Hijacked DNA repair proteins and unchained DNA polymerases

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Somatic hypermutation of immunoglobulin (Ig) genes occurs at a frequency that is a million times greater than the mutation in other genes. Mutations occur in variable genes to increase antibody affinity, and in switch regions before constant genes to cause switching from IgM to IgG. Hypermutation is initiated in activated B cells when the activation-induced deaminase protein deaminates cytosine in DNA to uracil. Uracils can be processed by either a mutagenic pathway to produce mutations or a non-mutagenic pathway to remove mutations. In the mutagenic pathway, we first studied the role of mismatch repair proteins, MSH2, MSH3, MSH6, PMS2 and MLH1, since they would recognize mismatches. The MSH2–MSH6 heterodimer is involved in hypermutation by binding to U:G and other mismatches generated during repair synthesis, but the other proteins are not necessary. Second, we analysed the role of low-fidelity DNA polymerases η , ι and θ in synthesizing mutations, and conclude that polymerase η is the dominant participant by generating mutations at A:T base pairs. In the non-mutagenic pathway, we examined the role of the Cockayne syndrome B protein that interacts with other repair proteins. Mice deficient in this protein had normal hypermutation and class switch recombination, showing that it is not involved.

Keywords: immunoglobulin genes; somatic hypermutation; class switch recombination; MSH2–MSH6; DNA polymerase η ; Cockayne syndrome B

1. ABERRANT DNA REPAIR GENERATES ANTIBODY DIVERSITY

The identification of the activation-induced cytidine deaminase (AID) protein by Honjo and colleagues (Muramatsu *et al.* 2000), and the discovery that it deaminates cytosine to uracil in immunoglobulin genes (Di Noia & Neuberger 2002), has exploded our knowledge of how antibodies become diversified. AID initiates both affinity maturation in variable (*V*) genes and class switch recombination (CSR) of heavy chain constant (*C_H*) genes. Deamination of cytosine is mutagenic, and it can be processed by a mutagenic pathway to generate mutations, or by a non-mutagenic pathway to remove mutations (figure 1). Both these pathways probably occur at the same time in activated B cells, so that the ultimate outcome is a balance between the mutation and the repair.

In the mutagenic pathway, uracil (U) can be processed by several enzymes that have been identified based on genetic and biochemical evidence (Di Noia & Neuberger 2002; Petersen-Mahrt *et al.* 2002; Rada *et al.* 2002). First, U could be replicated by high-fidelity DNA polymerases to produce C:G transitions. Second, U could be removed by uracil DNA glycosylase (UNG) to produce an abasic site, which when copied by low-fidelity DNA polymerases, would generate C:G

transitions and transversions. Third, U could be recognized as a U:G mismatch by the MSH2–MSH6 mismatch repair (MMR) proteins, which would generate a gap that could be filled in by the low-fidelity DNA polymerase (pol) η to produce mutations of A:T base pairs (bp; Zeng *et al.* 2001; Wilson *et al.* 2005). The last two steps are also associated with strand breaks, which produce substrates for recombination during heavy-chain class switching.

In the non-mutagenic pathway, which consists of base excision repair proteins, U is removed by UNG to produce an abasic site. The abasic site is cleaved by the apurinic/apyrimidinic endonuclease (APE) 1, and the deoxyribose phosphate group is removed by DNA pol β , which then fills in the one nucleotide gap with high fidelity. This pathway is present in every cell, and is used to remove uracils generated by spontaneous deamination. No mutations or strand breaks would be produced, and heavy-chain class switching would be diminished.

Thus, in B cells, AID generates many uracils in a defined region around the rearranged *V* gene and in the switch regions preceding each *C_H* gene. Presumably not all the uracils are repaired, and those that are left in the DNA would produce nucleotide substitutions by the mutagenic pathway described above. We have studied proteins that are involved in the mutagenic pathway by examining mice that are deficient in the proteins, and by analysing the biochemical interactions between the relevant proteins. Intriguingly, the hypermutation machinery has hijacked certain repair

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Figure 1. Mutagenic and non-mutagenic pathways for handling U. The mutagenic pathway involves either removal of U by uracil DNA glycosylase (UNG), or recognition of U:G by MSH2–MSH6. Mutations arise by DNA polymerases copying the abasic site or polymerase η synthesizing mutations opposite A and T nucleotides in a gap generated by exonuclease 1. Strand breaks occur when the abasic site is nicked by an APE, or by an endonuclease associated with MMR. The non-mutagenic pathway removes U with UNG and APE1, and polymerase β synthesizes the correct nucleotide (CSB, Cockayne syndrome B).

proteins from the MMR and base excision pathways to create mutations, rather than repair them.

2. MISMATCH REPAIR PROTEINS

In the mutagenic pathway, many mismatches could be generated before they are fixed as mutations after DNA replication. In eukaryotic MMR, the heterodimer MSH2–MSH6 binds to single-nucleotide mismatches, and MSH2–MSH3 binds to loops created by insertions, deletions or mispairing on one strand. The heterodimer PMS2–MLH1 is then recruited to the complex. PMS2 has recently been shown to function as an endonuclease (Kadyrov *et al.* 2006), which nicks the strand containing a discontinuity. Exonuclease 1 binds to MSH2 and MLH1, and creates a gap at the nick by excising the misincorporated nucleotide from the newly synthesized strand. This gap can then be filled in by a DNA polymerase. To see which of these proteins are involved in hypermutation and CSR, we and others have examined mice deficient in each of them. As summarized in table 1, all of the repair-deficient mice had lower frequencies of mutation than their wild-type counterparts, which may be due to direct (on hypermutation) or indirect (cells may divide slower) effects on the B cells. Most strikingly, two of the

proteins, MSH2 and MSH6, were responsible for the mutations of A:T bp (Frey *et al.* 1998; Phung *et al.* 1998; Rada *et al.* 1998; Wiesendanger *et al.* 2000; Li *et al.* 2004; Martomo *et al.* 2004). The number of mutations at A:T bp and G:C bp is roughly equal in normal mice and in mice deficient in MSH3, PMS2 and MLH1 (Frey *et al.* 1998; Winter *et al.* 1998; Kim *et al.* 1999; Phung *et al.* 1999; Wiesendanger *et al.* 2000; Ehrenstein *et al.* 2001; Li *et al.* 2004; Martomo *et al.* 2004), whereas mutations of A:T drop precipitously in mice deficient in MSH2 and MSH6. Scharff and colleagues (Bardwell *et al.* 2004) also showed that mice deficient in exonuclease 1 had a similar phenotype to MSH2 and MSH6 deficiency, i.e. fewer mutations of A:T bp. This is consistent with the notion that MSH2–MSH6 binds to a single mismatch, and recruits exonuclease 1 to create a gap. The gap can then be filled in by a DNA polymerase that would predominantly synthesize mutations of A:T bp. The endonuclease that is needed to generate an initial nick for the exonuclease to act on is not known. It is not PMS2, since mice deficient in this protein had relatively normal hypermutation.

However, for class switching, with the exception of MSH3, most of the MMR proteins are involved, since mice deficient in MSH2, MSH6, PMS2, MLH1 and exonuclease 1 had less class switching (Ehrenstein & Neuberger 1999; Schrader *et al.* 1999; Ehrenstein *et al.* 2001; Li *et al.* 2004; Martomo *et al.* 2004). Therefore, unlike hypermutation, intact MMR participates in resolving recombinational intermediates at the switch junctions.

In conclusion, not all the MMR proteins had an effect on hypermutation. MSH3, PMS2 and MLH1 were not involved in changing the spectra of mutations. Thus, canonical MMR to correct mismatches does not happen in the mutagenic pathway. In fact, three of the proteins, MSH2, MSH6 and exonuclease 1, are actually involved in an error-prone pathway that generates mutations rather than repairing them.

3. ERROR-PRONE DNA POLYMERASES

If U remains in the DNA, it could be replicated by high-fidelity DNA polymerases, such as polymerases δ and ϵ , to produce C:G to T:A transitions, as confirmed by Neuberger and colleagues in UNG-deficient mice (Rada *et al.* 2002). By convention, mutations are recorded from the non-transcribed strand, so equal frequencies of C and G transitions means that uracils are generated on both DNA strands. Low-fidelity DNA polymerases must then generate the C:G transversions and A:T mutations, which are abundant in immunoglobulin genes. Eight polymerases have been studied for their role in this process, using mice deficient in the polymerases. Polymerases ι (McDonald *et al.* 2003; Martomo *et al.* 2006), κ (Schenten *et al.* 2002), λ (Bertocci *et al.* 2002), μ (Bertocci *et al.* 2002) and θ (Masuda *et al.* 2007; Martomo *et al.* 2008) are either not involved or play a minor role; and pol η (Zeng *et al.* 2001; Delbos *et al.* 2005, 2007; Martomo *et al.* 2005) and Rev1 (Jansen *et al.* 2006) are involved. The role of pol ζ is less clear (Diaz *et al.* 2001) due to the non-viability of gene-deficient mice.

Table 1. Somatic hypermutation and switching in B cells from mice deficient in MMR proteins.

genotype	mutations/ bp $\times 10^{-3}$	percentage of mutations at A:T ^a	percentage of mutations at G:C ^a	percentage of switching to IgG1 ^b
<i>J_H4</i> intron from Peyer's patch ^c				
C57BL/6	6.3	54	46	13
<i>Msh3</i> ^{-/-}	1.6	54	46	13
<i>Msh6</i> ^{-/-}	0.8	15	85	4
<i>V_kOx1</i> from spleen ^d				
C57BL/6	7.0	58	42	n.d.
<i>Msh2</i> ^{-/-}	6.5	9	91	n.d.
<i>Pms2</i> ^{-/-}	4.5	49	51	n.d.
<i>Mlh1</i> ^{-/-}	3.5	40	60	n.d.

^a Data corrected for nucleotide base composition.^b Splenic B cells stimulated *in vitro* with lipopolysaccharide and IL-4 for 3 days. n.d., not determined.^c Data from Martomo *et al.* (2005).^d Data from Phung *et al.* (1998, 1999) and Winter *et al.* (1998). These clones came from immunized mice and were selected for high affinity. Figure 2. Hypermutation in mice deficient in DNA polymerases η , ι and θ . Mutations were measured in the *J_H4* intron region from Peyer's patch B cells. Both the spectra and range of mutation frequencies are shown. Data from Martomo *et al.* (2005, 2006, 2008).

The strongest evidence for participation of a polymerase is to see a difference in the types of mutations that are generated in the absence of the polymerase. This criterion has established a role for *pol η* and *Rev1*. For *pol η*, we first reported that it is an A:T mutator (Zeng *et al.* 2001), after observing that the frequency of mutations of A and T dropped fourfold in *V* genes from patients with xeroderma pigmentosum disease, who lack *pol η*. The frequency of mutations of G and C increased, so that the overall frequency of mutation was not changed compared to normal people, but the spectra were altered. This work was confirmed in mice deficient in *pol η* (Delbos *et al.* 2005; Martomo *et al.* 2005), where the frequency of A:T mutations was also diminished in non-coding regions around *V* genes. An analysis of the switch regions in humans and mice deficient in the polymerase showed that *pol η* synthesizes mutations of A and T there as well (Faili *et al.* 2004; Zeng *et al.* 2004; Delbos *et al.* 2005; Martomo *et al.* 2005).

As seen in figure 2, in wild-type C57BL/6 mice, the frequency of mutations of G and C are roughly equal, whereas the mutations of A are twice as frequent as

those of the complementary T nucleotide. This bias for more mutations of A compared with T has been well documented in the previous studies of hypermutation, but did not have an explanation. In *pol η*-deficient humans (Mayorov *et al.* 2005) and mice (Martomo *et al.* 2005; figure 2), there was significantly less of a bias for mutations of A compared with T, suggesting that *pol η* is responsible for the A:T strand asymmetry. *In vitro*, it has been shown that *pol η* inserts a mismatched nucleotide opposite template T more frequently than opposite templates A, G or C (Matsuda *et al.* 2001). Specifically, there is a fivefold increase in the incorporation of G opposite T, which would represent an A–G substitution, compared with incorporation opposite the other nucleotides. This is exactly the category that drops to virtually nothing in humans and mice deficient in *pol η*. These data support a hypothesis where MSH2–MSH6 and exonuclease 1 create a gap in the DNA more frequently on the non-transcribed strand. *Pol η* then synthesizes in the gap and puts in predominantly G opposite template T, which is located on the transcribed strand.

We have also examined mice deficient in polymerases ι (Martomo *et al.* 2006) and θ (Martomo *et al.* 2008) and found no significant difference in the spectra or frequency of mutation compared with wild-type mice (figure 2). Since pol η has such a dominant effect on A:T mutations, it may substitute in the absence of other polymerases to affect the pattern. We therefore bred mice that were doubly deficient in pol η and either pol ι or pol θ , and measured mutation in these mice. As summarized in figure 2, mice deficient in both polymerases η and ι , and mice deficient in both polymerases η and θ , had the same mutation spectra as pol η -deficient mice, i.e. fewer mutations of A and T. These findings confirm the major role of pol η in somatic hypermutation (SHM).

In mice deficient in polymerases η , ι and θ , CSR to different heavy chain classes was normal in splenic B cells stimulated *in vitro* with lipopolysaccharide and several cytokines (Martomo *et al.* 2006, 2008). Thus, polymerases η , ι and θ do not fill in the staggered ends generated during double-strand break repair, even though pol η is clearly present and generating mutations in this region.

For Rev1, several groups have shown that it generates G:C to C:G transversions in mice and cell lines, in accordance with its activity as a cytidyl transferase (Jansen *et al.* 2006; Ross & Sale 2006). However, other low-fidelity DNA polymerases must generate the G:C to T:A transversions, and the residual A:T mutations seen in the absence of pol η . These may be any of the polymerases studied previously, but the effect might become more obvious in multiple knockouts.

4. INTERACTION OF MSH2–MSH6 AND DNA POLYMERASE η

Mice deficient in MSH2, MSH6, exonuclease 1 and DNA pol η all have the same hypermutation phenotype, i.e. fewer mutations of A:T. To see if they operate in the same mutagenic pathway, we tested their interactions biochemically (Wilson *et al.* 2005). First, we showed that the MSH2–MSH6 heterodimer binds to a U:G mismatch, which suggests that it can enter the pathway right after deamination of C to U. Second, MSH2 and pol η physically interact in cells, as shown by an antibody pull-down assay from cell extracts. Third, and most significantly, MSH2–MSH6 stimulated the catalytic activity of pol η *in vitro*. These data support the hypothesis that these proteins work together during SHM to produce mutations downstream of the initial U to fill in a gap created by exonuclease 1. As shown in figure 3, AID generates a U:G mismatch, MSH2–MSH6 binds to U:G and exonuclease 1 and pol η are recruited to the site. It is not known what generates a nick for the exonuclease to act on. It is unlikely to be APE1, since mice deficient in UNG, which have no abasic sites, have a normal frequency of A:T mutations (Rada *et al.* 2002). As pol η synthesizes in the repair patch, mismatches are generated opposite A and T. Repeated cycles of MSH2–MSH6 binding to the mismatches would further stimulate pol η to make more mutations downstream of the original C deamination. The bias for more mutations of A than T

Figure 3. Generation of A:T mutations. MSH2–MSH6 binds to the U:G mismatch, and exonuclease 1 makes a gap primarily on the non-transcribed strand. DNA polymerase η is recruited to synthesize in the gap, and generates mutations opposite A and T.

is due to pol η predominantly synthesizing in a gap on the non-transcribed strand.

Although mice deficient in MSH2, MSH6 and pol η have fewer A:T mutations, their mutation signatures—where the mutations are located—are quite different (Martomo *et al.* 2005). The mutations in mice deficient in MSH2 and MSH6 occur predominantly in WGCW motifs (W is A or T), whereas the mutations in mice deficient in pol η are spread all over. AID may initially recognize and deaminate C in WGCW before it processively deaminates other cytosines. MSH2–MSH6 would then bind to the U:G mispair in WGCW and attract an endonuclease and exonuclease 1 to generate a gap, and pol η would insert bases downstream of the U:G mispair. In the absence of MSH2 or MSH6, there would be no gap, and mutations would be focused at the WGCW sites. In the absence of pol η , another polymerase would synthesize in the gap to disperse the mutations downstream of WGCW. Therefore, the observed phenotype of gene-deficient mice nicely supports the biochemical interactions of MSH2–MSH6 and pol η .

5. OTHER REPAIR PROTEINS

Nucleotide excision repair removes bulky adducts and lesions in DNA by using a plethora of repair proteins. We (Winter *et al.* 1998) and others examined mice deficient in many of these proteins, and found that hypermutation was normal. Thus, the nucleotide

Figure 4. SHM and CSR from CSB^{-/-} mice. (a) Mutation was measured in the J_H4 region from Peyer's patch B cells, in two experiments. Data are corrected to represent a sequence with equal amounts of the four nucleotides. (b) Splenic B cells from four to six mice were stimulated *in vitro* with lipopolysaccharide and several cytokines for 4 days. The data were normalized to the percentage of switching in wild-type mice, which is expressed as 100%.

excision repair pathway does not participate in generating SHM. However, we recently used this pathway to gauge the relative efficiency of DNA repair of cyclobutane pyrimidine dimers in *V* genes versus a housekeeping gene. The rate of repair in a rearranged *V* gene was less efficient than in the dihydrofolate reductase gene (Alrefai *et al.* 2007). These results suggest that *V* genes have inherent properties that affect their rate of repair, which might allow misinserted nucleotides to linger longer.

The canonical base excision repair pathway could remove U produced by AID. Using UNG, U is removed, and the abasic site is nicked by APE1. The one-nucleotide gap is then faithfully repaired by DNA pol β , so no mutations or strand breaks would occur (figure 1). If pol β is removed, the frequency of mutation should increase as more Us would be shunted down the mutagenic pathway. Confirming this hypothesis, Wu & Stavnezer (2007) have nicely showed that mice deficient in DNA pol β do indeed have an increased frequency of mutation and CSR.

The mutagenic pathway uses two proteins in base excision repair, UNG and APE. Mice deficient in UNG have less heavy-chain class switching and an altered spectra of mutations (Rada *et al.* 2002), and mice heterozygous for APE1 or deficient in APE2 also have less switching and an altered spectra (Guikema *et al.* 2007). Thus, similar to the scenario for MSH2–MSH6, some, but not all, proteins in base excision repair participate in generating mutations in immunoglobulin genes.

To see if other proteins in base excision repair had an effect on hypermutation and switching, we examined mice deficient in Cockayne syndrome B (CSB).

CSB has been shown to stimulate APE1 activity *in vitro*, and cells deficient in CSB were more sensitive to methylating agents that are usually repaired by base excision repair (Wong *et al.* 2007). If CSB was involved in repairing hypermutations, cells deficient in CSB should have more mutation and switching. As shown in figure 4a, an examination of mutations in Peyer's patch B cells showed that the frequency and spectra of mutations was similar to wild-type B cells. Switching induced *in vitro* with lipopolysaccharide and a variety of cytokines was also normal, compared with wild-type cells (figure 4b). These data and those of Jacobs *et al.* (1998) suggest that CSB is not recruited to the complex of base excision repair proteins assembled at the immunoglobulin locus.

6. FUTURE DIRECTIONS

The most profound enigma in hypermutation, and perhaps immunology, is how AID is targeted to a very small region of DNA in the immunoglobulin loci. Mutations are found in a 2 kb region downstream of the promoter preceding rearranged *V_H*, *V_K* and *V_L* genes, and in a 4 kb region downstream of intronic promoters preceding the switch regions for *C_H* genes. Why does mutation go up, and why does it come down? What cofactors guide AID specifically to these regions? Why is AID directed to both V and switch regions *in vivo*, but only to switch regions in cells stimulated *in vitro*? These are the burning questions that will direct future research efforts.

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REFERENCES

- Alrefai, R. H., Winter, D. B., Bohr, V. A. & Gearhart, P. J. 2007 Nucleotide excision repair in an immunoglobulin variable gene is less efficient than in a housekeeping gene. *Mol. Immunol.* **44**, 2800–2805. (doi:10.1016/j.molimm.2007.01.018)
- Bardwell, P. D., Woo, C. J., Wei, K., Li, Z., Martin, A., Sack, S. Z., Parris, T., Edelmann, W. & Scharff, M. D. 2004 Altered somatic hypermutation and reduced class-switch recombination in exonuclease 1-mutant mice. *Nat. Immunol.* **5**, 224–229. (doi:10.1038/ni1031)
- Bertocci, B., De Smet, A., Flatter, E., Dahan, A., Bories, J.-C., Landreau, C., Weill, J.-C. & Reynaud, C.-A. 2002 Cutting edge: DNA polymerases μ and λ are dispensable for Ig gene hypermutation. *J. Immunol.* **168**, 3702–3706.
- Delbos, F., De Smet, A., Faili, A., Aoufouchi, S., Weill, J.-C. & Reynaud, C.-A. 2005 Contribution of DNA polymerase η to immunoglobulin gene hypermutation in the mouse. *J. Exp. Med.* **201**, 1191–1196. (doi:10.1084/jem.20050292)
- Delbos, F., Aoufouchi, S., Faili, A., Weill, J.-C. & Reynaud, C.-A. 2007 DNA polymerase η is the sole contributor of A/T modifications during immunoglobulin gene hypermutation in the mouse. *J. Exp. Med.* **204**, 17–23. (doi:10.1084/jem.20062131)
- Diaz, M., Verkoczy, L. K., Flajnik, M. F. & Klinman, N. R. 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.
- Di Noia, J. & Neuberger, M. S. 2002 Altering the pathway of immunoglobulin hypermutation by inhibiting uracil-DNA glycosylase. *Nature* **419**, 43–48. (doi:10.1038/nature00981)
- Ehrenstein, M. R. & Neuberger, M. S. 1999 Deficiency in Msh2 affects the efficiency and local sequence specificity of immunoglobulin class-switch recombination: parallels with somatic hypermutation. *EMBO J.* **18**, 3484–3490. (doi:10.1093/emboj/18.12.3484)
- Ehrenstein, M. R., Rada, C., Jones, A.-M., Milstein, C. & Neuberger, M. S. 2001 Switch junction sequences in PMS2-deficient mice reveal a microhomology-mediated mechanism of Ig class switch recombination. *Proc. Natl Acad. Sci. USA* **98**, 14 553–14 558. (doi:10.1073/pnas.241525998)
- Faill, A., Aoufouchi, S., Weller, S., Vuillier, F., Sary, A., Sarasin, A., Reynaud, C.-A. & Weill, J.-C. 2004 DNA polymerase η is involved in hypermutation occurring during immunoglobulin class switch recombination. *J. Exp. Med.* **199**, 265–270. (doi:10.1084/jem.20031831)
- Frey, S., Bertocci, B., Delbos, F., Quint, L., Weill, J.-C. & Reynaud, C.-A. 1998 Mismatch repair deficiency interferes with the accumulation of mutations in chronically stimulated B cells and not with the hypermutation process. *Immunity* **9**, 127–134. (doi:10.1016/S1074-7613(00)80594-4)
- Guikema, J. E. J., Linehan, E. K., Tsuchimoto, D., Nakabeppu, Y., Strauss, P. R., Stavnezer, J. & Schrader, C. E. 2007 APE1- and APE2-dependent DNA breaks in immunoglobulin class switch recombination. *J. Exp. Med.* **204**, 3017–3026. (doi:10.1084/jem.20071289)
- Jacobs, H. *et al.* 1998 Hypermutation of immunoglobulin genes in memory B cells of DNA repair-deficient mice. *J. Exp. Med.* **187**, 1735–1743. (doi:10.1084/jem.187.11.1735)
- Jansen, J. G., Langerak, P., Tsaalbi-Shtylik, A., van den Berk, P., Jacobs, H. & de Wind, N. 2006 Strand-biased defect in C/G transversions in hypermutating immunoglobulin genes in Rev1-deficient mice. *J. Exp. Med.* **203**, 319–323. (doi:10.1084/jem.20052227)
- Kadyrov, F. A., Dzantiev, L., Constantin, N. & Modrich, P. 2006 Endonucleolytic function of MutL α in human mismatch repair. *Cell* **126**, 297–308. (doi:10.1016/j.cell.2006.05.039)
- Kim, N., Bozek, G., Lo, J. C. & Storb, U. 1999 Different mismatch repair deficiencies all have the same effects on somatic hypermutation: intact primary mechanism accompanied by secondary modifications. *J. Exp. Med.* **190**, 21–30. (doi:10.1084/jem.190.1.21)
- Li, Z. *et al.* 2004 Examination of Msh6- and Msh3-deficient mice in class switching reveals overlapping and distinct roles of MutS homologues in antibody diversification. *J. Exp. Med.* **200**, 47–59. (doi:10.1084/jem.20040355)
- Martomo, S. A., Yang, W. W. & Gearhart, P. J. 2004 A role of Msh6 but not Msh3 in somatic hypermutation and class switch recombination. *J. Exp. Med.* **200**, 61–68. (doi:10.1084/jem.20040691)
- Martomo, S. A., Yang, W. W., Wersto, R. P., Ohkumo, T., Kondo, Y., Yokoi, M., Masutani, C., Hanaoka, F. & Gearhart, P. J. 2005 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. (doi:10.1073/pnas.0501852102)
- Martomo, S. A., Yang, W. W., Vaisman, A., Maas, A., Yokoi, M., Hoeijmakers, J. H., Hanaoka, F., Woodgate, R. & Gearhart, P. J. 2006 Normal hypermutation in antibody genes from congenic mice defective for DNA polymerase ι . *DNA Repair* **5**, 392–398. (doi:10.1016/j.dnarep.2005.12.006)
- Martomo, S. A., Saribasak, H., Yokoi, M., Hanaoka, F. & Gearhart, P. J. 2008 Reevaluation of the role of DNA polymerase θ in somatic hypermutation of immunoglobulin genes. *DNA Repair* **7**, 1603–1608. (doi:10.1016/j.dnarep.2008.04.002)
- Masuda, K. *et al.* 2007 DNA polymerases η and θ function in the same genetic pathway to generate mutations at A/T during somatic hypermutation of Ig genes. *J. Biol. Chem.* **282**, 17 387–17 394. (doi:10.1074/jbc.M611849200)
- Matsuda, T., Bebenek, K., Masutani, C., Rogozin, I. G., Hanaoka, F. & Kunkel, T. A. 2001 Error rate and specificity of human and murine DNA polymerase η . *J. Mol. Biol.* **312**, 335–346. (doi:10.1006/jmbi.2001.4937)
- Mayorov, V. I., Rogozin, I. B., Adkison, L. R. & Gearhart, P. J. 2005 DNA polymerase η contributes to strand bias of mutations of A versus T in immunoglobulin genes. *J. Immunol.* **174**, 7781–7786.
- McDonald, J. P., Frank, E. G., Plosky, B. S., Rogozin, I. B., Masutani, C., Hanaoka, F., Woodgate, R. & Gearhart, P. J. 2003 129-derived strains of mice are deficient in DNA polymerase ι and have normal immunoglobulin hypermutation. *J. Exp. Med.* **198**, 635–643. (doi:10.1084/jem.20030767)
- Muramatsu, M., Kinoshita, K., Fagarasan, S., Yamada, S., Shinkai, Y. & Honjo, T. 2000 Class switch recombination and hypermutation require activation-induced cytidine deaminase (AID), a potential RNA editing enzyme. *Cell* **102**, 553–563. (doi:10.1016/S0092-8674(00)00078-7)
- Petersen-Mahrt, S. K., Harris, R. S. & Neuberger, M. S. 2002 AID mutates *E. coli* suggesting a DNA deamination mechanism for antibody diversification. *Nature* **418**, 99–103. (doi:10.1038/nature00862)
- Phung, Q. H., Winter, D. B., Cranston, A., Tarone, R. E., Bohr, V. A., Fishel, R. & Gearhart, P. J. 1998 Increased hypermutation at G and C nucleotides in immunoglobulin variable genes from mice deficient in the MSH2 mismatch repair protein. *J. Exp. Med.* **187**, 1745–1751. (doi:10.1084/jem.187.11.1745)
- Phung, Q. H., Winter, D. B., Alrefai, R. & Gearhart, P. J. 1999 Cutting edge: hypermutation in Ig V genes from mice deficient in the MLH1 mismatch repair protein. *J. Immunol.* **162**, 3121–3124.
- Rada, C., Ehrenstein, M. R., Neuberger, M. S. & Milstein, C. 1998 Hot spot focusing of somatic hypermutation in MSH2-deficient mice suggests two stages of mutational targeting. *Immunity* **9**, 135–141. (doi:10.1016/S1074-7613(00)80595-6)
- Rada, C., Williams, G. T., Nilsen, H., Barnes, D. E., Lindahl, T. & Neuberger, M. S. 2002 Immunoglobulin isotype switching is inhibited and somatic hypermutation perturbed in UNG-deficient mice. *Curr. Biol.* **12**, 1748–1755. (doi:10.1016/S0960-9822(02)01215-0)
- Ross, A.-L. & Sale, J. E. 2006 The catalytic activity of REV1 is employed during immunoglobulin gene diversification in DT40. *Mol. Immunol.* **43**, 1587–1594. (doi:10.1016/j.molimm.2005.09.017)
- Schenten, D., Gerlach, V. L., Guo, C., Velasco-Miguel, S., Hladik, C. L., White, C. L., Friedberg, E. C., Rajewsky, K. & Esposito, G. 2002 DNA polymerase κ deficiency does not affect somatic hypermutation in mice. *Eur. J. Immunol.* **32**, 3152–3160. (doi:10.1002/1521-4141(200211)32:11<3152::AID-IMMU3152>3.0.CO;2-2)
- Schrader, C. E., Edelmann, W., Kucherlapati, R. & Stavnezer, J. 1999 Reduced isotype switching in splenic B cells from mice deficient in mismatch repair enzymes. *J. Exp. Med.* **190**, 323–330. (doi:10.1084/jem.190.3.323)

- Wiesendanger, M., Kneitz, B., Edelmann, W. & Scharff, M. D. 2000 Somatic hypermutation in MutS homologue (MSH)3-, MSH6-, and MSH3/MSH6-deficient mice reveals a role for the MSH2-MSH6 heterodimer in modulating the base substitution pattern. *J. Exp. Med.* **191**, 579–584. (doi:10.1084/jem.191.3.579)
- Wilson, T. M., Vaisman, A., Martomo, S. A., Sullivan, P., Lan, L., Hanaoka, F., Yasui, A., Woodgate, R. & Gearhart, P. J. 2005 MSH2-MSH6 stimulates DNA polymerase η , suggesting a role for A:T mutations in antibody genes. *J. Exp. Med.* **201**, 637–645. (doi:10.1084/jem.20042066)
- Winter, D. B. *et al.* 1998 Altered spectra of hypermutation in antibodies from mice deficient for the DNA mismatch repair protein PMS2. *Proc. Natl Acad. Sci. USA* **95**, 6953–6958. (doi:10.1073/pnas.95.12.6953)
- Wong, H.-K., Muftuoglu, M., Beck, G., Iman, S. Z., Bohr, V. A. & Wilson III, D. M. 2007 Cockayne syndrome B protein stimulates apurinic endonuclease 1 activity and protects against agents that introduce base excision repair intermediates. *Nucleic Acids Res.* **35**, 4103–4113. (doi:10.1093/nar/gkm404)
- Wu, X. & Stavnezer, J. 2007 DNA polymerase β is able to repair breaks in switch regions and plays an inhibitory role during immunoglobulin class switch recombination. *J. Exp. Med.* **204**, 1677–1689. (doi:10.1084/jem.20070285)
- Zeng, X., Winter, D. B., Kasmer, C., Kraemer, K. H., Lehmann, A. R. & Gearhart, P. J. 2001 DNA polymerase η is an A-T mutator in somatic hypermutation of immunoglobulin variable genes. *Nat. Immunol.* **2**, 537–541. (doi:10.1038/88740)
- Zeng, X., Negrete, G. A., Kasmer, C., Yang, W. W. & Gearhart, P. J. 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. (doi:10.1084/jem.20032022)