Role for Mismatch Repair Proteins Msh2, Mlh1, and Pms2 in Immunoglobulin Class Switching Shown by Sequence Analysis of Recombination Junctions

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Abstract

B cells from mice deficient in mismatch repair (MMR) proteins show decreased ability to undergo class switch recombination in vitro and in vivo. The deficit is not accompanied by any reduction in cell viability or alterations in the cell cycle in B cells cultured in vitro. To assess the role of MMR in switching we examined the nucleotide sequences of $S\mu$ -S γ 3 recombination junctions in splenic B cells induced in culture to switch to IgG3. The data demonstrate clear differences in the sequences of switch junctions in wild-type B cells in comparison with Msh2-, Mlh1-, and Pms2-deficient B cells. Sequences of switch junctions from Msh2-deficient cells showed decreased lengths of microhomology between $S\mu$ and $S\gamma$ 3 relative to junctions from wild-type cells and an increase in insertions, i.e., nucleotides which do not appear to be derived from either the $S\mu$ or $S\gamma$ 3 parental sequence. By contrast, 23% of junctions from Mlh1- and Pms2-deficient cells occurred at unusually long stretches of microhomology. The data indicate that MMR proteins are directly involved in class switching and that the role of Msh2 differs from that of Mlh1 and Pms2.

Key words: splenic B cells • DNA recombination • DNA repair • antibody heavy chain isotypes • mismatch repair

Introduction

Upon activation, B cells expressing IgM and IgD undergo Ig isotype (class) switching to express IgG, IgE, or IgA. Class switching occurs by a DNA recombination event that results in exchanging the constant region of the Ig heavy chain, without changing the antibody variable region. This process changes the effector functions of the antibody but does not affect antigen-binding specificity. Class switch recombination (CSR) occurs by an intrachromosomal deletional recombination between switch (S) sequences located upstream of the constant region genes (for a review, see reference 1).

S sequences consist of tandem repeats of short (20–80 bp) consensus elements, extending from 1 to 10 kb in length, and CSR can occur at any site within the S regions (2). Although the S regions have short elements in common, e.g., GGGGT or GAGCT, the S regions of different heavy chain genes (isotypes) differ too much to undergo homologous recombination. Instead, CSR is thought to occur by a

type of nonhomologous end joining (NHEJ, reference 3). Ku70, Ku80, and DNA-PK, proteins known to be important for NHEJ, are essential for normal CSR (4–6). This hypothesis is supported by the fact that one often observes short bits of microhomology at the S-S junctions, which is typical of NHEJ. However, whether these microhomologies play a role in the recombination is unknown as their presence may simply be due to shared sequence elements among S regions (2).

An interesting feature of S recombination junctions is the presence of nucleotide substitutions, deletions and insertions, which has led to the proposal that CSR occurs by a process involving error-prone DNA synthesis (2, 7). The mutations appear quite similar to those due to somatic hypermutation of antibody variable regions, and these two processes have many other similarities. Both CSR and somatic hypermutation occur during antigen activation of B cells and require transcription; both appear to be initiated by double-strand breaks (8–10), and both require activation-induced deaminase (11).

Recently, mismatch repair (MMR) proteins have been shown to be involved in both CSR and somatic hypermutation. MMR proteins in eukaryotes fall into two classes: (i)

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the MutS homologs (Msh1-6) which recognize DNA mismatches, loops, and other distortions, and (ii) the MutL homologs (Pms1, Pms2, and Mlh1 in mammals) which bind to MutS homologs bound to DNA (for a review, see reference 12). It is well established that MMR proteins have additional roles besides the correction of nucleotide substitutions and small insertions or deletions created by DNA synthesis errors (12). Msh2, Msh6, Mlh1, and Pms2 are involved in, but not required for, somatic hypermutation (13-16). In the absence of these proteins, the frequency of somatic hypermutation is decreased. In addition, some MMR proteins have roles in homologous DNA recombination. MMR proteins have been shown to prevent recombination between homeologous sequences (sequences that are homologous, but not identical) (for a review, see reference 17). Msh 2 and 6 have been shown to bind to Holliday junctions (18) and Mlh1 and Pms2 are found bound to chromosomes undergoing meiosis in spermatogonia. In addition, Mlh1 mice and male Pms2 mice are sterile (19, 20). DSB repair in yeast requires removal of nonhomologous DNA segments adjacent to the break before the break can be repaired. Msh2 and Msh3 are required for this end-processing if 30 nts or more of such heterologous sequences are present, and their role is to recruit an endonuclease complex (Rad1/XPF and Rad 10/ ERCC1) to excise the heterologous 3' single-strand tail (21, 22).

By testing the ability of splenic B cells from mice deficient in three MMR proteins, Msh2, Mlh1 and Pms2, to undergo CSR in culture, we have previously shown that MMR proteins are required for optimal switching in these cultures, although they are not essential (23). Depending on the particular isotype, switch recombination is reduced by two to fourfold. MMR-deficient B cells proliferate as well as wild-type B cells and are no more susceptible to apoptosis than wild-type B cells in these cultures. Experiments in which the effect of Msh2 deficiency was examined during in vivo immune responses also showed a deficit in class switching, and the deficit was somewhat greater using this approach (24, 25).

To begin to determine the role of MMR in switch recombination, we examined the $S\mu$ - $S\gamma3$ junctions in B cells induced to switch to IgG3 in culture. We have compared the junctions obtained from Msh2-, Mlh1- and Pms2-deficient mice with junctions obtained from wild-type littermates. Our results demonstrate that all three MMR proteins are involved in CSR, but that Msh2 appears to be involved at a different step from Mlh1 and Pms2. Msh2, but not Mlh1 and Pms2, may be involved in processing the ends after DSB formation, while Mlh1 and Pms2 may be involved in stabilizing the recombination complex before DNA ligation.

Materials and Methods

Mice. Mice made deficient in Pms2 or Mlh1 by gene targeting were obtained from R.M. Liskay, Oregon Health Sciences University, Portland, OR (19, 20). Msh2-deficient mice were obtained from W. Edelmann and R. Kucherlapati, Albert Einstein College of Medicine, Bronx, NY (26). Mouse strains were carried as heterozygotes and wild-type littermates were used as controls. The background strains are 129 and C57Bl/6.

B Cell Isolation and Cultures. B cells were isolated from spleens by depletion of RBCs by lysis in Gey's solution for 5 min on ice and by depletion of T cells with a cocktail of anti-T cell reagents, anti-CD4 (GK1.5), anti-CD8 (3.168), and anti-Thy1 (HO13.4 and J1J10), followed by anti-rat κ-chain mAb (MAR18.5) and guinea pig complement (Pelfreeze Biochem). Viable cells were isolated by flotation on Ficoll/Hypaque gradients ($\delta = 1.09$). 10⁶ B cells were cultured at 2 × 10⁵ cells per milliliter in 6-well plates for 4 d in RPMI 1640 (BioWhittaker), with 10% FCS (Hyclone), 2 mM L-glutamine, 100 U/ml penicillin, 100 µg/ml streptomycin (all from GIBCO BRL), and 1X MEM nonessential amino acid solution, 1 mM sodium pyruvate, and 5 × 10⁻⁵ M 2-mercaptoethanol (all from Sigma-Aldrich). LPS (50 µg/ml, *Escherichia coli* 055:B5; Sigma-Aldrich) was added at the initiation of culture.

PCR Amplification of S μ -S γ 3 Junctions and Germline S μ and $S\gamma3$ Segments. Genomic DNA was isolated from B cells, either resting or cultured with LPS for 4 d. Cell pellets were incubated with proteinase K (0.5 mg/ml), RNaseA (100 µg/ml), and SDS (0.5%) in STE (0.1 M NaCl, 20 mM Tris, 1 mM EDTA) for 2 h at 37°C, followed by 3-4 extractions with phenol/chloroform (1:1) and precipitation with 0.3 M sodium acetate, pH 7, and ethanol. DNA was wound out on glass rods and resuspended in TE, pH 8. The germline $S\gamma3$ segment was amplified by PCR from resting purified B cells from WT(129 \times B6) mice for comparison to S μ -S γ 3 junctions from cells induced to switch to IgG3. Expand HiFidelity Taq polymerase (Roche Laboratories) was used with the following primers: g3-1 (5'CAGGCTAAGATGGATG-CTACAGGG-3') (MUSIGHANA 404-427) and g3-2 (5'TAC-CCTGACCCAGGAGCTGCATAAC-3') (MUSIGHANA 2603-2628) to amplify the 2.22-kb fragment of germline $S\gamma 3$. $S\mu$ - $S\gamma 3$ junctions were amplified by PCR using Expand Long Template Taq polymerase (Roche Laboratories) and the primers µ3-H3 (5'AACAAGCTTGGCTTAACCGAGATGAGCC-3') and g3-2 (above). The germline $S\mu$ sequence was deduced by comparing the sequences of a large number of $S\mu$ -Sy3 junctions from wildtype mice. For the sequence analyses, the wild-type sequences from the corresponding littermates were used.

Cloning, Identification, and Sequence Analysis of PCR Products. PCR products were cloned into the vector pGEM®-T Easy (Promega) using blue/white screening for inserts. DNA was isolated from white colonies using QIAprep spin miniprep kit (QIAGEN). Inserts of the proper size for germline $S\mu$ and $S\gamma3$ segments were sequenced and compared with the corresponding germline 129 × B6 or BALB/c sequences. Clones containing Sµ-Sy3 junction inserts of varying sizes were chosen to optimize the identification of unique junctions. For Msh2 clones, colonies containing inserts were identified by colony hybridization using the germline BALB/c Sµ 1.8-kb HindIII fragment. Sequence analysis of the cloned inserts was performed, using standard T7 and SP6 primers, by the UMass Nucleic Acid 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 repetitious nature of the repeats and occasional internal deletions. In these cases, alignments were optimized to reduce nucleotide differences between the germline and recombinant sequences. The sequences which had to be aligned by minimizing mutations are WT28 (Sy3), WT44 (Sµ), Msh195 (Sy3), and Mlh124 (Sµ).

Results

To obtain $S\mu$ - $S\gamma3$ junctions for nucleotide sequence analysis, splenic B cells from wild-type or MMR-deficient mice were cultured with LPS to induce switching to IgG3 (23). On day 4, genomic DNA was isolated and $S\mu$ - $S\gamma3$ junctions were amplified by PCR using a primer located at the 5' end of $S\mu$ and a primer located at the 3' end of $S\gamma3$. PCR products were cloned and plasmids containing inserts of various sizes were chosen for sequence analysis. The nucleotide sequences of the junctions obtained from wildtype littermates of Mlh1- and Pms2-deficient mice are shown in Fig. 1. The upper sequence in each set is the corresponding unrearranged, or germline, $S\mu$ sequence (labeled 129 × B6 Sm), or if not available for the particular junction shown, then from the BALB/c $S\mu$ sequence (GenBank locus MUSIGCD09). The third sequence of

	i i
MUSICCD09 807 TGAGCTGAGCTGAGCTGAGCTGAGCTGGGGTGAGCTGA	129XB6 Sm 420 GAGCTGGGCTGAGCTGGGGTGAGCTGGGGTGAGCTGAGC
WT5 463 TGAGCTGAGCTGAGCGGTGAGCGTAAGGGGTATGGGGACCAG 0 nt identity	NT 44 255 GAGCTGGGGCTGGGGCTAGGGGTAGGAGTATAG 0 nt identity
129xB6 Sg3 592 CAGCTCTGGAGGGAOCTAGGGTAAGTGAGGGTATGGGGACCAG	129xB6 Sq3 249 GCTGGGCAGCTCTCAAGTGAATTGGGGTAGGTTGGAATATAG
129XB6 Sm 533 CTGAGCTGAGCTGGGGTGAGCTGAGCTGAGCTGAGCTGA	129XB6 Sm 223 ACTGAGCTGAGCTGAGCTGAGCTGAGCTGAGCTGAGCTG
WT21 553 CTGAGCTGAGCTGAGCTGGGGTAGGTT 2 nt identity	WT 45 223 ACTGAGCTGAGCTAGGGTGAGCAGGCTGGGTAGCTCTGG 1 nt identity
129xB6 Sg3 335 GTGGGGACCAAGCTGGGGGGGGGGGGGGGGGGGGGGGGG	Sg3-Balb/c 1120 CTGGGGTAGGTGGGGTATACCAGCCTGGGTAGCTCTGG
NUSIGCE09 562 GAOCTEAGCTEGAGCT	129XB6 5m 306 AGCTOGGCTGAGCTGGGGTGAGCTGAGCTGGGGTAAG
WT26 366 GAGCTGAGCTGGGGTGAGCTGGGGAGGTGGAGCTA 1 nt identity	WT 46 306 ASCTGGGCTGAGCTGGGCTGACTGGGGGAGCC 0 nt identity
Sg3-Balb/c 580 ACCAGGCTGAGCAGCTCTCAGDGAGCTGGGGAGGTGGAGCTA	Sg3-Balbc 1127 AGGTGGGGTATAGGAACCAGGTGGGGTAGCTCTGGGGGAGCC
129XB6 Sm 570 GAGCTGAGCTGGGGGTGAGCTGAGCTGAGCTGAGCTGAG	129XB6 Sm 391 GCTGGGCTGAGCTGGGGTGAGCTGGGCTGAGCTGGGCTGAGC
WT 27 570 GAGCTGAGCTGGGGTGAGCTGCTGGAGCATGGGAAACAGGC 1 nt identity	WT 50 391 GCTGGGCTGAGCTGGGGTGATTGGGCAGCTACAGGTGAGCTG 0 nt identity
Sg3-Balb/c 1303 CTCTCGGGGGAGCTAGGGTAGGTAGGGAGCATGGGAAAACAGGC	129xB6 Sg3 798 GAGGGAGTATGAGGACTAGGTGGGCASCTACAGGTGAGCTG
129XB6 Sm 280 GAGCTGAGCTTGGCTBAGCTAGGGTGAGCTGGGCTGAGCTGG	129XB6 Sm 246 GAOCTGGGTGAGCTGAGCTGAGCTGAGCTGAGCTGAGCTG
WT 28 280 GAGCTGAGCTTGGCTGAGCTCAGGAGAGGGTAAGGGTATGGGG 4 nt identity	WT 51 246 GAGCTGGGTGAGCTGAGCTAAGCTGGGCAGCTCTCAGGGAG 2 nt identity
Sg3-Balb/c 1149 TOGGTAGCTUTOGGGSBAGCCCAGGAGAGGTAAGGGTGTGGGGG	129xB6 Sg3 613 TAAGTGAGGGTATGGGGACCAGCTGGGCAGCTCTCAGGGAG
MUSIGCD09 778 GCTGAGCTGGGGTGAGCTGAGCTGAGC	129XB6 Sm 351 GGATGAGCTGGGGTGAGCT AGCTGAGCTGAGCTGAGCT
WT29 562 GCTGAGCTGGGGTGAGCTGAACTATGTGGGGTTGTT 1 nt identity	wr 53 351 GGATGAGCTGGGGTGAGCTDAGGGAGTATGAGGACTAGGTTG 3 nt identity
sg3-Balb/c 728 GGCTGGGACAGCTCTGGAAGGAGATGAGATATGTGGGGGTTGTT	129xB6 Sg3 779 TACAGGTGAGCTGGGGTAGAAGGGAGTATGAGGACTAGGTTG
129XB6 Sm 198 GOTGAGCTGAGCTGGGCTGAGCTAGACTGAGCTGAGCTAGGGT	129XB6 Sm 448 GTGAGCTGAGCTGGGGTGAGCTGAGCTGAGCTGGGGTGAGCT
wT30 198 GOTGAGCTGAGCTGGGCTGGGTGAGGATGTGGGGGACCAAGCTG 2 nt identity	WT 56 448 GTGAGCTGAGCTGGGGTGAGCTACAOGTGAGCTGOGTTAGAT 4 nt identity
129xB6 sg3 297 GCTGGAGGGAGCTAGGATAGGTGAGGATGTGGGGACCAAGCTG	129xB6 Sg3 806 ATGAGGACTAGGTTOSSCREETACAGGTGAGCTGGGTTAGAT
129X86 Sm 390 TGAGCTGGGCTGAGCTGGGCTGAGCTGGGCTG	129XB6 Sm 304 GAGCTGGGGCTGAGCTGGGGTSAGCTGAGCTGAGCTGGGGTA
wr35 390 tgagctgggctgagctgggcgaccaggctgggaaactette 2 nt identity	WT 59 304 GAGCTGGGCTGAGCTGGGGTGCAGCTACAGGTGAGCCAGGG 1 nt identity
129xB6 Sg3 175 TGGGGTACATGGGGTTGTDCDGACCAGGCTGGGAAACTCTTG	129xB6 5g3 668 GGTTGTGAGGACCAGGCTGGGCAGCTACAGGTGAGCCAGGG
	# 00000000000000000000000000000000
129XB6 Sm 594 TGAGCTGAGCTGAGCTGAGCTGGGGTGGAGCTGAGCTGA	MUSIGCD09 486 CTGAGCTGGGGTGAGCTGAGCTGAGCTGGGCGC MEMO 633 CTTAACTGGGGTAACTGGGTGAACTGGGCGGACAGG 0 nt identity
WT36 594 TGAGCTGAGCTGAGCTGAGCCAGGCTGGGGAGCTCTCAGGG 1 nt identity	W160 633 CTGAGCTGGGGTGAGCTGGGGTGAGCTGAGCAGGGTACAGG 0 nt identity 129x86 9g3 467 GTAGGTGGAGCATAGGATATTAAGCTGAGCAGC-TACAGG
129xB6 Sg3 611 GGTAAGTGAGGGTATGGGGCCAGGCTGGGCAGGCTCTCAGGG	
129XB6 Sm 373 CTGGAGTGAGCTGAGCTGAGCTGAGCTGGGGCT	129X86 Sm 174 AGTAGCTGAGATGGGGTGAGATGGGGTGAGCTGAGCTGGGC
WT1-87 373 CTGGAGTGAGCTGAGATGCGCTGAGCAGGTACAGGTGAGTTG 7 nt identity	WT 61 174 AGTAGCTGAGATOGGGTGAGAGGGGGGGACCAGGCT 2 nt identity
129xB6 Sg3 473 TGGAAGCATAGGATATTAADCTGAGCIGCTACAGGTGAGCTG	129xB6 Sg3 501 TACAGGTGAGCTGGGGTAG AGGGGGGGGGGGGGGGGGCCAGGCT
129XB6 Sm 393 CTGGGCTGAGCTGGGGGTGAGCTGAGCTGAGCTGAGCTG	129XB6 Sm 490 GAGCTGAGCTGGGGTGAGCTGGGGTGAGCTGAGCTGGGGTG
WT 38 393 CTGGGCTGAGCTGGGGTGGGGTGGGGTGGGGTGGGGTAGGT 4 nt identity	WT 62 490 GAGCTGAGCTGGGGTGAGCGGGTGAGCTGGGGTA 0 nt identity
129xB6 Sg3 47 ATGGGAAACAGGCTGGACAGCTCTGGGGGGACCTGGGGTAGGT	129xB6 Sg3 478 CATAGGATATTAAGCTGABCAGCTACAGGTGAGCTGGGGTA
	129хв6 Sm 256 АССТОЛОСТАЛОСТОВОСТОЛОСТОЛОСТООСТАЛО
129XB6 Sm 253 GAGCTGAGCTAAGCTGGGGTGAGCTGAGCTGAGCTTGGCTGAG	WT 63 256 AGCTGAGCTAAOCTGOGGTGAGGTGGGGGGAGTCCAGGAGAGGT 0 nt identity
VT 42 253 GAGCTGAGCTAAGCTGAGGTTAGACTTGGACAGCTCTGGGGAGC 2 nt identity Sg3-Balb/c 753 ATATGTGGGGTTGTGGGGAGC 2 nt identity	Sg3-Balb/c 1137 TAGGAACCAUGCTGGGTAGCTCGGGGAGCCCAGGAGGGT
Sg3-Balb/c 753 ATATGTGGGGTTGTGGGGGAGCAGCTGGGGGAGC	
129XB6 Sm 206 GAGCTGGGGCTGAGCTAGAGTGAGCTAGGGTGAGCTGA	129XB6 Sm. 595 GAGCTGAGCTGAGCTGAGCTGAGCTGAGCTGAGCTGA
WT 43 206 GAGCTGOGCTGAGCTAGACTGAGGACTAGGTTGGGCAGCTAC 4 nt identity	WT 65 595 GAGCTGAGCTGAGCTGAGCTGAGCTGAGCTGAGCTGAGC
129xB6 593 788 GCTGGGGTAGGAGGGAGTA <mark>FGAGBACTAGGTTGGGCAGCTAC</mark>	129xB6 5g3 613 TAAGTGAGGGTATGGGGACCAG
129XB6 Sm 549 TGAGCTGAGCTGGGGTGAGCTGAGCTGGGGTGAGCTG	129XB6 Sm 211 GGGCTGGGACTGGACTGAGCTGAGCTGAGCTGAGCTGAG
WT1-93 546 TGAGCTGAGCTGGGGTGAGCTGGTTAGATGGAAATGTGAATA 1 nt identity	WT2-28 211 GGCTGAGCTGAGCTGAGCTGAGCTGAGCTGGGCCAGACTGGGC 3 nt identity
129xB6 Sg3 819 TOGGCAGCTACAGGTGAGCTGGGTTGGAAATGTGAAATGTGAAATA	129xB6 Sg3 407 GGGGCAGCTGAGGTTAGTGG <mark>LAGT</mark> GTAGGGACCAGACTGGGC
	129XB6 Sm 320 GGGGTGAGCTGAGCTGAGCTGCGGATGAGCTGGGATGAGCTGG
129xB6 Sm 349 T3GGATGAGCTGAGCTGAGCTGAGCTGAGCTGAGCTGAGC	WT1-32 320 0G0GTGAGTTGAGCTGAGCTGGGGTAGGAGGGGGT 1 nt identity
WT1-4 349 TOGGATGAGCTOGGGTGAGCTOGGGTAAGTGOGAATATOOAGA 0 nt identity	129xB6 sg3 486 ATTAAGCTGAGCAGCTACAGCTGAGCTGGGGTAGGAGGGAG
129xB6 Sg3 684 CTGGGCAGCTACAGGTGAGCCAGGGTAAGTGGGAGTATGGAGA	TEXTOR OF ALTRACTOROGOUND Provention State
129XB6 Sm 195 TGGGGTGAGCTGAGCTGGGCTGGGCTGAGCTGAGCTGAG	129xB6 Sm 194 ATGGGGTGAGCTGAGCTGAGCTGAGCTGAGCTGAGCT
WT3-16 195 TEGESTGAGCTGAGCTGGGCTATGCGGATATTAAGCTGAGCAG 4 nt insert or	WT2-6 194 ATGGGGTGAGCTGAGCTGGGCTCAGGTGGGTTGGAT 3 nt identity
129xB6 Sg3 744 GGGAGCTGAGGTGGAAGCATAGGATATTAAGCTGAGCAG	129xB6 Sg3 806 ATGAGGACTAGGTTGGGCACTTCAGGTGAGCTGGGTTGGAT

each set is the unrearranged Sy3 sequence from IgM^+ cells of wild-type littermates (labeled $129 \times B6$ Sg3), or if not available, the BALB/c Sy3 sequence (GenBank locus MU-SIGHANA). The middle sequence shows the segment surrounding the S μ -S γ 3 junction, with the junction either marked as a vertical line (if there is no microhomology at the junction) or enclosed with a box to indicate nucleotides that may have been derived from either the S μ or S γ 3 segments, i.e., the microhomology at the junction. One junction appears to have a short insert that does not correspond to either parental sequence (WT3-16), although alternatively, it could have two mutated nucleotides adjacent to a junction with 0 nucleotides of microhomology. As shown in Fig. 1, the sequences from wild-type B cells generally show 0 to 4 nucleotides of identity at the Sµ-Sy3 junctions, although one sequence (WT1-87) has 7 nucleotides of identity.

Although the recombinant S junctions have nucleotide substitutions and small deletions or insertions typical of switch recombination junctions, these clones are PCR prod-

> Figure 1. Nucleotide sequences surrounding Sµ-Sy3 junctions from wild-type mice. PCR products obtained from 12 individual cultures from two mice were cloned and sequenced. DNA from each culture was amplified individually. The sequences are aligned with the Sµ sequence cloned from these mice, numbered from the first nucleotide of the segments cloned in pGEM®-Teasy (GenBank/EMBL/DDBJ AF446347; nt 1 = nt 5206 in MUSIGCD07) and with the sequenced region of the 129 \times B6 Sy3 segment. If the $129 \times B6$ sequence has not been determined in the region at the junction, the sequence is aligned with the BALB/c Sµ sequence MUSIGCD09 (numbering from nt 1), which is located 3' to MUSIGCD07, or the $S\gamma3$ sequence, MUSIGHANA (numbering from nt 404 in MUSIGHANA). The Sy3 consensus tandem repeats begin at nt 195 and terminate at nt 2203 (repeat 42) in the BALB/c Sy3 sequence. The BALB/c MUSIGCD09 and 129 \times B6 Sµ sequenced region (760 nts) do not overlap, whereas nucleotide position 1245 in the BALB/c 2.2-kb Sy3 sequence corresponds to nt position 1 in the 129 \times B6 sequence. There are a few nt differences between the 129 imes B6 and BALB/c sequences. To the right of each sequence is indicated the number of nucleotides (nts) of this identity at the junction. Underlined nts at the junction do not appear to be derived from either S μ or S γ 3 and may represent nts inserted during recombination. Differences between the recombinant sequence and the parental sequence may be due to mutations introduced during CSR (2, 7) or to PCR errors.

ucts so it is not clear if all of the mutations were introduced during switching. Furthermore, we did not observe any clear differences in mutation frequency among the sequences obtained from WT or the three MMR-deficient B cells.

Sμ-Sγ3 junctions obtained from Msh2-deficient B cells are shown in Fig. 2. These sequences show shorter elements of microhomology at the junctions than WT (P =0.004), usually 1 or 2 nts of identity, ranging up to 3 nts at most. In addition, 19% (6 of 32) have short inserts or nucleotide mutations at the junctions. Although WT junctions also have inserts (Fig. 1, and reference 2), the frequency of inserts in Msh2^{-/-} junctions was significantly higher than the WT frequency (P = 0.002).

The Sµ-S γ 3 junctions obtained from Mlh1-deficient B cells, shown in Fig. 3, differ from the sequences of both Msh2^{-/-} and of wild-type B cells. Although 78% of these sequences have junctional microhomologies of 4 nts or less, similar to wild-type junctions, 22% of them show \geq 5 nts of microhomology at the S junctions, extending up to 14 nts of identity. The junctions from Pms2-deficient B cells have the same feature, with 24% showing junctional micro-

homology of 5 or more nts, extending up to 11 nts (Fig. 4). Table I presents a summary of the microhomology analyses. These data suggest that Mlh1 and Pms2 are also involved in CSR, but that their role differs from the role of Msh2.

Discussion

The finding that the sequences of $S\mu$ - $S\gamma3$ junctions differ between Msh2-deficient and wild-type B cells suggests that Msh2 is involved in the recombination process itself. One attractive possibility is that Msh2 is involved in DNA end-processing, similar to its role in DSB repair in yeast. In this model, single-strand DNA ends produced after DSB formation and during the alignment of the donor and acceptor S regions would be clipped off by an endonuclease recruited by Msh2 (presumably as a heterodimer with either Msh3 or Msh6). In yeast DSB repair, the recruited endonuclease is a complex of Rad 1 and 10 (homologs of mammalian XPF and ERCC1). We have previously described this model (Fig. 5 in reference 23). In the absence of Msh2, lack of this type of end-processing might

129XB6 Sm	226 GAGCTGAGCTAGGTGAGCTGAGCTGAGCTGAGCTGAGCT	129XB6 Sm 174 ASTAGCTGAGATGGGGTGAGCTGAGCTGGGCTGAGCTGGGCT
Hsh20	226 GASCTGAGCTAGOGTGAGCTGCGGGCAGCTCTCAGGGAGCT 1 nt identity	Heb102 174 AGTAGCTGAGATGOOGTGAADGCCTOSGCAGCTCTCAGGGAG 1 nt identity
129×B6 5g3	615 AGTGAGGGTATGGGGACCAGCTGGOGCAGCTCTCAGGGAGCT	129×B5 Sg3 517 TAGGAGGGAGTGTGGGGGACCEDOCTGGGCAGCTCTCAGGGAG
129XB6 Sm	390 GAGCTGGGCTGAGCTGGGGTGAGCTGGGCTGA	NUSIGCD39 791 CTGAGCTGGGGTGAGCTGAGCTGAGCTGGGGTGAGCTGAGCT
Nsh24	390 GAGCTGGGCTGAGCTGGGGTGGGGTAGGAGGAGTATGA 1 nt identity	#sh195 727 CTGACCT0000TGAUCTUAQUITTGAAGTATA-GAACAGGCT 0 nt identity
129xB6 Sg3	768 AGCTGAGCAGCTACAGGTGAGCTGGGGGTAGGAGGGAGTATGA	129x86 Sg3 758 CTCTCAAGTGAACTGGGGTAGGTTGGAATATAGGAGCAGGCT
MUSIGCD09	646 CTGAGCTGGGGTGAGCTGAGCTGAGCTGAGCTGGGGTGAGCT	129XB6 Sm 574 TGAGCTGGGGTGAGCTGAGCTGAGCTGAGCTGAGCTGAG
Msh28	730 CTGAGCTGGGGTTAGCTGGAGGTTGGAGTATAG-AGCAGGC 2 nt insert	Ksh388 494 TGAGCTGGGGTGAGCTGAGGGAGGTGAGCTG3GGTAGGTTGG 1 nt insert
129xB6 Sg3	257 GCTCTCAAGTGAACTOGGGTAGGTTGGAATATAGGAGCAGGC	129xB6 Sg3 98 GOGACCAOGCTGGGCAGCTAGAGGTGAGCTGGGGTAGGTTGG
129XB6 Sm	279 TEAUCTEASCTTESCTEASCTASGETEASCTESSETE	MUSIGCD09 820 GGGTGAGCTGAGCTGAGCTGGGGTGAGCTGAGCTGAGCT
Mah43	279 TGAGCTGAGCTTGGCTGAGCTGGCGGACGTGGCGGA 0 nt identity	weh410 639 GAGTGAGCTGAGCTGGGCTAGGTATGGGGACCAGGCTGGGCA 3 nt identity
Sg3-Balb/c	965 TGSSCASCTCTGGGGGAACTRGGGTAGGTGGGG-TGTGGGGA	129xB6 Sg3 601 AGGGAGCTAGGGTAAGTGADGGTATGGGGACCAGGCTGGGCA
129XB6 Sm	532 GCTGAGCTGOGGTGAGCTGGGGTGAGCTGAGCTGGAA	129XB6 8m 527 GGTGAGCTGAGCTGAGCTGAGCTGAGCTGAGCTGAGCTG
Msh62	411 GCTGGGCTGGGCTGAGCTGGGCAGCTCT 2 nt identity	Mab538 522 GGTGAGCTGAGCTGAGCTGGGCTGGGGTGGGGTGGGGTTGTGA 1 nt identity
129xB6 Sg3	510 GCTGGGGTAGGAGGGAGTGTGGGCAGCTCT	129xB6 Sg3 634 GGCTGGGCAGCTCCAGGGAGCTGGGGTGGGTGGGTGGGTTGTGA
129XB6 Sm	197 GGGTGAGCTGAGCTGGGCTGAGCTGAGCTGAGCTAGG	129XB6 Sm 364 TGAGCTGAGCTGAGCTGGAGCTGAGCTGAGCTGGGCTGG
Msh73	197 GGGTGAGCTGAGCTGAGCTGAGCAGC 0 nt identity	Meh542 364 TGAGCTGAGCTGGAGCTGGAGGGAGCTGGGGGGGGGGGG
129xB6 Sg3	737 GAGCTGAGGTAGGTGGAAGCATAGGATATTAAGCTGAGCAGC	129xB6 Sg3 573 CTGTGGGGACCAGGCTGGGCCAGGCTGGGGGGGGGGGGG
129XB6 Sm	194 ATGOGGTGAGCTGAGCTGAGCTGAGCTGAGCTGAGCTGAG	129xB6 sm 197 GGGGTGAGCTGAGCTGGGGCTGGGCTGGGCTGGGCTGGG
	194 ATOGOGTGAGCTGAGCTGGGCTGAGCTGGGTTGGAT 3 nt identity	Neb567 250 0000TGAGCTGAGCTGG <u>TCTATGC</u> GGATATTAAGCTGAGCAG <u>6 nt insert</u>
129xB6 Sg3	806 ATGAUGACTAGOTTGGGCA	129xB6 Sg3 736 GGAGCTGAGGTAGGTGGAAGCATAGGATATTAAGCTGAGCAG
129XB6 Sm	252 GOTGAGCTGAGCTAGCTGGGCTGAGCTGAGCTGGGC	129XB6 Sm 498 TEGESTERGETERGETERGETERGETERGETERGETERGE
Msh2-21	252 OGTGAGCTGAGCTAACCTGGGRTTAAGCTGAGTAGTTATAGG 0 nt identity	Mab687 375 TGCCGTCAGCTGGGGTGAGCTCGGGGTAGGTTCGAGT 1 nt identity
129xB6 Sg3	743 AGGTAGGTGGAAGCATAGGATATTAAGCTGAGCAGCTACAOG	129xB6 Sg3 341 ACCAAGCTGGGCAGCTCTGGGGAAGCTGGGGTAGGTTCUAGT
129XB6 Sm 3	242 AGCTGAGCTGGGTGAGCTGAGCTAAGCTGGGGTGAGCTGAGC	129xB6 sm.r 463 ccccacercaceccacercaceccacercacec
Msh2-46	242 AGTTGAGTTGGGTGAGTTGAGATGAGGACTAGGTTGGGCAGC 0 nt identity	Mahl-8.rev 412 CCTCAGCTCAGCCCAGTTCABCTGGGCAGATTTCAGGGAGCT Sµ inverted
129×B6 Sg3 1	785 TGAGCTGGGGTAGGAGGGAGTATGAGGACTAGGTTGGGCAGC	129xB6 Sg3 519 GGAGGGAGTGTGGGGACCAG <mark>DCT</mark> GGGCAGCTCTCAGGGAGCT
MUSIGCD09	700 AGCTGAGCTGGGGTGAGCTGAGCTGGGGTGAGCTGAGCT	MUSIGCD09 784 GCTGAGCTGAGCTGGGGTGASCTGAGCTGAGCTGGGGTGAGC
	100 AGCTGAGCTGGGGTGAGCTGAACTACAGGTGAGCCGGGGTAA	Meh1-64 -1450 OCTODOCTORGCTOGGCTORGTORGCCOGGGGTARGTOGGART 1 nt identity
	670 TTOTGAGGACCAGGCTOGGCAGCTACAGGTGAGCCAGGGTAA	129хв6 5g3 676 ассадостворскостасновтраесскоооталоторонот
	757 TOGGETGAGCTGAGCTGAGCTGAGCTGAGCTGAGCTG	129XB6 Sm 264 TAAGCTGAGCTGAGCTGAGCTGAGCTGAGCTGAGCTGAG
	608 TGGGGTGAGCTGAGCTGAGCTATGGGGGGGGGGGGGGGG	Msh2-3 264 TAAGCTGGGGTGAGCTGAGCTGAGCTGAGCAGTTATAGSTG 1 nt identity
	768 ACCTGACCACCTACAGGTGACCTGGCGTAGGAGGGAGTATGA	129xB6 Sq3 745 GTAGGTGGAAGCATAOGATA
	***************************************	129XB6 Sm 296 AGCTAGGGTGAGCTGAGCTGAGCTGAGCTGAGCTGAGCT
	347 GCTG3GATGAGCTG3GGTGAGSGTGAGSGTGAGGTGTG3GGA 0 nt identity 009 GCTG3GGACTCTG3GGGAGCTGGGGTGGGTGAGGTGTG3GGA	129x86 Sg3 775 CAGCTACAGGTGAGCTGGGGTAGAGGGAGTATGAGGACTAG
	705 GCTGAGCTGAGCTGGGGTGAGCTGAGCTGAGCTGGGG	129XB6 Sm 300 AGGGTGAGCTGGACTGAGCTGGGCTGAGCTGAGCTGAGC
Msh3-24	602 GCTGGGCTGAACTGCGCTGGGGGGGGGGGGGGGGGAGTAT 1 nt identity	Mab2-17 300 AGGGTGAGCTGGAGCTGCAGCTCCGGACAGCTCCGGAGGGAG
129xB6 Sg3	766 TAAGCTGAGCAGCTACAGGTEAGCTGGGGTAGGAGGGAGTAT	129x86 Sg3 277 AGGTTGGAATATAGGAGGAGCAGGTCTGGACAGCTCTGGAGGGAG
129XB6 Sm	332 GCTGAGCTGGGGTAAGCTGGGATGAGCTGGGGTGAGCTGAGC	129XB6 Sm 249 CTGGGTGAGCTGAGCTAAGCTGGGGTGAGCTGAGCTGAG
	332 GTTGAGCTGGGGTAAGCTGGGAGCTAGGTGGGAACATAGOGT 2 nt identity	Hab4-36 249 CTGGGTGAGCTGAGCTGAGCTTGGGAGCATGGGAAACAGGCTG 1 nt identity
129xB6 Sg3	722 GGAGACCTGGCTGGGGGGGGGGGGGGGGGGGGGGGGGGG	129xB6 Sg3 20 CTC00003GAGCTAGGGTAGGTTGGAGCATGGGAAACAGGCTG
	818 GGGTGAGCTGAGCTGAGCTGGGGTGAGCTGAGGTGA	MUSIGCE09 851 CTAGGGTGAGCTGGGCTGGGTGAGCTGGAGTGAGCTGAGCTG
Msh4-2 - 1	1000 GGGGTGAGCTGAGCTGAGCTGAGCGGAGCGGAGCTGGAGCTGAGCTGGAGCTGAGCGGGGGGGG	Mah4-40 ~1300 TTGGGGTGAGCTG3GCTGAACTGATAGGGTAAGTGAGGGTAT 4ntinsert
129xB6 Sg3	750 TOGAAGCATAOGATATTAAGCTGAGCAGCTACAOGTGAGCTG	129xB6 Sg3 584 AGGCTGGGCAGCTCTGGAGGGAGCTAGGGTAAGTGAGGGTAT
MUSIGCD09	843 GAGCTGAGCTAGGGTGAGCTGGGTGAGCTGGAGTGAG	MUSICCD09 420 CCTGAGCTGAGCTGAGCTGGGGTGAGCTGAGCTGAGCTG
Msh4-3 -	900 GAGCTGAGCTGGGGTGAGCTGCTTAGATGGAAATGTGAATAA 2 nt identity	Hsb4-65 -1200 GCTGAGCTGAGTTGAGTTGGGTGAAGGGAGCTAGGTAAGTGA 0 nt identity
129x86 Sg3	820 GGGCAGCTACAGGTGAGCTGGGTTGGATGGAAATGTGAATAA	129xB6 Sg3 577 GOGGACCAGOCTGOGCAGCTGGGAGGGAGCTAGGGTAAGTG

Figure 2. Nucleotide sequences surrounding $S\mu$ -Sy3 junctions from Msh2-deficient mice. Methods and notation identical to Fig.1. Sequences were obtained from 21 cultures of B cells from 5 mice.

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129XB6 Sm 280 GAGCTGAGCTGAGCTGGGCTGAGCTGGGGTGAGCTGGGCTGA		_
MLH 30 280 GACCTGACCTGACCTGGCCTGACTGAGGTGAGCTGAGCT	0 nt identity	129x86 зм 501 ааатиласталастиластиластиластиластиласти
129XB6 Sm 251 GCTOGGGTBAGCTGAGCTGAGCTAGGCTGAGCTAGGGTGAGC		129×86 Sm 172 GEAGTAGCTGAGATGGGGGGAAATGGGGGTGAGCTGAGCT
MLb 37 251 GCTGGGGTGAGCTGAGCTGAGCCCAGGAGAGGGTAAGGGTGTG		M1h 84 172 003.07300/that.astroggotta.as. Discrete Topological Control of the identity 129x86 515 007.00030.070700000.0007000000.000707000000.000707000000
Sg3-Balb/c 1146 состовотляетствоос <u>рласт</u> словляетляеватого 129XB6 sm 552 остолостовоютслостолост <mark>р</mark> ластовоютолостолостоло		129XB6 Sm 216 GAOCTAGACTGAOCTGAOCTGAOCTGAOCTGAOCTGAOCT
M1h 53 552 GCTGAGCTGGGGTGAGCTGAGCTGTTAGGAGTGTAGGGACCAG	1 nt identity	129xB6 Sg3 65 AGCTCTGGGGGAGCTGGGGTGGGGGTGGGGGGTGGGGGGCCAGG
Sg3-Balb/c 442 TGGGCAGCTCTGGGGCAGCTGGGGTTTTAGGAGTGTAGGGACCAG		123836 Sm 525 адааталасталасталасталасталасталастаявитаа N3h 89 525 аваөталасталасталастазосталасталастатааса 13 nt identity
MUSIGCD09 550 GTGAGCTGAGCTGAGCTGAGCTGAGCTGAGCTGAGCTGA	6 nt identity	129xB6 Sg3 493 ТОЛОСЛАСТЛА <mark>АОТОЛОСТОВООТ</mark> ЛООЛОСОЛОТОТООООЛ
129х86 5g3 567 атоалостатасаалсскар <u>ствос</u> расстстволоодаастлада		129X86 8m 552 GAOCTOAGCTODOOTDAOCTOAGCTOROGTOAGCTO M1h 93 552 GAOCTOAGCTODOOTDAOCTOAGCTATATATATATATATATATATATATATATATATATAT
129XB6 Sn 555 GAGCTGGGGTGAGCTGAGCTGAGCTGAGCTGAGCTGAGC	14 nt identity	Sg3-Balb/c 732 GGACAGCTCTGGAAGAGCTGAG
129xB6 Sg3 103 CAGGCTGGGCAGCTA <u>DAGGTGAGCTGGGGT</u> GGGTTGGAGTATGGG	in identity	129X86 Sm 206 GACCTGGGCTGAACTGCTGACGTGGGTGAGCTAGGGGGAGCTGA x1b 97 286 GAGCTGGGCTGAACTGACTGGCGGAAGCTAGGATAGTAA 1 nt identity
129x86 Bm 301 GCGTGAGCTGGGCTGAGCTGAGCTGAGCTGAGCTGAGCT		129xB6 Sg3 449 AGCTCTGGGGGAGCTAGGGGAAGCATAGGATATTAA
M1h 62 301 GGGTGAGCTGGGGCTGAGCTGGGGCTGGGCGGGCTGGGCAGCTACAGGTGAGCCA 129xB6 5g3 660 TGGGGGGGGTGGGAGGAGCACCAGCTGGGCAGCTACAGGTGAGCCA	2 nt identity	129XB5 Sm 256 AGCTGAGCTAAGCTGGGGTGAGCTGAGCTGGGCTGGGCT
129×86 REV 460 AGGTCAGGTCAGGTCAGGTCAGGTCAGGCCAGGTCAG	1 nt identity	Balb/c Sg3 640 GCCCTGGGGGGTAGCTAGGGTGAGGATGTGGGGACCAGG
Klb69 286 AGETCAGETCAGETCAGETCAGETCAGETCAGETCAGETC	Sµ inverted at junction	NUSIGCD09 690 GCTGAGCTGAGCTGAGCTGAGCTGAGCTGAGCTGAGCTG
_		N1h 124 3+:228 GCTGAGCTGGGGCTGGGGGTGGGGGGGGGGGGGGGGGGG
129XB6 Sm 490 GAGCTGAGCTGAGCTGAGCTGAGCTGAGCTGAGCTGAGC	1 nt identity	129XB6 Sm 267 GCTG3GGTGAGCTGAGCTGAGCTTGGCTGAGCTAGGTGAGC
129xB6 Sg3 14 GOOGAGCTAGGGTAGGTTCGALCATGGGAAACAGOCTOGACAGCT		Mih 127 267 GCTGGGGTGAGCTGAGCTGAGCTGAGGTAGGAGGAGTATGG 129xB6 Sg3 490 ACCTGAGCAGCTACAGGTAGAGCTGGGGAGTAGGAGGGAG
129XB6 Sm 231 GASCTAGOGTGAGCTGAGCTGAGCTGAGCTGAGCTGAGCTAGCGGG 8Lb76 231 GASCTAGGGTGAGCTGAGCTGGGGGGCAGTTAGAGGTGAGCTG	0 nt identity	125×86 8# 174 AGTAGCTGAGATGGGGGGGGGGGGGGGGGGGGGGGGGGG
129xB6 Sg3 86 GGTGGGGTTGTGGGGGACCAGCTAGAGGTGAGCT	,	N1h 130 174 AGTAGCTGAGATGSGGGTGAL Aggregogcagggggggggggggggggggggggggggggggggg
129XB6 Sm 439 TGACCTGGGGTGACCTGGCGTGACCTGACCTGACCTGAC		129XB6 Sm 213 GCTGAGCTAGACTGAGCTGAGCTGAGCTGAGCTGGGTG
x1h 79 439 TGAGCTGGGGTGAGCTGAGCTGAGCTGGGGTAGGTTGGAGG 129x86 \$g3 101 ACCAGGCTGGGGCAGCTAGAGTTGAGCTGGGGTAGGTTGGAGT	0 nt identity	N1h 134 213 GETGAGETAAGETGAGETGAGETCECAGETATGGAGETCET 129xB6 8g3 885 GETGGGGETATCAGATCACAGEGETCCCAGGETATGGAGETCET
129XB6 Sm 201 TAGGGTGAGCTGAGCTGAGCTGAGCTGAGCTGAGCTGAG		129X86 5m 532 GCTGAGCTGAGCTGGGGTGAGCTGGGGTGAGCTGAGCT
N1b 80 201 TAGGSTGAGCTGAGCTGAGCTGAGGGGAGCTAGGGGAGCTAGG 129xB6 Sg3 442 ACTGGGCAGCTCTGGGGGAGCTAGGTGGAGGCTAGGTGGAGCTAGGGGGAGCTAGGGGAGCTAGGGGGAGCTAGGGGGAGCTAGGGGGAGCTAGGGGGAGCTAGGGGGAGCTAGGGGGAGCTAGGGGGAGCTAGGGGGAGCTAGGGGGAGCTAGGGGGAGCTAGGGGGAGCTAGGGGGAGCTAGGGGGAGCTAGGGGGGGG	2 nt insert	N1h 135 512 SCTGAGCTGAGCTGGGGTGAACCATAGGTGGAGCATAGGATATT 1 nt identity 129x86 sg3 446 GOCAGCTCTGGGGGGGCTGAGCTAGGTGGAGCATAGGATATT T
129XB6 Sm 504 TGAGCTGGGGTGAGCT-DAGCTGGGGTDAGCTGAGCTG		129XB6 Sm 92 GTTCTGAGCTGAGATGAGCTGAGGTGAGCTCAGCTATGCTA
Pms 2 504 TGAGCTGGGGTGAGCTCGAGCTGGGGTAGGTTCGAGTATGGGG	10 nt identity	Pms 39 92 GTTCTGAGCTGAGATGAGCTGGGCAGATTTGGGGCAGCTGA 5 nt identity
129xB6 Sg3 354 GCTGGGCAGCTCTGGGG <mark>DAGCTGGGGTAGGCTGAGTTCGAGTATGGGG</mark> 129xB6 Sm 195 TGGGGTGAGCTGAGCTGGGCTGGGCTGAGCTGAGCTGAG		129xB6 5g3 377 TCGAGTATGGGGACTAACTTGGCCCTCTGGGGCCAGCTGA
129XB6 Sm 195 T0G0GTGAGCTGAGCTGAGCTGAGCTGAGCTGAGCTGAGC	0 nt identity	129XB6 Sm 466 TGAGCTGAGCTGAGCTGGGDTGGGDTGGGGTGGGGGT Pms 42 466 TGAGCTGAGCTGAGCTGGGDTGGGTTGGGTGAAGGGAAATGTGA 1 nt identity
129xB6 Sg3 735 GGGAGCTGAGGTAGGTGGAAGCATAGGATATTAAGCTGAGCAG		129×86 5g3 817 OTTOGOCAGCTACAGGTGADCTGGGTTAGATGGAAATGTGA
129XB6 Sm 211 GGGCTGAGCTAGACTGAGCTGAGCTGAGCTGAGCTGAGC	2 nt identity	129XB6 Sm 260 GAGCTAACTGGGGGGGACCTGACTTGACCTGGCCTGGCC
129xB6 Sg3 882 GAGCTGGGGCTATCAGATCAGAGGGTCCCAGGTTATGCAGCTC		129x86 5g3 220 AGCTGGGGAGGTGGGGGTGGGGGAGCTGGGGCAGCTCT
129XB6 Sm 90 CTGTTCTGAGCTGAGTGAGCTGAGGTGAGCTCAGGTATGCTA Pms 19 90 CTGTTCTGAGCTGAGATGAGCTAGGCGAGGCGCGAGGCCTGGGGAG	0 nt identity	129XB6 Sm 384 GAGCTGAGCTGAGCTGAGCTGGGGTBAGCTGGGCTGAGCTGA
sg3-Balb/c 1031 GOGTGGGTGAGGTGTGGGGGAAChGGCTGGGCAGCTCGGGGAG	o ne identity	
		129x86 5g3 493 TGAGCAGCTACAGGTGAGCTGOGGTAGGACGGAGTGTGGGGA
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	<u>5. nt. insert</u>	129×86 Sg3 493 TGAGCAGCTACAGG <mark>TGAGCTGGGGTACGACGGAGTGTGGGGA</mark>
Pms 20 270 GAGCTTGGCTGAGCTAGGGTGA ANTOTTGGGCAGCTCTGGAGG	<u>5 nt insert</u>	129x86 893 493 толостослостислостралоствородитородилостородил 129x86 8m 369 толостолосториотели, Пластородстородото Рев 50 369 толостолосториотели, Пластородстанислосскит 1 nt identity
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Figure 3. S μ -S γ 3 junctions from Mlh1-deficient B cells. Note that the boxes include single nts that are not identical with both the S μ and S γ 3 sequences which are included only if they are preceded by 2 or more identical nts. The numbers of identical nts indicated to the right of each sequence do not count these non-identical nts within the boxed regions. In the Mlh 69 sequence, the S μ segment adjacent to S γ 3 had undergone inversion. This has been observed previously (reference 2). Sequences were obtained from 5 cultures from 2 mice.

Figure 4. S μ -S γ 3 junctions from Pms2-deficient B cells. Sequences were obtained from 4 cultures from 2 mice.

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Percentage of junctions with indicated length of microhomology					
≥2 bp	≥5 bp	≥8 bp	≥10 bp	P value ^a	Nbr of sequences
%					
44	3	0	0		32
41	22	13	9	0.035	23
40	24	16	16	0.047	25
19	0	0	0	0.004	32
	2 bp $2 bp$ $6 44$ 41 40	$2 bp \geq 5 bp$ $2 bp \geq 5 bp$ $\frac{\%}{44} \qquad 3$ $41 \qquad 22$ $40 \qquad 24$	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	$\begin{array}{c c c c c c c c c c c c c c c c c c c $	$\begin{array}{c c c c c c c c c c c c c c c c c c c $

Table I. Lengths of Microhomologies at Sµ-Sy3 Junctions in MMR-deficient Cells Differ from Junctions in Wild-Type Cells

^aSignificance of difference in length of microhomology from WT, using Student's one-tailed *t* test.

lead to reduced microhomology at the junctions. We found a decrease in microhomology at the junctions from Msh2-deficient cells compared with wild-type: twofold fewer junctions with 2 or more nts of identity (19 vs. 44%, Table I) and an increase in frequency of insertions at the junctions (19 vs. 3%, Figs. 1 and 2). Perhaps the insertions and mutations observed at the S μ -S γ 3 junctions in the absence of Msh2 are due to a lack of normal processing of ends. Inability to properly process DNA ends could reduce the efficiency of switching, and this reduction could vary depending on the sequence of the particular downstream S region.

The sequences of the S μ -S γ 3 junctions from Mlh1- and Pms2-deficient mice are similar to each other. About onefourth of the junctions from these B cells show unusually long microhomologies, which suggests that these proteins are not performing the same function as Msh2. Mlh1 and Pms2 form a heterodimer and therefore it is reasonable that a deficiency in either of these proteins has the same phenotype. The Mlh1-Pms2 heterodimer is known to bind to Msh2-Msh6 and to Msh2-Msh3 heterodimers bound to DNA mismatches. The Mlh1-Pms2 heterodimer has been shown to greatly increase the affinity and thereby stabilize the binding of Msh2-Msh3 when bound to mismatches (27). In addition, it has been recently shown that the yeast Mlh1-Pms1 heterodimer (yeast Pms1 is equivalent to mammalian Pms2) can directly bind DNA in the absence of the Msh2 heterodimer. Interestingly, the heterodimer has two DNA binding sites and thus can bind to two different DNA molecules simultaneously (28). Consistent with these data, our sequencing results suggest that Mlh1-Pms2 might stabilize a recombination intermediate and that in the absence of this heterodimer, increased stability might be provided by increased lengths of microhomology. The increased lengths of microhomology also suggest that Mlh1 and Pms2 are probably not involved in processing the single-strand ends. If they were, one might predict that in their absence the lengths of microhomologies might decrease. This conclusion is in agreement with the lack of requirement for yeast Mlh1 and Pms1 (equivalent of mammalian Pms2) in DNA end-processing in DSB repair (21).

It was previously reported that $S\mu$ -S γ 3 and $S\mu$ -S α junctions in Msh2-deficient B cells occur more frequently at the consensus elements GAGCT and GGGGT than do junctions from wild-type cells (24). We could not examine this in our data set, because nearly all of the wild-type and Msh2^{-/-} switch junctions we obtained occurred in or near the S μ tandem repeats, presumably because our 5' S μ primer is located near the beginning of the tandem repeats. We also examined the location of junctions within the S γ 3 consensus repeats and found no difference in the frequency of recombination within the S γ 3 SNIP and SNAP elements (8) in wild-type and MMR-deficient mice (data not shown).

In conclusion, the sequences of the $S\mu$ - $S\gamma3$ junctions from MMR-deficient mice indicate that these proteins are involved in the recombination process itself and that Msh2 has a different role from Mlh1 and Pms2. It is possible that Msh2 is present at the stage of alignment of the two S regions, perhaps recruiting an endonuclease to process the DNA ends and also attracting Mlh1-Pms2 to stabilize the recombination intermediate. In the absence of Mlh1 or Pms2, we hypothesize that Msh2 could still recruit endonuclease for end-processing, but the recombination complex would be less stable, with the result that recombination intermediates with longer stretches of microhomology would be favored.

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Note Added in Proof: Similar data showing an increase in microhomology length at switch junctions in Pms2-deficient mice have recently been reported by Ehrenstein, M.R., C. Rita, A.-M. Jones, C. Milstein, and M.S. Neuberger. 2001. *Proc. Natl. Acad. Sci. USA*. 98:14553–14558.

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