD = cyclobutane pyrimidine dimers
 dNTP = deoxyribonucleotide triphosphate
 dRP = deoxyribose phosphate
 DSB = double-strand break
 DSBP = double-strand break repair
 DSG = damage-specific glycosylase
 ERCC1 = excision repair cross-complementing
 rodent repair deficiency protein
 FEN1 = flap endonuclease 1
 HR = homologous recombination
 Lig = DNA ligase
 NER = nucleotide excision repair
 NHEJ = nonhomologous end-joining
 PCNA = proliferating cell nuclear antigen
 Pol = DNA polymerase
 RFC = replication factor C
 RPA = replication protein A
 SSB = single-strand break
 SSBP = single-strand break repair
 TLS = translesion synthesis
 VDJ = variable, diversity and joining gene segments
 XPV = xeroderma pigmentosum variant
 XRCC1 = X-ray repair cross-complementing
 protein 1