DNA polymerases and somatic hypermutation of immunoglobulin genes

Mineaki Seki¹, Patricia J. Gearhart² & Richard D. Wood^{1*}

¹University of Pittsburgh Cancer Institute, Pennsylvania, USA, and ²National Institutes of Health, Baltimore, Maryland, USA

Somatic hypermutation of immunoglobulin variable genes, which increases antibody diversity, is initiated by the activation-induced cytosine deaminase (AID) protein. The current DNA-deamination model posits that AID deaminates cytosine to uracil in DNA, and that mutations are generated by DNA polymerases during replication or repair of the uracil residue. Mutations could arise as follows: by DNA replicating past the uracil; by removing the uracil with a uracil glycosylase and replicating past the resulting abasic site with a low-fidelity polymerase; or by repairing the uracil and synthesizing a DNA-repair patch downstream using a low-fidelity polymerase. In this review, we summarize the biochemical properties of specialized DNA polymerases in mammalian cells and discuss their participation in the mechanisms of hypermutation. Many recent studies have examined mice deficient in the genes that encode various DNA polymerases, and have shown that DNA polymerase H (POLH) contributes to hypermutation, whereas POLI, POLK and several other enzymes do not have major roles. The low-fidelity enzyme POLQ has been proposed as another candidate polymerase because it can efficiently bypass abasic sites and recent evidence indicates that it might participate in hypermutation.

Keywords: AID; bypass; DNA polymerase; POLQ; somatic hypermutation; translesion

EMBO reports (2005) 6, 1143–1148. doi:10.1038/sj.embor.7400582

Introduction

Antibody variable genes are initially formed by assembling immunoglobulin variable (V), diversity (D) and joining (J) gene segments by non-homologous end joining. To produce antibodies with higher specificity, a second cycle of diversification takes place by somatic hypermutation (SHM). During this process, base substitutions are introduced into a region of ~1 kb surrounding the antibody-coding sequence (Lebecque & Gearhart, 1990). The

process of SHM seems to increase the antibody repertoire enough to limit the frequency of environmentally acquired infections and thereby increases the individual fitness of many vertebrate species, including humans and mice. In one of his lectures on this subject, Myron Goodman proposed SHM as the answer to the question “Why don’t you die when I sneeze?” Indeed, it might be argued that the demise of the invaders in the *War of the Worlds* could be attributed to their lack of an adequate mechanism for diversifying their antibodies.

The base-substitution mechanism is initiated by the activation-induced cytidine deaminase (AID) protein, which is encoded by the *AICDA* gene (Honjo *et al*, 2005; Neuberger *et al*, 2005) and is expressed only in B lymphocytes. AID deaminates cytosine to uracil in DNA and has a strong preference for WRC (W is A or T and R is A or G) hotspot motifs in single-stranded DNA (ssDNA; Pham *et al*, 2003). Such ssDNA might arise transiently during gene transcription and SHM is, in fact, dependent on the active transcription of antibody genes to create the target for cytosine deamination by AID (Barreto *et al*, 2005).

Subsequent steps of SHM have long been postulated to involve a low-fidelity DNA polymerase, and the history of this concept has been reviewed (Gearhart & Wood, 2001). As it is now known that SHM is initiated by AID, it is possible to formulate models involving not only DNA polymerase errors, but also mutations produced during replication or repair of the lesion. U:G mispairs are formed in immunoglobulin DNA by the action of AID, which acts preferentially on a cytosine in the non-transcribed strand (Martomo *et al*, 2005a). As first envisaged by Neuberger and colleagues, there are several possible pathways to produce mutations from uracil (Fig 1; Neuberger *et al*, 2005). One pathway (or ‘phase’ as it is sometimes called) mainly influences events at C:G base pairs, and a second pathway affects substitutions at A:T pairs. After cytosine deamination by AID to generate uracil, copying of this base by a high-fidelity polymerase during chromosomal replication will cause a C:G to T:A transition (C:G mutagenesis type I). In human cells, uracil in DNA is normally removed after incision by a uracil-DNA glycosylase and repaired by base-excision repair (Fig 1). A uracil-DNA glycosylase, such as the enzyme UNG, eliminates the uracil base to produce an apyrimidinic (AP) site. Such sites can be cleaved by several mechanisms, either by an AP endonuclease in duplex DNA or by an AP lyase activity in ssDNA. During error-free base-excision repair in cells, the gap of one or a

¹Suite 2.6, Research Pavilion, Hillman Cancer Center, University of Pittsburgh Cancer Institute, 5117 Centre Avenue, Pittsburgh, Pennsylvania 15213, USA

²Laboratory of Molecular Gerontology, National Institute on Aging, National Institutes of Health, 5600 Nathan Shock Drive, Baltimore, Maryland 21224, USA

*Corresponding author. Tel: +1 412 623 7766; Fax: +1 412 623 7761; E-mail: rdwood@pitt.edu

Submitted 2 September 2005; accepted 17 October 2005

few nucleotides is usually filled by DNA polymerase β . In SHM, error-prone base-excision repair can occur if other low-fidelity DNA polymerases are used in this step. Alternatively, mutations at C:G base pairs might arise (C:G mutagenesis type II; Fig 1) if a bypass DNA polymerase incorporates a base opposite an AP site before repair is initiated.

By contrast, mutations of A:T base pairs seem to be generated by a genetically distinct process. This pathway involves the MSH2–MSH6 mismatch-recognition complex, which can bind to a U:G mismatch (Wilson *et al*, 2005). A current hypothesis is that the low-fidelity DNA polymerase H (POLH; also known as pol η) generates mutations at A:T pairs during a mismatch-repair event that is initiated at a U:G base pair, but involves a larger gap-filling reaction that might use exonuclease I (Bardwell *et al*, 2004; Wilson *et al*, 2005). This process could also generate mutations at C:G base pairs.

Bypass polymerases and translesion synthesis

Damage to DNA alters its structure and so DNA polymerases responsible for chromosome replication often stall at sites of lesions. Fortunately, in addition to DNA-repair pathways, cells can tolerate damage by bypassing it. Specialized bypass DNA polymerases can insert a nucleotide opposite a lesion and, in some cases, continue DNA synthesis a few nucleotides further. In mammalian cells, six enzymes—pol ζ , POLH, POLI (pol ι), POLK (pol κ), REV1 and POLQ (pol θ)—have been shown to bypass DNA damage. POLH, POLI, POLK and REV1 belong to the Y-family polymerase group and share some protein sequence similarity. The catalytic subunit of pol ζ , REV3L, belongs to the B-family of polymerases, which includes DNA polymerases α , δ and ϵ . POLQ belongs to the A-family of enzymes, which are similar to *Escherichia coli* DNA pol I. Translesion DNA-synthesis reactions consist of at least two steps (Fig 2A; Wang, 2001; Prakash *et al*, 2005): the first is insertion of a base opposite the lesion by a bypass polymerase and the second is extension from this inserted base by another polymerase. In yeast, pol ζ has some ability to extend past misincorporations (Prakash *et al*, 2005). There are human counterparts of the pol ζ subunits (Lin *et al*, 1999a; Gibbs *et al*, 2000), so the enzyme might also extend mispairs in higher eukaryotes. After extension, a switch back to a high-fidelity replicative DNA polymerase (for example, pol δ) must take place so that normal DNA synthesis can continue.

Bypass DNA polymerases and SHM

POLH. There is good genetic evidence that mammalian POLH (Table 1) is involved in SHM. POLH efficiently bypasses cyclobutane pyrimidine dimers induced by ultraviolet radiation, and its absence in humans causes the variant form of xeroderma pigmentosum (XP-V; Johnson *et al*, 1999; Masutani *et al*, 1999). POLH is a low-fidelity enzyme that generates replication errors at an average frequency of $\sim 3 \times 10^{-2}$ mutations per base pair (Table 1). In XP-V patients, the frequency of SHM is normal but the mutation pattern changes; the frequency of mutations at A:T bases is decreased and there is a concomitant increase in C:G base mutations (Zeng *et al*, 2001; Failli *et al*, 2004; Zeng *et al*, 2004; Mayorov *et al*, 2005). *In vitro* experiments show that POLH often makes errors at A:T base pairs, particularly at WA sequences (W is A or T), which are potential hotspots for SHM in immunoglobulin genes (Rogozin *et al*, 2001). In the mouse, SHM hotspots *in vivo*

The DNA deamination model

Fig 1 | The DNA deamination model. According to the DNA deamination model, the somatic hypermutation process begins with deamination of cytosine residues in DNA by activation-induced cytosine deaminase (AID) (Neuberger *et al*, 2005). Deamination of cytosine generates uracil and copying of the uracil residue without repair would cause a cytosine to thymine transition (C:G mutagenesis type I). The uracil residue in DNA would be eventually recognized by a uracil-DNA glycosylase, such as UNG, and excised. A U:G mismatch can be recognized by the mismatch-repair system (dependent on MSH2 and MSH6) and a repair-patch process can be initiated (A:T mutagenesis). A gap would presumably be generated by an exonuclease, and mutations would be generated if a gap-filling reaction were catalysed by a low-fidelity DNA polymerase. C:G mutagenesis type II could occur by either translesion synthesis opposite an apyrimidinic (AP) site or by repair of an AP site using a low-fidelity DNA polymerase.

and *in vitro* are strongly correlated (Pavlov *et al*, 2002). Recent analyses of *Polh*-deficient mice have established that POLH is involved in A:T mutagenesis during SHM (Delbos *et al*, 2005; Martomo *et al*, 2005b). POLH physically interacts with the MSH2–MSH6 complex, which is also implicated in mutagenesis at A:T base pairs (Wilson *et al*, 2005). However, POLH might not be involved in C:G mutagenesis type II, as mice deficient in this polymerase still have a high frequency of mutations of C:G. POLH does not efficiently insert a base opposite AP sites, and extension from a primer opposite an AP site is limited (Table 1; Wang, 2001; Kusumoto *et al*, 2002; Prakash *et al*, 2005).

Pol ζ and REV3L. The role of pol ζ in SHM has also been considered. In the yeast *Saccharomyces cerevisiae*, the enzyme consists of the catalytic subunit Rev3 (a 173-kDa protein) and the accessory subunit Rev7. In mammalian cells, a 350-kDa protein homologous to Rev3 is known as REV3L (Murakumo *et al*, 2001). REV3L is expressed in germinal centre B cells, which suggests that it might be involved in SHM (Winter *et al*, 2000; Poltoratsky *et al*, 2001; Zan *et al*, 2001; Zeng *et al*, 2001). When *Rev3L* expression was inhibited using antisense oligonucleotides in the mutating CL-01 cell line, there was a moderate reduction of both A:T and G:C mutations in an immunoglobulin variable gene (Zan *et al*, 2001). A similar effect was reported in transgenic mice expressing *Rev3L* antisense RNA (Diaz *et al*, 2001). The modest decrease in SHM in these mice is difficult to explore further because the complete disruption of *Rev3L* causes lethality during embryonic development (Bemark *et al*, 2000; Esposito *et al*, 2000a; Wittschleben *et al*, 2000; O-Wang *et al*, 2002; Van Sloun *et al*, 2002). Presumably, the antisense inhibition is incomplete and enough pol ζ remains in the mice to allow viability. Biochemical studies using yeast pol ζ suggest that it might extend the mismatched primers generated by POLH in A:T mutagenesis and complete the gap-filling reaction, or extend a primer opposite AP sites generated by bypass in C:G mutagenesis type II (Wang, 2001; Prakash *et al*, 2005).

POL1 and other Y-family and X-family DNA polymerases. POL1 is a paralogue of POLH that also has low fidelity (Table 1). POL1 can efficiently insert a base opposite an AP site (Wang, 2001; Vaisman *et al*, 2002; Prakash *et al*, 2005). When the POL1 gene was knocked out in the Burkitt's lymphoma cell line BL2, most SHM (which in this cell line is largely at C:G base pairs) was dependent on POL1 (Faili *et al*, 2002). However, mice from the 129 strain, which have an inactivating nonsense mutation in *Poli*, had normal SHM (McDonald *et al*, 2003). Addition of the 129 strain *Poli* mutation to a *Polh*- or *Polk*-knockout mouse did not change the phenotype (Delbos *et al*, 2005; Shimizu *et al*, 2005). Currently, the reason for the different results in *Poli*-deficient 129 mice and the POL1-knockout cell line is not understood; this issue might be clarified by experiments with mice that have a complete or inactivating knockout of the *Poli* gene in another mouse strain.

Speculation that POLK might participate in SHM arose because mutation-spectrum analysis indicates that POLK preferentially mutates the SHM hotspot sequence RGYW (R is a purine, G is a guanine, Y is a pyrimidine and W is A or T; Rogozin *et al*, 2001). However, disruption of *Polk* has no significant effects on SHM in the mouse (Schenten *et al*, 2002; Shimizu *et al*, 2003). Purified POLK can bypass an AP site by a primer/template-slippage mechanism (Ohashi *et al*, 2000a), but such frameshift mutations are uncommon events in SHM.

Mammalian cells also have several DNA polymerases in the X family: POLB (pol β), POLL (pol λ) and POLM (pol μ). POLB functions in error-free base-excision repair of DNA, whereas POLL and POLM have roles in non-homologous end-joining of DNA during double-strand-break repair (Bertocci *et al*, 2003; Ma *et al*, 2004; Nick McElhinny & Ramsden, 2004). No alterations in the SHM process have been found in mice with knockouts of *Polb* (Esposito *et al*, 2000b), *Poll* (Bertocci *et al*, 2002) or *Polm* (Bertocci *et al*, 2002; Lucas *et al*, 2005). However, POLL and POLM do have a role in generating antibody diversity during VDJ joining (Ma *et al*, 2004; Nick McElhinny & Ramsden, 2004). Mice carrying a specific defect

A Two-polymerase model for insertion and extension at an AP site

B POLQ insertion and extension at an AP site

Fig2 | Multistep models for bypass of apyrimidinic (AP)-site damage. (A) Several different Y-family DNA polymerases can insert bases opposite an AP site; however, none can efficiently extend past an AP site, and so a second DNA polymerase, such as pol ζ , is needed to extend the primer after insertion. (B) POLQ can catalyse both the insertion and extension reactions opposite an AP site.

in the proofreading 3' to 5' exonuclease function of the replicative DNA polymerase δ (a B-family polymerase) also lack defects in SHM (Longacre *et al*, 2003).

REV1. REV1 has deoxycytidine monophosphate (dCMP) transferase activity that can insert a cytosine opposite an AP site (Table 1). Experiments with budding yeast show that REV1 is involved in the bypass of AP sites and other lesions, but its exact role remains to be determined (Nelson *et al*, 2000; Prakash *et al*, 2005). The dCMP transferase activity is dispensable for its *in vivo* role in translesion synthesis opposite an AP site (Nelson *et al*, 2000). REV1 interacts with other Y-family member polymerases as well as REV7, which is a subunit of pol ζ (Murakumo *et al*, 2001; Guo *et al*, 2003; Ohashi *et al*, 2004; Tissier *et al*, 2004), and might be a docking protein for translesion DNA-synthesis proteins in SHM. Disruption of the REV1 gene in chicken DT40 B cells indicates that REV1 might be involved in SHM by producing C:G to G:C transversions (Simpson & Sale, 2003). No significant effects on SHM were found in a mouse lacking the BRCT domain of REV1, which is important for protein-protein interactions (Jansen *et al*, 2005). The SHM spectrum in hypermutating DT40 cells could be explained by the insertion of cytosine opposite abasic sites, but this explanation cannot account for the SHM spectrum in mice. Specific analysis of the role of the dCMP transferase activity in SHM needs to be performed using a dCMP transferase mutant *Rev1* mouse or cell line.

POLQ. The POLH, POLI, POLK and REV1 enzymes belong to the Y family, and are characterized by their ability to manipulate highly distorted templates. However, POLQ is a low-fidelity bypass polymerase that belongs to the A family (Table 1; Seki *et al*, 2003; Seki *et al*, 2004). A-family polymerases are generally high-fidelity enzymes that operate during DNA repair, recombination and

Table 1 | Low-fidelity DNA synthesis and AP-site-bypass activity of mammalian lesion-bypass enzymes

Enzyme*	Gene	Amino acids	Mr [†] (kDa)	Family	Fidelity (mutations/base pair)	AP site bypass (insertion)	AP site bypass (extension)	References
POLH (pol η)	<i>POLH</i>	713	78.4	Y	~10 ⁻² –10 ⁻³	Yes	Not efficient	Johnson <i>et al</i> , 2000c Masutani <i>et al</i> , 1999 Matsuda <i>et al</i> , 2000
POLI (pol ι)	<i>POLI</i>	715	80.4	Y	~10 ⁻¹ –10 ^{-5§}	Yes	No	Johnson <i>et al</i> , 2000a Tissier <i>et al</i> , 2000 Zhang <i>et al</i> , 2001
POLK (pol κ)	<i>POLK</i>	870	98.8	Y	~10 ⁻³ –10 ⁻⁴	(Slippage)	(Slippage)	Johnson <i>et al</i> , 2000b Ohashi <i>et al</i> , 2000b Fischhaber <i>et al</i> , 2002
REV1	<i>REV1</i>	1,251	138	Y	dCMP transferase	Yes	No	Lin <i>et al</i> , 1999b Gibbs <i>et al</i> , 2000 Masuda <i>et al</i> , 2001
Pol ζ	<i>REV3L[‡]</i>	3,130	353	B	~10 ^{-4#}	No	Yes	Gibbs <i>et al</i> , 1998 Lin <i>et al</i> , 1999a Johnson <i>et al</i> , 2000a
POLQ (pol θ)	<i>POLQ</i>	2,590	290	A	~10 ⁻² –10 ⁻³	Yes	Yes	Seki <i>et al</i> , 2003 Seki <i>et al</i> , 2004

*Human Genome Organisation (HUGO)-approved names are shown (<http://www.gene.ucl.ac.uk/nomenclature>) with the alternative Greek letter designations in parentheses.

[†]Molecular mass of the catalytic subunit. [§]With a template T, POLI misincorporates a guanosine tenfold better than it incorporates an adenosine. ^{||}A version of human REV1 that is one amino acid shorter has been reported by Masuda *et al* (2001). [‡]REV3L, the catalytic subunit of mammalian pol ζ, is listed. Other subunits include MAD2L2 (REV7). [#]The fidelity of human pol ζ has not been determined so that of yeast pol ζ is listed. AP, apyrimidinic.

replication, and do not normally engage in the bypass of lesions (Seki *et al*, 2004). POLQ is unusual in this respect, as it can efficiently insert an adenine opposite an AP site and subsequently extend the DNA (Fig 2B). So far, it is the only polymerase in human cells that can efficiently perform both insertion and extension reactions. POLQ is involved in the maintenance of genome integrity, as reticulocytes from mice deficient in this polymerase show abnormal nuclear division or increased chromosome breakage (Shima *et al*, 2003; Shima *et al*, 2004).

As POLQ is highly expressed in lymphoid tissues, including the spleen and germinal centres, the enzyme might be involved in SHM (Kawamura *et al*, 2004; Shima *et al*, 2004). Extension of a primer from a misincorporated adenine opposite an AP site would stably generate a C:G to T:A transition mutation, which is common in SHM. POLQ might also be a candidate for involvement in A:T mutagenesis, as a low-fidelity DNA polymerase is proposed to be involved in a gap-filling reaction in this process. Recent experiments with gene-deficient mice have directly tested whether POLQ is involved in SHM. One series of experiments from Casali, Schimenti and colleagues used mice carrying a gene disruption that completely eliminates the POLQ enzyme (Zan *et al*, 2005). The overall frequency of SHM was reduced by 60–80%. The spectrum of mutations was moderately shifted towards more transitions at both A:T and C:G base pairs, but there was no overall change in the proportion of events at A:T and C:G positions. An independent study from the laboratory of O-Wang (Masuda *et al*, 2005) used mice carrying an in-frame deletion of part of the DNA polymerase domain, so that a catalytically inactive POLQ protein was still produced. In this mouse, the frequency of SHM was only reduced slightly, with normal levels of A:T mutations but fewer mutations of C:G base pairs. In follow-up studies, O-Wang and collaborators constructed a second *Polq*-null mouse, which did not synthesize *Polq* mRNA or protein. In this mouse, a mild overall reduction in mutation frequency was seen with little shift in the

mutation spectrum (J. O-Wang, personal communication). The effects in all of these studies were relatively moderate and it is not certain whether POLQ has a major role in SHM.

Future directions

As the mechanism of SHM emerges, it is proving to be a fascinating intersection of biochemistry associated with DNA repair, transcription and replication enzymes. However, there are many unanswered questions about the pathway. For example, once an AP site is generated in DNA through the sequential activities of AID and UNG, why does the active base-excision-repair pathway fail to repair the AP site in an error-free manner? Moreover, what is the mutagenic pathway that involves the activities of MSH2 and MSH6? The latter does not seem to be normal mismatch repair, as it does not involve other mismatch-repair proteins, such as MLH1 and PMS2. The MRE11–RAD50–NBS1 (MRN) nuclease complex also accelerates SHM, but the biochemical mechanism is unknown (Yabuki *et al*, 2005).

In summary, it seems that several DNA polymerases are involved in different stages of the hypermutation process. Further experiments using double-gene disruptions (for example, *Polq*–*Polh* double-mutant mice) should be informative in exploring which DNA polymerases can participate in the process and at which steps. It is also possible that specialized DNA polymerases might substitute for one another in SHM when one enzyme is completely deleted. One way to explore this subject might be to examine mice or cell lines in which DNA polymerase catalytic sites are specifically inactivated, without total deletion of the enzyme.

ACKNOWLEDGEMENTS

We thank G. Gan for assistance with editing, and members of our laboratories for comments on the manuscript. This work was supported by US National Institutes of Health grants CA098675 and CA101980 to R.D.W., by the NIH Intramural Research Program and by the University of Pittsburgh Cancer Institute.

REFERENCES

- Bardwell PD, Woo CJ, Wei K, Li Z, Martin A, Sack SZ, Parris T, Edelman W, Scharff MD (2004) Altered somatic hypermutation and reduced class-switch recombination in exonuclease 1-mutant mice. *Nature Immunol* **5**: 224–229
- Barreto VM, Ramiro AR, Nussenzweig MC (2005) Activation-induced deaminase: controversies and open questions. *Trends Immunol* **26**: 90–96
- Bemark M, Khamlichi AA, Davies SL, Neuberger MS (2000) Disruption of mouse polymerase ζ (Rev3) leads to embryonic lethality and impairs blastocyst development *in vitro*. *Curr Biol* **10**: 1213–1216
- Bertocci B, De Smet A, Flatter E, Dahan A, Bories JC, Landreau C, Weill JC, Reynaud CA (2002) Cutting edge: DNA polymerases μ and λ are dispensable for Ig gene hypermutation. *J Immunol* **168**: 3702–3706
- Bertocci B, De Smet A, Berek C, Weill JC, Reynaud CA (2003) Immunoglobulin κ light chain gene rearrangement is impaired in mice deficient for DNA polymerase μ . *Immunity* **19**: 203–211
- Delbos F, De Smet A, Faili A, Aoufouchi S, Weill JC, Reynaud CA (2005) Contribution of DNA polymerase η to immunoglobulin gene hypermutation in the mouse. *J Exp Med* **201**: 1191–1196
- Diaz M, Verkoczy LK, Flajnik MF, Klinman NR (2001) Decreased frequency of somatic hypermutation and impaired affinity maturation but intact germinal center formation in mice expressing antisense RNA to DNA polymerase ζ . *J Immunol* **167**: 327–335
- Esposito G, Godindagger I, Klein U, Yaspo ML, Cumano A, Rajewsky K (2000a) Disruption of the Rev3l-encoded catalytic subunit of polymerase ζ in mice results in early embryonic lethality. *Curr Biol* **10**: 1221–1224
- Esposito G, Texido G, Betz UA, Gu H, Muller W, Klein U, Rajewsky K (2000b) Mice reconstituted with DNA polymerase β -deficient fetal liver cells are able to mount a T cell-dependent immune response and mutate their Ig genes normally. *Proc Natl Acad Sci USA* **97**: 1166–1171
- Faili A, Aoufouchi S, Flatter E, Gueranger Q, Reynaud CA, Weill JC (2002) Induction of somatic hypermutation in immunoglobulin genes is dependent on DNA polymerase τ . *Nature* **419**: 944–947
- Faili A, Aoufouchi S, Weller S, Vuillier F, Sary A, Sarasin A, Reynaud CA, Weill JC (2004) DNA polymerase τ is involved in hypermutation occurring during immunoglobulin class switch recombination. *J Exp Med* **199**: 265–270
- Fischhaber PL, Gerlach VL, Feaver WJ, Hatahet Z, Wallace SS, Friedberg EC (2002) Human DNA polymerase κ bypasses and extends beyond thymine glycols during translesion synthesis *in vitro*, preferentially incorporating correct nucleotides. *J Biol Chem* **277**: 37604–37611
- Gearhart PJ, Wood RD (2001) Emerging links between hypermutation of antibody genes and DNA polymerases. *Nature Rev Immunol* **1**: 187–192
- Gibbs PE, McGregor WG, Maher VM, Nisson P, Lawrence CW (1998) A human homolog of the *Saccharomyces cerevisiae* REV3 gene, which encodes the catalytic subunit of DNA polymerase ζ . *Proc Natl Acad Sci USA* **95**: 6876–6880
- Gibbs PE, Wang XD, Li Z, McManus TP, McGregor WG, Lawrence CW, Maher VM (2000) The function of the human homolog of *Saccharomyces cerevisiae* REV1 is required for mutagenesis induced by UV light. *Proc Natl Acad Sci USA* **97**: 4186–4191
- Guo C, Fischhaber PL, Luk-Paszyc MJ, Masuda Y, Zhou J, Kamiya K, Kisker C, Friedberg EC (2003) Mouse Rev1 protein interacts with multiple DNA polymerases involved in translesion DNA synthesis. *EMBO J* **22**: 6621–6630
- Honjo T, Nagaoka H, Shinkura R, Muramatsu M (2005) AID to overcome the limitations of genomic information. *Nature Immunol* **6**: 655–661
- Jansen JG, Tsaalbi-Shtylik A, Langerak P, Calleja F, Meijers CM, Jacobs H, de Wind N (2005) The BRCT domain of mammalian Rev1 is involved in regulating DNA translesion synthesis. *Nucleic Acids Res* **33**: 356–365
- Johnson RE, Kondratik CM, Prakash S, Prakash L (1999) hRAD30 mutations in the variant form of xeroderma pigmentosum. *Science* **285**: 263–265
- Johnson RE, Washington MT, Haracska L, Prakash S, Prakash L (2000a) Eukaryotic polymerases τ and ζ act sequentially to bypass DNA lesions. *Nature* **406**: 1015–1019
- Johnson RE, Prakash S, Prakash L (2000b) The human DINB1 gene encodes the DNA polymerase Pol θ . *Proc Natl Acad Sci USA* **97**: 3838–3843
- Johnson RE, Washington MT, Prakash S, Prakash L (2000c) Fidelity of human DNA polymerase η . *J Biol Chem* **275**: 7447–7450
- Kawamura K et al (2004) DNA polymerase θ is preferentially expressed in lymphoid tissues and upregulated in human cancers. *Int J Cancer* **109**: 9–16
- Kusumoto R, Masutani C, Iwai S, Hanaoka F (2002) Translesion synthesis by human DNA polymerase η across thymine glycol lesions. *Biochemistry* **41**: 6090–6099
- Lebecque SG, Gearhart PJ (1990) Boundaries of somatic mutation in rearranged immunoglobulin genes: 5' boundary is near the promoter, and 3' boundary is approximately 1 kb from V(D)J gene. *J Exp Med* **172**: 1717–1727
- Lin W, Wu X, Wang Z (1999a) A full-length cDNA of hREV3 is predicted to encode DNA polymerase ζ for damage-induced mutagenesis in humans. *Mutat Res* **433**: 89–98
- Lin W, Xin H, Zhang Y, Wu X, Yuan F, Wang Z (1999b) The human REV1 gene codes for a DNA template-dependent dCMP transferase. *Nucleic Acids Res* **27**: 4468–4475
- Longacre A, Sun T, Goldsby RE, Preston BD, Storb U (2003) Ig gene somatic hypermutation in mice defective for DNA polymerase δ proofreading. *Int Immunol* **15**: 477–481
- Lucas D, Lain de Lera T, Gonzalez MA, Ruiz JF, Dominguez O, Casanova JC, Martinez AC, Blanco L, Bernad A (2005) Polymerase μ is up-regulated during the T cell-dependent immune response and its deficiency alters developmental dynamics of spleen centroblasts. *Eur J Immunol* **35**: 1601–1611
- Ma Y, Lu H, Tippin B, Goodman MF, Shimazaki N, Koizumi O, Hsieh CL, Schwarz K, Lieber MR (2004) A biochemically defined system for mammalian nonhomologous DNA end joining. *Mol Cell* **16**: 701–713
- Martomo SA, Fu D, Yang WW, Joshi NS, Gearhart PJ (2005a) Deoxyuridine is generated preferentially in the nontranscribed strand of DNA from cells expressing activation-induced cytidine deaminase. *J Immunol* **174**: 7787–7791
- Martomo SA, Yang WW, Wersto RP, Ohkumo T, Kondo Y, Yokoi M, Masutani C, Hanaoka F, Gearhart PJ (2005b) Different mutation signatures in DNA polymerase η - and MSH6-deficient mice suggest separate roles in antibody diversification. *Proc Natl Acad Sci USA* **102**: 8656–8661
- Masuda K, Ouchida R, Takeuchi A, Saito T, Koseki H, Kawamura K, Tagawa M, Tokuhisa T, Azuma T, O-Wang J (2005) DNA polymerase θ contributes to the generation of C/G mutations during somatic hypermutation of Ig genes. *Proc Natl Acad Sci USA* **102**: 13986–13991
- Masuda Y, Takahashi M, Tsunekuni N, Minami T, Sumii M, Miyagawa K, Kamiya K (2001) Deoxycytidyl transferase activity of the human REV1 protein is closely associated with the conserved polymerase domain. *J Biol Chem* **276**: 15051–15058
- Masutani C, Kusumoto R, Yamada A, Dohmae N, Yokoi M, Yuasa M, Araki M, Iwai S, Takio K, Hanaoka F (1999) The XPV (xeroderma pigmentosum variant) gene encodes human DNA polymerase η . *Nature* **399**: 700–704
- Matsuda T, Bebenek K, Masutani C, Hanaoka F, Kunkel TA (2000) Low fidelity DNA synthesis by human DNA polymerase- η . *Nature* **404**: 1011–1013
- Mayorov VI, Rogozin IB, Adkison LR, Gearhart PJ (2005) DNA polymerase η contributes to strand bias of mutations of A versus T in immunoglobulin genes. *J Immunol* **174**: 7781–7786
- McDonald JP, Frank EG, Plosky BS, Rogozin IB, Masutani C, Hanaoka F, Woodgate R, Gearhart PJ (2003) 129-derived strains of mice are deficient in DNA polymerase τ and have normal immunoglobulin hypermutation. *J Exp Med* **198**: 635–643
- Murakumo Y, Ogura Y, Ishii H, Numata S, Ichihara M, Croce CM, Fishel R, Takahashi M (2001) Interactions in the error-prone postreplication repair proteins hREV1, hREV3, and hREV7. *J Biol Chem* **276**: 35644–35651
- Nelson JR, Gibbs PE, Nowicka AM, Hinkle DC, Lawrence CW (2000) Evidence for a second function for *Saccharomyces cerevisiae* Rev1p. *Mol Microbiol* **37**: 549–554
- Neuberger MS, Noia JM, Beale RC, Williams GT, Yang Z, Rada C (2005) Opinion: Somatic hypermutation at A-T pairs: polymerase error versus dUTP incorporation. *Nature Rev Immunol* **5**: 171–178
- Nick McElhinny SA, Ramsden DA (2004) Sibling rivalry: competition between Pol X family members in V(D)J recombination and general double strand break repair. *Immunol Rev* **200**: 156–164
- O-Wang J, Kajiura K, Kawamura K, Kimura M, Miyagishima H, Koseki H, Tagawa M (2002) An essential role for REV3 in mammalian cell survival: absence of REV3 induces p53-independent embryonic death. *Biochem Biophys Res Commun* **293**: 1132–1137
- Ohashi E, Ogi T, Kusumoto R, Iwai S, Masutani C, Hanaoka F, Ohmori H (2000a) Error-prone bypass of certain DNA lesions by the human DNA polymerase κ . *Genes Dev* **14**: 1589–1594

- Ohashi E, Bebenek K, Matsuda T, Feaver WJ, Gerlach VL, Friedberg EC, Ohmori H, Kunkel TA (2000b) Fidelity and processivity of DNA synthesis by DNA polymerase κ , the product of the human DINB1 gene. *J Biol Chem* **275**: 39678–39684
- Ohashi E, Murakumo Y, Kanjo N, Akagi J, Masutani C, Hanaoka F, Ohmori H (2004) Interaction of hREV1 with three human Y-family DNA polymerases. *Genes Cells* **9**: 523–531
- Pavlov YI, Rogozin IB, Galkin AP, Aksenova AY, Hanaoka F, Rada C, Kunkel TA (2002) Correlation of somatic hypermutation specificity and A–T base pair substitution errors by DNA polymerase η during copying of a mouse immunoglobulin κ light chain transgene. *Proc Natl Acad Sci USA* **99**: 9954–9959
- Pham P, Branstetter R, Petruska J, Goodman MF (2003) Processive AID-catalysed cytosine deamination on single-stranded DNA simulates somatic hypermutation. *Nature* **424**: 103–107
- Poltoratsky V, Woo CJ, Tippin B, Martin A, Goodman MF, Scharff MD (2001) Expression of error-prone polymerases in BL2 cells activated for Ig somatic hypermutation. *Proc Natl Acad Sci USA* **98**: 7976–7981
- Prakash S, Johnson RE, Prakash L (2005) Eukaryotic translesion synthesis DNA polymerases: specificity of structure and function. *Annu Rev Biochem* **74**: 317–353
- Rogozin IB, Pavlov YI, Bebenek K, Matsuda T, Kunkel TA (2001) Somatic mutation hotspots correlate with DNA polymerase η error spectrum. *Nature Immunol* **2**: 530–536
- Schenten D, Gerlach VL, Guo C, Velasco-Miguel S, Hladik CL, White CL, Friedberg EC, Rajewsky K, Esposito G (2002) DNA polymerase κ deficiency does not affect somatic hypermutation in mice. *Eur J Immunol* **32**: 3152–3160
- Seki M, Marini F, Wood RD (2003) POLQ (Pol θ), a DNA polymerase and DNA-dependent ATPase in human cells. *Nucleic Acids Res* **31**: 6117–6126
- Seki M, Masutani C, Yang LW, Schuffert A, Iwai S, Bahar I, Wood RD (2004) High-efficiency bypass of DNA damage by human DNA polymerase ζ . *EMBO J* **23**: 4484–4494
- Shima N, Hartford SA, Duffy T, Wilson LA, Schimenti KJ, Schimenti JC (2003) Phenotype-based identification of mouse chromosome instability mutants. *Genetics* **163**: 1031–1040
- Shima N, Munroe RJ, Schimenti JC (2004) The mouse genomic instability mutation chaos1 is an allele of Pol θ that exhibits genetic interaction with Atm. *Mol Cell Biol* **24**: 10381–10389
- Shimizu T, Shinkai Y, Ogi T, Ohmori H, Azuma T (2003) The absence of DNA polymerase κ does not affect somatic hypermutation of the mouse immunoglobulin heavy chain gene. *Immunol Lett* **86**: 265–270
- Shimizu T, Azuma T, Ishiguro M, Kanjo N, Yamada S, Ohmori H (2005) Normal immunoglobulin gene somatic hypermutation in Pol κ -Pol θ double-deficient mice. *Immunol Lett* **98**: 259–264
- Simpson LJ, Sale JE (2003) Rev1 is essential for DNA damage tolerance and non-templated immunoglobulin gene mutation in a vertebrate cell line. *EMBO J* **22**: 1654–1664
- Tissier A, McDonald JP, Frank EG, Woodgate R (2000) pol ι , a remarkably error-prone human DNA polymerase. *Genes Dev* **14**: 1642–1650
- Tissier A, Kannouche P, Reck MP, Lehmann AR, Fuchs RP, Cordonnier A (2004) Co-localization in replication foci and interaction of human Y-family members, DNA polymerase pol η and REVI protein. *DNA Repair (Amst)* **3**: 1503–1514
- Vaisman A, Frank EG, McDonald JP, Tissier A, Woodgate R (2002) pol ι -dependent lesion bypass *in vitro*. *Mutat Res* **510**: 9–22
- Van Sloun PP, Varlet I, Sonneveld E, Boei JJ, Romeijn RJ, Eeken JC, De Wind N (2002) Involvement of mouse Rev3 in tolerance of endogenous and exogenous DNA damage. *Mol Cell Biol* **22**: 2159–2169
- Wang Z (2001) DNA damage-induced mutagenesis: a novel target for cancer prevention. *Mol Interv* **1**: 269–281
- Wilson TM, Vaisman A, Martomo SA, Sullivan P, Lan L, Hanaoka F, Yasui A, Woodgate R, Gearhart PJ (2005) MSH2–MSH6 stimulates DNA polymerase η , suggesting a role for A:T mutations in antibody genes. *J Exp Med* **201**: 637–645
- Winter DB, Phung QH, Wood RD, Gearhart PJ (2000) Differential expression of DNA polymerase epsilon in resting and activated B lymphocytes is consistent with an *in vivo* role in replication and not repair. *Mol Immunol* **37**: 125–131
- Wittschleben J, Shivji MK, Lalani E, Jacobs MA, Marini F, Gearhart PJ, Rosewell I, Stamp G, Wood RD (2000) Disruption of the developmentally regulated Rev3l gene causes embryonic lethality. *Curr Biol* **10**: 1217–1220
- Yabuki M, Fujii MM, Maizels N (2005) The MRE11–RAD50–NBS1 complex accelerates somatic hypermutation and gene conversion of immunoglobulin variable regions. *Nature Immunol* **6**: 730–736
- Zan H, Komori A, Li Z, Cerutti A, Schaffer A, Flajnik MF, Diaz M, Casali P (2001) The translesion DNA polymerase ζ plays a major role in Ig and bcl-6 somatic hypermutation. *Immunity* **14**: 643–653
- Zan H, Zhong Y, Xu Z, Evinger III AJ, Shima N, Schimenti JC, Casali P (2005) The translesion DNA polymerase θ plays a dominant role in Ig gene somatic hypermutation. *EMBO J* **24**: 3757–3769
- Zeng X, Winter DB, Kasmer C, Kraemer KH, Lehmann AR, Gearhart PJ (2001) DNA polymerase η is an A–T mutator in somatic hypermutation of immunoglobulin variable genes. *Nature Immunol* **2**: 537–541
- Zeng X, Negrete GA, Kasmer C, Yang WW, Gearhart PJ (2004) Absence of DNA polymerase η reveals targeting of C mutations on the nontranscribed strand in immunoglobulin switch regions. *J Exp Med* **199**: 917–924
- Zhang Y, Yuan F, Wu X, Taylor JS, Wang Z (2001) Response of human DNA polymerase ι to DNA lesions. *Nucleic Acids Res* **29**: 928–935

Mineaki Seki & Richard D. Wood

Patricia J. Gearhart