**Mlh1 Can Function in Antibody Class Switch Recombination Independently of Msh2**

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**Abstract**

Mismatch repair proteins participate in antibody class switch recombination, although their roles are unknown. Previous nucleotide sequence analyses of switch recombination junctions indicated that the roles of Msh2 and the MutL homologues, Mlh1 and Pms2, differ. We now asked if Msh2 and Mlh1 function in the same pathway during switch recombination. Splenic B cells from mice deficient in both these proteins were induced to undergo switching in culture. The frequency of switching is reduced, similarly to that of B cells singly deficient in Msh2 or Mlh1. However, the nucleotide sequences of the S(H)9262-S(H)9253 junctions resemble junctions from Mlh1— but not from Msh2-deficient cells, suggesting Mlh1 functions either independently of or before Msh2. The substitution mutations within S regions that are known to accompany switch recombination are increased in Msh2- and Mlh1 single-deficient cells and further increased in the double-deficient cells, again suggesting these proteins function independently in class switch recombination. The finding that MMR functions to reduce mutations in switch regions is unexpected since MMR proteins have been shown to contribute to somatic hypermutation of antibody variable region genes.

Key words: B cells • immunoglobulin isotypes • mismatch repair • switch region mutations • switch junctions

**Introduction**

Antibody class switching occurs in B cells after activation by antigen and results in a switch from IgM and IgD isotype expression to IgG, IgE, or IgA isotype expression, thereby changing the antibody effector functions while maintaining the identical antigen specificity. Isotype switching occurs by an intrachromosomal deletional recombination within tandemly repeated switch (S) region sequences located upstream of each Ig heavy chain constant region gene. Recombination occurs anywhere within each S region segment, which differ in sequence from each other and also vary in length from 2 to 10 kb (for a review, see reference 1). Activation-induced cytidine deaminase (AID) is required for class switch recombination (CSR) and for somatic hypermutation (SHM) of antibody variable region genes (2, 3). Recent data from Neuberger et al. (4, 5) indicate that AID directly deamimates genomic DNA. A current model for the role of AID in CSR is to deaminate dC residues in S regions, which generates U:G mismatches that are repaired by base-excision repair, resulting in single-strand DNA breaks (6). Consistent with this model, mice found deficient in uracil DNA glycosylase (UNG), which is the first enzyme in the base excision repair pathway, have greatly impaired (~10-fold reduced) CSR (5). One possible explanation for the low levels of CSR remaining is that mismatch repair (MMR) proteins, specifically Msh2 and Msh6, might recognize the U:G mismatch created by AID and initiate MMR by introducing DNA breaks. Fibroblasts transfected with an AID expression plasmid can recombine plasmid switch recombination substrates, indicating that AID is the only B cell–specific component essential for CSR (7).

After creation of DNA breaks, recombination of Sp with downstream S regions appears to occur by nonhomologous end-joining (NHEJ), because S-S junctions often occur at very short microhomologies (0 to 2 nts) between the upstream and downstream S regions (8). Furthermore, the Ku protein complex, thought to be essential for NHEJ, is required for CSR (9, 10). Interestingly, the kinase activity of DNA-PKcs is not required for CSR, as shown by examination of sad mice (11); however, when the DNA-PKcs gene is deleted by gene targeting, CSR to all isotypes except IgG1 is eliminated (12). Examination of mice deficient in the mismatch repair (MMR) proteins Msh2, Mlh1, and Pms2 has shown that...
these proteins have roles in CSR (13–16). Splenic B cells from mice deficient in Msh2, Mlh1, and Pms2 show two- to fivefold reductions in CSR, compared with wild-type B cells when stimulated in culture, and also show altered switch recombination junctions. MMR proteins in eukaryotes fall into two classes: (a) the MutS homologues (Msh1–6), which recognize DNA mismatches, loops, and other distortions, and (b) the MutL homologues (Pms2, Mlh1, and Mlh3 in mammals), which bind to MutS homologues bound to DNA and are thought to recruit endonuclease, exonuclease, and helicase activities (17). In addition to roles in post-replicative MMR, i.e., the correction of mutations introduced during DNA replication, MMR proteins have roles in homologous recombination during meiosis and in double-strand break repair (18, 19).

Switch recombination junctions obtained from both IgG- and IgA-expressing MMR-deficient B cells differ from junctions from wild-type B cells, suggesting that MMR proteins are directly involved in CSR (13, 14, 16). Interestingly, the characteristics of S junctions from Msh2-deficient B cells differ from those of Mlh1- and Pms2-deficient cells. Junctions formed during switching from IgM to IgG3 (Sp-Sy3 junctions) in Msh2-deficient B cells show decreased lengths of the microhomology normally observed at S junctions from wild-type cells and increased occurrence of small insertions at the junctions which do not correspond to the sequences of either the Sp or Sy3 regions (16). In addition, the junctions are restricted to the portion of the Sp region containing tandem consensus repeats, although CSR normally also occurs both 5′ and 3′ to this segment (13). By contrast, about one-fourth of the junctions from Mlh1- and Pms2-deficient B cells showed increased lengths of microhomology in comparison to wild-type junctions and no obvious restriction in location of recombination sites (14, 16). These data indicate that the role of Msh2 differs from the role of Mlh1 and Pms2 in CSR. However, these data do not indicate whether Msh2 is acting in a different pathway from Mlh1 or Pms2.

In this study we investigated whether Msh2 and Mlh1 function in the same or in different pathways in CSR. We reasoned that if they were to function in different pathways, splenic B cells deficient in both Msh2 and Mlh1 might show a greater deficiency in CSR than either mutant alone. In addition, the nucleotide sequences of the S junctions should indicate if the proteins function in the same or different pathways. If the proteins function in the same pathway, it seemed likely that the junction sequences would resemble those from cells deficient in the protein which acts earlier in the pathway, which presumably would be Msh2.

Materials and Methods

Mice. Mlh1-deficient mice were made by gene targeting and were obtained from R.M. Liskay, Oregon Health Sciences University, Portland, OR (20). Msh2-deficient mice were obtained from T. Mak, University of Toronto, Toronto, CA (21). Mice heterozygous for Mlh1 were bred to Msh2-heterozygotes to obtain mice heterozygous for both genes. These double heterozygotes were bred to generate mice deficient in both Mlh1 and Msh2, as well as wild-type and single-deficient msh2+/− and msh2−/− mice used as littermate controls. For the analysis of switching in cultured B cells, all the cells were from littermates. However, the sequence analysis of msh2/msh1−/− mice was initiated after the analysis of the wild-type and single-deficient junctions was completed, and thus these mice are from the same colony but are not true littermates.

B Cell Isolation and Cultures. Splenic B cells were isolated and cultured as described (15, 16).

PCR Amplification of Sp-Sy3 Junctions and Germline Sp and Sy3 Segments. Genomic DNA was isolated as described (16). Sp-Sy3 junctions were amplified from genomic DNA by PCR using the Expand Long Template Taq and Pfu polymerase mix (Roche) and the primers µ3-H3 (5′-AACAGCTTGGCTTAAACGGAGATGAGCC-3′) and g3–2 (5′-TACCCTGACCAAGGACTGATAACA-3′) (16) for sequences M-M-4–138. Different primers were used for sequences M-M-201–262, Smu1 (5′-TAGTAAGCGAGGCTCTAAAAAGCAT-3′), nts 5031–5055 of MUSIGCD07; reference 13) and Sg3–4 (5′-CTCTAGAATCTGGCTTAACAAAGCAT-3′), nts 1378–1405 of TIB114, like other plasmacytomas does not have AID and does not undergo SHM (22).

Cloning, Identification, and Sequence Analysis of PCR Products. PCR products were cloned into the vector pGEM®-T Easy (Promega) or into pCR4-TOPO (Invitrogen) using blue/white screening for inserts. DNA was isolated from white colonies using QIAprep spin miniprep kit (QIAGEN). Clones containing Sp-Sy3 junction inserts of varying sizes were chosen to optimize the identification of unique junctions. Nucleotide sequencing was performed by the University of Massachusetts Medical School Nucleic Acids Facility, using an ABI 377 DNA sequencer and Big Dyes. Sequences were aligned using the Clustal program of MacVector 6.5.3. Alignments were generally obvious, although in a few cases more than one alignment was possible due to the repetitive nature of the sequences and occasional internal deletions. In these cases, alignments were optimized according to the number of nts of identity at each junction: 0, 1 and 2, 3 and 4, and 5 or more and the Fisher-Freeman-Halton test was applied. Analysis of the whole table found significant differences between strains in the distribution of nts of junctional identity (P = 0.005).

Results

To examine the ability of msh2/msh1−/− B cells to undergo CSR, splenic B cells were cultured with inducers of

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isotype-specific CSR for 4 d and then analyzed by FACS® for surface Ig expression. The wild-type mice and mice deficient in Msh2, Mlh1, or both Msh2 and Mlh1 were tested in the same experiment and were always littermates. Fig. 1 presents two examples of FACS data showing surface isotype expression of wild-type and MMR-deficient B cells stimulated to switch to IgG2a and IgG1. Fig. 2 A presents the average amount of switching to each isotype as a percentage of switching in WT cells. CSR to all of the isotypes assayed was reduced in msh2/mlh1−/− B cells to 22 to 45% of WT, but the reduction was no more than was observed in cells from msh2−/− or mlh1−/− single-deficient mice.

To ensure that the reduced switching was not due to problems in cell proliferation or the cell cycle, we examined proliferation in B cells from the wild-type and 3 strains of MMR-deficient mice cultured to induce isotype-specific CSR. Proliferation, assayed by 3H-thymidine incorporation on day 3 of culture, was identical in wild-type and all MMR-deficient cells under all induction conditions (Fig. 2 B). The cell cycle, assayed by propidium iodide incorporation, was also not altered in MMR-deficient B cells induced with LPS and anti-δ-dextran for 65 h (Fig. 2 C).

As S3 junctions from msh2−/− B cells are strikingly different from mlh1−/− S3 junctions, we reasoned that the nucleotide sequence of the junctions in msh2/mlh1−/− B cells would indicate whether these proteins are functioning together in CSR. Genomic DNA was prepared from cells induced to switch to IgG3 with LPS and anti-δ-dextran for 4 d. S3 junctions were amplified by PCR, cloned and sequenced (Fig. 3). We compared the lengths of overlap, or identity, between the S and Sy3 sequences (boxed nucleotides) and found that junctions from msh2/mlh1−/− mice have significantly longer overlaps than those from msh2−/− mice ($p = 0.034$) and are similar but not identical to those from mlh1−/− mice ($p = 0.173$; references 14 and 16). Like Mlh1-deficient cells, there are junctions with extended microhomology (5–11 nts); however, the frequency of junctions with extended microhomology seems to be lower (Table I). It is clear that cells from msh2/mlh1−/− B cells have junctions that differ from msh2−/− B cells, and therefore that Mlh1 must have a role in CSR that does not require Msh2.

Nucleotide Mutations in S Regions Are Increased in MMR-deficient B Cells. Frequent nucleotide substitutions are observed in the S regions surrounding switch recombination.

Figure 1. Class switching is similarly reduced in Mlh1− and Msh2-single-deficient and double-deficient mice. Representative FACS® analysis of splenic B cells induced as indicated for 4d, surface stained with anti-IgM and anti-IgG2a or anti-IgG1 and analyzed by flow cytometry.

Figure 2. (A) In vitro class switching in splenic B cells from Mlh1− and Msh2-single-deficient and double-deficient mice expressed as percent of switching in B cells from wild-type littermates. Culture conditions for each isotype have been described (reference 16), except for IgG2a which was induced by treatment with LPS + interferon-γ (10 U/ml). The average of three experiments is shown (except n = 2 for Mlh1− IgG1, IgG2a, IgG2b, and IgG3) and error bars represent the SEM. (B) DNA synthesis measured by 3H-thymidine incorporation in 3d cultures with the same inducers used in panel A. Bars show the average of triplicate cultures plus 2 SD, and are representative of two experiments. (C) Cell cycle analysis by propidium iodide staining of cells induced with LPS and anti-δ-dextran for 65 h. The markers estimate the percent of cells in G0/G1, S, and G2/M (left to right) phases of cell cycle.
Figure 3. Nucleotide sequences surrounding the switch recombination junctions cloned from Mlh1/Msh2-double-deficient cells induced with LPS and anti-β-dextran. The sequences are of PCR products amplified from 13 independent cultures from two mice. Each cloned sequence (designated M-M) is shown aligned with the sequence of germline S4 (AF446347, upper sequence) and germline S3 (lower sequence) from 129/C57BL/6 mice (16), or occasionally from BALB/c mice (MUSIGCD09) as necessary. The boxed nucleotides represent the overlap in sequence between S4 and S3, with the number of these nucleotides shown to the right of each sequence. A vertical line represents no overlap (no identity). Underlined, boldface nucleotides are designated as insertions as these nucleotides do not appear to be templated by either S region.
The role of Mlh1 in the absence of Msh2 appears to affect mutated. These data indicate that Mlh1, in the absence of Msh2b/ Pms2 (24, 25). We asked if MMR deficiency also those observed during Ig V gene SHM, which have been indicating that error-prone DNA synthesis is involved in creating S-S junctions (23). The mutations are reminiscent of cating that error-prone DNA synthesis is involved in creat-

| Mouse                | Percentage of junctions with indicated length of microhomology | No. of sequences | Percentage w/inserts |
|----------------------|---------------------------------------------------------------|-----------------|----------------------|
|                      | ≥2 bp | ≥5 bp | ≥8 bp | ≥10 bp |
| WT                   | 44    | 3     | 0     | 0     | 32    | 3     |
| Mlh1−/−              | 49    | 30    | 17    | 11    | 47    | 2     |
| Pms2−/−              | 40    | 24    | 16    | 16    | 25    | 4     |
| Msh2−/−              | 19    | 0     | 0     | 0     | 32    | 19    |
| Msh2b/Pms2−/−        | 38    | 13    | 9     | 9     | 32    | 6     |

Mutations Are Increased in Recombined Sμ and Sy3 Segments from Mismatch Repair-deficient Cells

|          | WT   | msh2−/− | msh1−/− | msh2/msh1−/− |
|----------|------|---------|---------|--------------|
| Sμ mutations freq (×10^6) | 25.0 | 44.6 | 36.3 | 63.7 |
| Sy3 mutations freq (×10^6) | 19.2 | 34.3 | 26.9 | 44.9 |

Signif. of diff. from:

|          | WT | msh2−/− | msh1−/− | msh2/msh1−/− |
|----------|----|---------|---------|--------------|
| WT       | 0.046 | NS | 0.0001 |   |
| msh2−/−  | NS | NS |      | 0.002 |
| msh1−/−  | 0.021 |    |     |   |

NS, not significant.

Discussion

We had hypothesized that a combined deficiency of Msh2 and Mlh1 would have the phenotype of Msh2-deficiency in CSR, as binding of a Msh2 heterodimer with either Msh3 or Msh6 to DNA is the first step in the postreplicative MMR pathway, and as Msh2 functions in double-strand break repair in yeast without the MutL homologues (27). Instead, we found that a portion of the switch recombination junctions from Msh2/Mlh1 double-deficient cells showed a phenotype similar to Mlh1- or Pms2 deficiency, i.e., increased junctional microhomology relative to WT junctions, which is significantly different from Msh2 deficiency. From these data, we conclude that the presence of Mlh1/Pms2 heterodimers prevents the formation of switch junctions with extended microhomologies, whether or not Msh2 is present.

Atomic force microscopy has shown that yeast Mlh1/ Pms1 can directly bind two different DNA molecules (28), suggesting it might participate in synopsis of S region junctions in the absence of Msh2 or other MutS homologues. In addition, the NH2-terminal domain of human Pms2 can bind DNA as a monomer (29). Perhaps the Mlh1/Pms2 heterodimer is involved in forming the synaptic structure between the Sμ and Sy3 sequences. In its absence, the increased microhomology we observe at the junction may provide stability to the structure. The Msh2 heterodimer might function subsequently to process DNA ends before ligation. Perhaps Msh2 excises single-strand ends that if not removed result in mutations that resemble inserts at the junction. It is notable that the lengths of microhomology and presence of inserts in junctions from the msh2/ msh1−/− cells seem somewhat intermediate between msh2−/− and msh1−/− junctions (Table I), suggesting that Msh2 also has a function in the absence of Mlh1. Although comparison of junctions from wild-type and msh2−/− cells supports a role for Msh2 in end-processing, the msh2/ msh1−/− junctions clearly show that Msh2 is not needed to obtain extended microhomologies.

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Alternatively, it is possible that Mlh1 may be functioning with another MutS homologue, instead of directly binding to DNA itself. Mlh1 is thought to function in meiosis with Msh4 and 5, and Msh4 and Msh5 have been shown to bind Holliday junctions and to be required for crossing over during meiosis (18, 20, 30). However, the process of meiosis involves homologous recombination, which is not involved in CSR, as different S regions do not share the required lengths of homology. In addition, the sites of recombination used indicate that the repeat units do not align in register (8). Whether Mlh1/Pms2 functions alone or with other MutS homologues, our data suggest that it directs switching to an end-joining type of pathway and prevents an alternative short-homology pathway. This activity clearly does not depend on Msh2.

The use of an alternate pathway may explain why class switching in vitro is not further reduced in the Mlh1/Msh2-deficient mice, relative to those deficient in Mlh1 or Msh2 alone, as the frequency of switching would be governed by the rate of this alternate pathway. This alternate pathway may also be used when the kinase Ataxia-Telangiectasia Mutated (ATM) is absent, since these switch junctions have been shown to have increased lengths of microhomology as well (31). Our data suggest that the Mlh1/Pms2 heterodimer is involved during normal CSR, perhaps to hold the DNA ends together in a particular structure such as the synaptic complex, perhaps inhibiting the generation of relatively long stretches of junctional homology, and focusing the recombination to the DNA ends. The Mlh1/Pms2 heterodimer may function alone or with a MutS heterodimer that does not include Msh2. The data also suggest that Msh2 acts subsequently or independently to recruit end-processing machinery that trims DNA ends, resulting in Sµ-Sγ3 junctions that normally manifest one to two nucleotides of microhomology and infrequent appearance of sequence insertions.

We thank Drs. Wei Yang, Qiang Pan, and Denise Kaminski for very helpful suggestions. We thank Ms. Sofia Mochegova and Ms. Erin Flanagan for outstanding technical assistance. We thank Dr. Stephen Baker and Erin Flanagan for statistical analysis. We thank Drs. R.M. Liskay and T. Mak for mice deficient in Mlh1 and Msh2, respectively.

The research was supported by a grant from the National Institutes of Health, AI23283.

Submitted: 19 December 2002
Revised: 23 March 2003
Accepted: 23 March 2003

References
1. Stavnezer, J. 1996. Antibody Class Switching. Academic Press, San Diego. 79–146
2. Muramatsu, M., K. Kinoshita, S. Fagarasan, S. Yamada, Y. Shinkai, and T. Honjo. 2000. Class switch recombination and hypermutation require activation-induced cytidine deaminase (AID), a potential RNA editing enzyme. Cell. 102:553–563.
3. Revy, P., T. Muto, Y. Levy, F. Geissmann, A. Plebani, O. Sanal, N. Catalan, M. Forveille, R. Dufourc-Labelouse, A. Gennery, et al. 2000. Activation-induced cytidine deaminase (AID) deficiency causes the autosomal recessive form of the Hyper-IgM syndrome (HIGM2). Cell. 102:565–575.
4. Petersen-Mahrt, S.K., R.S. Harris, and M.S. Neuberger. 2002. AID mutates E. coli suggesting a DNA deamination mechanism for antibody diversification. Nature. 418:99–104.
5. Rada, C., G.T. Williams, H. Nilsen, D.E. Barnes, T. Lindahl, and M.S. Neuberger. 2002. Immunoglobulin isotype switching is inhibited and somatic hypermutation perturbed in UNG-deficient mice. Curr. Biol. 12:1748–1755.
6. Storb, U., and J. Stavnezer. 2002. Immunoglobulin genes: generating diversity with AID and UNG. Curr. Biol. 12: R725–R727.
7. Okazaki, I.M., K. Kinoshita, M. Muramatsu, K. Yoshikawa, and T. Honjo. 2002. The AID enzyme induces class switch recombination in fibroblasts. Nature. 416:340–345.
8. Dunnick, W., G.Z. Hertz, L. Scappino, and C. Gritzmaczer. 1993. DNA sequences at immunoglobulin switch region recombination sites. Nucleic Acids Res. 21:365–372.
9. Casellas, R., A. Nussenzweig, R. Wuerffel, R. Pelanda, A. Reichlin, H. Suh, X.F. Qin, E. Besmer, A. Kenter, K. Rajewsky, and M.C. Nussenzweig. 1998. Ku80 is required for immunoglobulin isotype switching. EMBO J. 17:2404–2411.
10. Manis, J.P., Y. Gu, R. Lansford, E. Sonoda, R. Ferrini, L. Davidson, K. Rajewsky, and F.W. Alt. 1998. Ku70 is required for late B cell development and immunoglobulin heavy chain switching. J. Exp. Med. 187:2081–2089.
11. Bosma, G.C., J. Kim, T. Urich, D.M. Fath, M.G. Cotticelli, N.R. Ruetsch, M.Z. Radic, and M.J. Bosma. 2002. DNA-dependent protein kinase activity is not required for immunoglobulin class switching. J. Exp. Med. 196:1483–1495.
12. Manis, J.P., D. Dudley, L. Kaylor, and F.W. Alt. 2002. IgH class switch recombination to IgG1 in DNA-PKcs-deficient B cells. Immunity. 16:607–617.
13. Ehrenstein, M.R., and M.S. Neuberger. 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.
14. Ehrenstein, M.R., C. Radz, A.M. Jones, C. Milstein, and M.S. Neuberger. 2001. Switch junction sequences in PMS2-deficient mice reveal a microhomology-mediated mechanism of Ig class switch recombination. Proc. Natl. Acad. Sci. USA. 98:14553–14558.
15. Schrader, C.E., W. Edelmann, R. Kucherlapati, and J. Stavnezer. 1999. Reduced isotype switching in splenic B cells from mice deficient in mismatch repair enzymes, J. Exp. Med. 190:323–330.
16. Schrader, C.E., J. Vardo, and J. Stavnezer. 2002. Role for mismatch repair proteins Msh2, Mlh1, and Pms2 in immunoglobulin class switching shown by sequence analysis of recombination junctions. J. Exp. Med. 195:367–373.
17. Kolodner, R.D., and G.T. Marsischky. 1999. Eukaryotic
DNA mismatch repair. *Curr. Opin. Genet. Dev.* 9:89–96.

18. Borts, R.H., S.R. Chambers, and M.F. Abdullah. 2000. The many faces of mismatch repair in meiosis. *Mutat. Res.* 451: 129–150.

19. Evans, E., and E. Alani. 2000. Roles for mismatch repair factors in regulating genetic recombination. *Mol. Cell. Biol.* 20: 7839–7844.

20. Baker, S.M., A.W. Plug, R.A. Prolla, C.E. Bronner, A.C. Harris, X. Yao, D.-M. Christie, C. Monell, N. Arnheim, A. Bradley, et al. 1996. Involvement of mouse *Mlh1* in DNA mismatch repair and meiotic crossing over. *Nat. Genet.* 13: 129–150.

21. Reitmair, A.H., J.C. Cai, M. Bjerknes, M. Redston, H. Cheng, M.T. Pind, K. Hay, A. Mitri, B.V. Bapat, T.W. Mak, and S. Gallinger. 1996. MSH2 deficiency contributes to accelerated APC-mediated intestinal tumorigenesis. *Cancer Res.* 56:2922–2926.

22. Ma, L., H.H. Wortis, and A.L. Kenter. 2002. Two new isotype-specific switching activities detected for Ig class switching. *J. Immunol.* 168:2835–2846.

23. Dunnick, W., M. Wilson, and J. Stavnezer. 1989. Mutations, duplication, and deletion of recombined switch regions suggest a role for DNA replication in the immunoglobulin heavy-chain switch. *Mol. Cell. Biol.* 9:1850–1856.

24. Kim, N., G. Bozek, J.C. Lo, and U. Storb. 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.

25. Rada, C., M.R. Ehrenstein, M.S. Neuberger, and C. Milstein. 1998. Hot spot focusing of somatic hypermutation in MSH2-deficient mice suggests two stages of mutational targeting. *Immunity.* 9:135–141.

26. Petersen, S., R. Casellas, B. Reina-San-Martin, H.T. Chen, M.J. Difilippantonio, P.C. Wilson, L. Hanitsch, A. Celeste, M. Muramatsu, D.R. Pilch, et al. 2001. AID is required to initiate Nbs1/gamma-H2AX focus formation and mutations at sites of class switching. *Nature.* 414:660–665.

27. Paques, F., and J.E. Haber. 1997. Two pathways for removal of nonhomologous DNA ends during double-strand break repair in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* 17:6765–6771.

28. Hall, M.C., H. Wang, D.A. Erie, and T.A. Kunkel. 2001. High affinity cooperative DNA binding by the yeast Mlh1-Pms1 heterodimer. *J. Mol. Biol.* 312:637–647.

29. Guarne, A., M.S. Junop, and W. Yang. 2001. Structure and function of the N-terminal 40 kDa fragment of human PMS2: a monomeric GHL ATPase. *EMBO J.* 20:5521–5531.

30. Hunter, N., and R.H. Borts. 1997. Mlh 1 is unique among mismatch repair proteins in its ability to promote crossing-over during meiosis. *Genes Dev.* 11:1573–1582.

31. Pan, Q., C. Petit-Frere, A. Lähdesmäki, H. Gregorek, K.H. Chrzanowska, and L. Hammarstrom. 2002. Alternative end joining during switch recombination in patients with ataxia-telangiectasia. *Eur. J. Immunol.* 32:1300–1308.