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

Carol E. Schrader, Joycelyn Vardo, and Janet Stavnezer

Department of Molecular Genetics and Microbiology and Program in Immunology and Virology, University of Massachusetts Medical School, Worcester, MA 01655

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)

Address correspondence to Janet Stavnezer, Dept. of Molecular Genetics and Microbiology and Program in Immunology and Virology, University of Massachusetts Medical School, 55 Lake Ave. North, Worcester, MA 01655-0122. Phone: 508-856-4100; Fax: 508-856-1789; E-mail: janet.stavnezer@umassmed.edu

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 Sy3 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 $129 \times B6$ 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

MUSIGCD09	807	TGAGCTGAGCTGGGGTGAGC	TGAGCTGAGCTGGGGTGAGCTGA			129XB6	Sn	420	GAGCTGGGCTGAGCTGGGCT	SAGCTGGGGTGAGCTGAGCTGG		
WT5	463	TGAGCTGAGCTGGGGTGAGC	GTAAGTGAGGGTATGGGGACCAG	0 nt	identity	NT 44		255	GAGCTGGGCTGAGCAGGGCT	CTGGGGTAGGTTGGAGTATAG	0 n	t identity
129×B6 Sq3	592	CAGCTCTGGAGGGAGCTAGG	STAAGTGAGGGTATGGGGACCAG			129xB6	Sg3	245	GCTGGGCAGCTCTCAAGTGA	TGOOGTAGGTTGGAATATAG		
130784 6**	623			,						1		
125780 20	000			,		129XB6 :	Sm	223	ACTGAGCTGAGCTAGGGTGA	TGAGCTGGGTGAGCTGAGCT		
WT21	553	CTGAGCTGAGCTGGGGTGAG	3BTCTGGGGGGAGCTGGGGTAGGT	1 2 nt	identity	WT 45		223	ACTGAGCTGAGCTAGGGTGAG	BACCAGGCTGGGTAGCTCTGG	1 nt	identity
129xB6 Sg3	335	5 GIGGGGACCAAGCIGGGCAG	GCTCTGGGGGAGCTGGGGTAGGT	r		Sg3-Bal	b/c :	1120	CTOGOGTAGGTGGGGTATAG	ACCAGGCTGGGTAGCTCTGG		
		-	-									
MUSIGCD09	562	GAOCTGAOCTGGGGTGAOCTG	RGCTGAGCTGAGCTGGGGTGA			129XB6	Sm	306	AGCTGGGCTGAGCTGGGGTG	CCTGAGCTGAGCTGGGGTAAG		
WT26	366	GAGCTGAGCTGGGGTGAGCTG	SGAGCTGGGGAGGTGGAGCTA	1 nt	identity	WT 46		306	AGCTGGGCTGAGCTGGGGTG.	CTGOGTAGCTCTGGGGGAGCC	0 n	t identity
Sg3-Balb/c	580	ACCAGGCTGAGCAGCTCTCA	BGAGCTGGGGAGGTGGAGCTA			Sg3-Bal	8 - 1	127	AGGTGGGGTATAGGAACCAG	TGGGTAGCTCTGGGGGAGCC		
			-			- ,						
129XB6 Sm	570	GAGCTGAGCTGGGGTGAGCT	SAGCIGAGCIGAGCIGAGCIGA			129XB6	Sm	391	GCTGGGCTGAGCTGGGGTGA	SCTGGGCTGAGCTGGGCTGAGC		
WT 27	570	GAGCTGAGCTGGGGTGAGCT	GTTGGAGCATGGGAAACAGGC	1 nt	identity	WT 50		391	GCTGGGCTGAGCTGGGGTG/	TTGGGCAGCTACAGGTGAGCTG	0	nt identity
Sg3-Balb/c	1303	CTCTCGGGGGGGGGGGGGGGGG	GTTGGAGCATGGGAAACAGGC			129×B6	Sg3	798	GAGGGAGTATGAGGACTAGG	TGGGCAGCTACAGGTGAGCTG		
			u									
129XB6 Sm	280	GAGCTGAGCTTGGCTGAGCI	PAGGGTGAGCTGGGCTGAGCTOG			129XB6	Sm	246	GAGCTGGGTGAGCTGAGCTF	AGETGGGGTGAGCTGAGCTGAG		
WT 28	280	GAGCTGAGCTTGGCTGAGCT	PCAGGAGAGGTAAGCGTATGGGG	4 nt	identity	WT 51		246	GAGCTGGGTGAGCTGAGCTA	AGGCTGGGCAGCTCTCAGGGAG	2	nt identity
Sg3-Balb/c	1149	TOGGTAGCTCTOGGGDAGC	CAGGAGAGGTAAGGGTGTGGGG			129×B6	Sg3	613	TAAGTGAGGGTATGGGGACC	AGUCTGGGCAGCTCTCAGGGAG		
									r	7		
MUSIGCD09	770	GCTGAGCTGGGGTGAGCTGAG	SCTGAGCTGGGGTGAGCTGAGC			129XB6	Sm	351	GGATGAGCTGGGGGTGAGCT	ACCTGAGCTGGAGTGAGCTGAG		
WT29	562	GCTGAGCTGGGGTGAGC1GGA	ACCTGAAATATGTGGGGTTGTT	1 nt	identity	WT 53		351	GGATGAGCTGGGGTGAGCT	AGGASTATGAGGACTAGGTTG	3 1	nt identity
Sg3-Balb/c	728	GGCTGGACAGCTCTGGAAGGA	AGCTGAGATATGTGGGGTTGTT			129×86	Sg3	779	TACAGGTGAGCTGGGGTAG	AGGGAGTATGAGGACTAGGTTG		
		-							-	_		
129XB6 Sm	198 0	JGTGAGCTGAGCTGGGCTGAG	TAGACTGAGCTGAGCTAGGGT			129XB6	Sm	448	GTGAGCTGAGCTGGGGTGAG	CT3AGCTGAGCTGGGGTGAGCT		
WT30	198 0	GTGAGCTGAGCTGGGCTGAG	GAGGATGTGGGGGGCCAAGCTG	2 nt	identity	WT 56		446	GTGAGCTGAGCTGGGGTGM	CTACAOGTGAGCTGOGTTAGAT	4	nt identity
129xB6 Sg3	297 0	SCTOGAGOGAGCTAOGATAAG	GAGGATGTOGGGACCAAGCTG			129×B6	Sg3	806	ATGAGGACTAGGTTOGGCAG	CTACAGGTGAGCTGGGTTAGAT		
									-			
129XB6 Sm	390	TGAGCTGGGCTGAGCTGGG	TGAGCTOGGCTGAGCTGGGCTG			129XB6	Sm	304	GAGCTGGGCTGAGCTGGGGT	AGCTGAGCTGAGCTGGGGTA		
WT35	390	TGAGCTGGGCTGAGCTGGGG	GACCAGGCTGGGAAACTCTTG		Identity	WT 59		304	GAGCTGGGCTGAGCTGGGGT	CAGCTACAGGTGAGCCAGGG	1 r	it identity
120-06 0-2				2 111	identity	129×B6	Sa3	668	GGTTGTGAGGACCAGGCTGG	CAGCTACAGGTGAGCCAGGG		
129XB0 593	1/5	100001ACA100001101pd	SACCASSCI OSSANACICI I S							-		
129X86 Sm	594	TGAGCTGAGCTGAGCTGAGC	TGGGGTGAACTGAGCTGAGCTG			MUSIGCE	0.9	486	CTGAGCTGGGGTGAGCTGGG	TGAGCTGGGGTGAGCTGGGC		
			******			WT60		633	CTGAGCTGGGGTGAGCTGGG	PTAAGCTGAGCAGGGTACAGG	0 1	nt identity
WT36	074	10400104001040010400		1 m	identity	129×86	543	467	GTAGGTGGAAGCATAGGATA	PTAAGCTGAGCAGC-TACAGG		
129xB6 Sg3	611	OGTAACTGACCGTATCOCH	CENGLETGGGEAGEICTEAGGG			** 7825	545					
129XB6 Sm	373 0	TEGAGTGAGCTGAGCTGGC	TGAGCTGOGGTGAGCTGGGCT			129XB6	Sm	174	AGTAOCTGAGATGGGGTGA	TGGGGTGAGCTGAGCTGGGC		
WT1-87	373.0	TOGACTGACCTGACATOCOC	TOACCACCTACACCTGACTTG	7 nt i	dentity	WT 61		174	AGTAGCTGAGATGGGGTGAG	AGGAGTGTGGGGGACCAGGCT	2 1	nt identity
	5,5 6	• •• • •				179v86	541	501	TACAGGTGAGCTGGGGTAG	ANGGAGTGTGGGGGGGGGGGGGGGG		
129xB6 Sg3	473 1	IGGAAGCATAGGATATTAA	TGAGCLGCTACAGGTGAGCTG			103400	095	501	Inchastander dabbithop			
129XB6 Sm.	393	CTGGGCTGAGCTGGGGTGAGC	TIGGCTGAGCTGGGCTGAGCT			129XB6	Sm	490	GAGCTGAGCTGGGGTGAGCT	GGGGTGAGCTGAGCTGGGGTG		
WT 38	393	CTGGGCTGAGCTGGGGTGAGC	TTTGGGGGAGCTGGGGTAGGT	4 nt	identity	WT 62		490	GAGCTGAGCTGGGGTGAGGG	AGTTACAGGTGAGCTGGGGTA	0 1	nt identity
120×86 5/3	47	ATGGGAAACAGGCTGGACAGC	TTTGGGGGAGCTGGGGTAGGT			129×86	503	478	CATAGGATATTAAGCTGABC	AGCTACAGGTGAGCTGGGGGTA		
105400 040										1		
129XB6 Sm	253	GAGCTGAGCTAAGCTGGGGTG	AGCTGAGCTGAGCTTGGCTGAG			129XB6	Sm	256	AGCTGAGCTAAGCTGGGGTGA	GETGAGETGAGETTGGETGAG		
WT 42	253	GAGCTGAGCTAAGCTGGGGTG	AGDTTGGACAGCTCTCGGGAGC	2 nt	identity	WT 63		256	AGCTGAGCTAAGCTGGGGTGA	GTGGGGGAGTCCAGGAGAGGT	0 r	t identity
sal-Balb (a	752	ATATOTOGOGIA	ACCOUNTS ACAGE TO			Sg3-Ball	b/c :	1137	TAGGAACCAGGCTGGGTAGCT	CTGGGGGGAGCCCAGGAGAGGT		
										-		
129XB6 Sm	206	GAGCTGGGCTGAGCTAGACTG	AGETGAGCTAGGGTGAGCTGA			129XB6	Sn.	595	GAGCTGAGCTGAGCTGAGCTG	osetgaactgagctgagctga		
WT 43	206	GAGCTGOGCTGAGCTAGACEG	BAGBACTAGGTTGGGCAGCTAC	4 nt i	identity	WT 65		595	GAGCTGAGCTGAGCTGAGCTG	SCTOOGCAGCTCTCAGUGAG	1 n	t identity
170-04 603	700	· · · · · · · ·	INCRACTAGOTTOGGCAGCTAC			129×B6	Sq3	613	TAAGTGAGGGTATGGGGACCA	GCTGGGCAGCTCTCAGGGAG		
1637000 393	00		and and a resolution of the			100000 (COCTON CONCERNMENT	ACT RECOGNO ACT GALOTTOR		
129XB6 Sm	549	TGAGCTGAGCTGGGGTGAGCT	GAGCTGAGCTGGGGTGAGCTG			173VB0 20			****************	•• •• ••		
WT1-93	546	TGAGCTGAGCTGGGGTGAGCT	GOTTAGATGGAAATGTGAATA	1 nt ide	entity	WT2-28	2	11 G	GGCTGAGCTGGACTGAGCTG	AGTUTAGOGACCAGACTOGOC	3 nt	identity
129v86 6~2	819	** ** * ** TOGCACCTACAGGTOACCTC	THE TOGATOGAAATGTGAATA			129xB6 Sg	3 4	07 G	OGGCAGCTGAGGTTAGTGG	AGTOTAGGGACCAGACTGGGC		
123400 203	019	10000000100001000010	Π						-	_		
129XB6 Sm	349 1	COGGATGAGCTGGGGTGAGCTC	AGCTGAGCTGGAGTGAGCTGA			129XB6 Sm	1 3	20 0	3GGGTGAGCTGAGCTGAGCT	DGGTAAGCTGGGATGAGCTGG		
						WT1-32	3	20 0	GGGTGAGTTGAGCTGAGCT	GAGCTGGGGTAGGAGGGAGT	1 1	t identity
WT1-4	349 1	IGGGATGAGCIGGGGTGAGCTC	SOUSTAAGTUUGAGA	0 nt i	dentity	129486 00	a 4	86.1	TAAGCTGAGCAGCTACAG	CAGCTOGGGTAGGAGGAGT		
129xB6 Sg3	684 0	TOGGCAGCTACAGGTGAGCCA	DOGTAAGTGGGAGTATGGAGA			193404 97				J		
129XB6 Sm	195 1	REGESTERECTERECTERECT	GAGCTGGACTGAGCTGAGCTAG			129XB6 94		94	ATGOGGTGAGCTGAGCTGO	TRAGCTOGACTGAGCTGAGCT		
xm2 16	105		** * * * * **	4 at 10	east or					** ** **	3-	tidentify
HI3-10	192 /		***************	0 nt id	lentity	WT2-6		.94 .	A TUUUGTGAGCTGAGCTGGG	THE MUSTER CTOUSTINGAT	ar	allocation
129xB6 Sg3	744 0	CGAGCTGAGGTAGGTGGAAG	CATAGGATATTAAGCTGAGCAG			129x86 Sg	3 E	106 i	ATGAGGACTAGGTTGGGCAD	TACAGGTGAGCTGGGTTGGAT		

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 Sµ-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 $129 \times B6 \text{ Sy3}$ 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 Sy3 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 Sr	n 2	26 GAGCTGAGCTAGGGTGAGCTGAGCTGAGCTGAGCTAAG	129XB6 Sm	174	ASTAGCTGAGATGGGGTGAGTGAGCTGAGCTGAGCTGGGGTGAGCTGAGCTGAGCTGAGATGGGGGTGAGTGA	
Hsh20	2	26 GAGCTGAGCTAGGGGAGCTCTCAGGGAGCT 1 nt identity	Mah102	174	AGTAGCTGAGATGOOGTGAAADGCTOOGCAGCTCTCAGGGAG	1 nt identity
129xB6 5ç	73 6	5 AGTGAGGGTATGGGGACCAGCTGCGGGGGGGGGGGGGGG	129×B6 Sg3	517	TAGGAG3GAGTGTGCGGGACCAGCTGGGCAGCTCTCAGGGAG	
129XB6 Sn	n 31	0 GAGCEGGGCTGAGCEGGGGTGAGCEGGGCTGA	MUSIGCD09	791	CTGAGCTGGGGTGAGCTGAGCTGGGGTGAGCTGAGCT	
Nah24	3	0 GAGCTGGGCTGAGCTGGGGTGCTGGGGTAGGAGGGAGTATGA 1 nt identity	Msh195	727	CTGACCTGGGGTGAGCTGAGGGTTGAAGTATA-GAACAGGCT	0 nt identity
129xB6 Sg	3 76	8 AGCTGAGCAGCTACAGGTGAGCTGGGGTACGAGGGAGTATGA	129xB6 Sg3	258	CTCTCAAGTGAACTGGGGTAGGTTGGAATATAGGAGCAGGCT	
MUSIGCD09	64	6 CTGAGCTGGGGTGAGCTGAGCTGAGCTGGGGTGAGCT	120786 0-	624		
Msh28	73	0 CTGAGCTGGGGTTAGCTGGAGGGTTGGAGTATAG_AGCAGGC	1297.00 50	274	ISAGETGGGGTGAGETGAGETGAGETGAGETGAGETGAGET	
129xB6 Sg	3 25	7 OCTCTCAAGTGAACTGGGGTAGGTTGGAATATAGGAGCAGGC	120×86 003	979		1 nt insert
129VD4 0m	27		117,000 040	20		
Hab43	27		MUSIGCD09	820	GGGTGAGCTGAGCTGAGCTGAGCTGAGCTAGGGTGAG	
Sel Balb (the state of the s	Msh410	639	GAGTGAGCTGAGCTGGGCTGGGCAGGCGAGCCAGGCTGGGCA	3 nt identity
595-D81070	. 50	1030CHSCTC100300AWC100301A0610306-T010300A	129xB6 Sg3	601	AGGGAGCTAGGGTAAGTGADGGTATGGGGACCAGGCTGGGCA	
129XB6 Sm	632	GCTGAGCTOOGGTGAGCTGAGCTGAGCTGGAGCTGGAA	129XB6 Sm	527	GOTGAGCTGAGCTGGGCTGGGCTGGGCTGGGCTGGGCTGG	
Msh62	411	GCTGGGCTGGGCTGAGCTGGCAGCTGGGCAGCTCT 2 nt identity	Msb538	522	GGTGAGCTGAGCTGAGCTGGGTGGGTGGGTGGGTTGGG	1 nt identity
129xB6 Sg3	510	GCTGGGGTAGGAGGAGTGTGGGCAGGCTGGGCAGCTCT	129x86 Sg3	634	GGCTGGGCASCTCTCAGGGASCTGGGGTGGGGTGGGGTTGTGA	
129XB6 Sm	197	GGGTGAGCTGAGCTGAGCTGAGCTGAGCTGAGCTGAGCT	129XB6 Sm	364	TGAGCTGAGCTGAGCTGGAGCTGGGCTGGGCTGGGCTGG	
Msh73	197	GGGTGAGCTGAGCTGGGCT ATGCGGATATTAAGCTGAGCAGC 4 ntinsert or	Msh542	364	TGAGCTGAGCTGAGCTGGAGAGATCTGGAGGGAACTAGGGTA	0 nt identity
129xB6 Sg3	737	GAGCTGAGGTAGGTGGAAGCATAGGATATTAAGCTGAGCAGC	129×B6 Sg3	573	CIGIGGGGACCAGGCTGGGCCAGGGAGGCAGGGAGGCAGGGAG	
129XB6 Sm	194	ATGOGGTGAGCTGAGCTGAGCTGAGCTGAGCTGAGCT	129X86 Sm	197	DOOTTS OF TGACTTGOOTTGACT TGACTTGACTTAC	
Msh81	194	ATODGGTGAGCTGAGCTGGGCTGAGCTGGGGTGAGCTGGGTTGGAT 2 pt identity	Nab567	250		
129xB6 Sg3	806	ATGAGGACTAGGTTGGGCASCTBCAGGTGAGCTGGGGTTGGAT	129×86 \$#3	736		o ni inseri
129XB6 Sm	252	GGTGAGCTGAGCTAGCTGGGCTGAGCTGGGC	129XB6 Sm	498	TGGGGTGAGCTGGGGTGAGCTGAGCTGAGCTGAGCTG	
Msh2-21	252	OUTGAGCTGAGCTAACCTGGGRTTAAGCTGAGTAGTTATAGG 0 nt identity	Mah687	375	TGGGGTGAGCTGGGGTGAGCTGGGGTAGGTTCGAGT	1 nt identity
129xB6 Sg3	743	AGGTAGGTGGAAGCATAGGAT	129xB6 Sg3	341	ACCAAGCTGGGCAGCTCTGGGGBAGCTGGGGTAGGTTCGAGT	
129XB6 Sm	242	AGCTGAGCTGGGTGAGCTGAGCTGAGCTGGGGTGAGCTGAGC	129XB6 Sm.r	463	CCCCAGCTCAGCCCAGCTCAGCCCAGCTCACC	0-414-44
Msh2-46	242	AGTTGAGTTGGGTGAGTTGAGGACTAGGTTGGGCAGC 0 nt identity	Msh1-8.rev	412	CCTCAOCTCAGCCCAGTTCASCGGGGCAGATTTCAGGGAGCT	Sµ inverted
129×B6 Sg3	785	TGAGCTGGGGTAGGAGGGGGGGGGGGGGGGGGGGGGGGG	129xB6 Sg3	519	GGAGGGAGTGTGGGGACCAG <mark>EC</mark> GGGCAGCTCTCAGGGAGCT	
MUSIGCD09	700	AGCTGAGCTGGGGTGAGCTGAGCTGGGGTGAGCTGAGCT	MUSIGCD09	784	GCTGAGCTGAGCTGGGGTGA	
Msh2-62 -	1100	AGCTGAGCTGGGGTGAGCTGAACTACAGGTGAGCCGGGGTAA 1nt insert	Msh1-64	-1450	OCTODOCTORSCTGGGCTGAPTGAGCCGGGGTAAGTGGGAAT	1 nt identity
129xB6 Sg3	670	TTGTGAGGACCAGGCTGGGCAGCTACAGGTGAGCCAGGGTAA	129×B6 Sg3	676	ACCAGGCTGGGCAGCTACAG	
MUSIGCD09	757	TOGGGTGAGCTGAGCTGAGCTGAGCTGAGCTGAGCTG	129XB6 Sm	264	TANGCTOGOOTGAGCTGAGCTGAGCTGGGCTGAGCTGGGGTG	
Msh2-70	608	TGGGGTGAGCTGAGCTGAGCTATOGGGTAGGAGGGAGTATGA 1nt insert	Msh2-3	264	TAAGCTGGGGTGAGCTGAGCTTAAGCTGAGCAGTTATAGGTG	1 nt identity
129xB6 Sg3	768	AGCTGAGCAGCTACAGGTGAGCTGGGGTAGGAGGGAGTATGA	129xB6 Sq3	745	STAGGTGGAAGCATAGSATA	
129XB6 Sm	347	GCTGGGATGAGCTGGGGTGAGCTGAGCTGGAGTGAGC	129XB6 Sm	296	AGCTAGGGTGAGCTGGACTGAGCTGGGGTGAGCTGAGCT	
Mah3-18	347	CCTODGATGAGCTOGGGTGAGAGGGGTGGGGTGGGGGGA 0 nt identity	Msh2-5	296	AGCTAGGGTGAGCTGGACTGARGGAGGGAGTATGAGGACTAG	0 nt identity
Sg3-Balb/c	1009	GCTGGGCACTCTGGGGAGCTAGGGTGGGGTGAGGTGTGGGGA	129xB6 Sg3	775	CAGCTACAGGTGAGCTGGGGTGGGAGGAGTATGAGGACTAG	
MUSICCD09	705	GCTGAGCTGAGCTGGGGTGAGCTGAGCTGGGGTGGGGTG	129XB6 Sm	300	AGGTGAGETGGAETGAGET	
Msh3-24	602	GCTGGGCTGAACTGGGGTGGGGGAGGAGGAGGAGTAT 1 nt identity	Mah2-17	300	AGGGTGAGCTGGACTGAGCTGCTGGACAGCTCTGGAGGAGC	1 nt identity
129xB6 Sg3	766	TAAGCTGAGCAGCTACAGOTCAGCTOGGGGTAGGAGGGAGTAT	129xB6 Sg3	277	AGGTTEGAATATAGGAGCAGETGGACAGCTCTGGAGGGAGC	
129XB6 Sm	332	GCTGAGCTGGGGTAAGCTGGGAGGTGAGCTGGGGTGAGC	129XB6 Sm	249	CTGGGTGAGCTGAGCTGAGCTGAGCTGAGCTGAGCTGAG	
Mab3-32	332	GTTGAGCTGGGGTAAGCTGGGAAGCTGGGAACATAGGGT 2 stidentity	Mah4-36	249	CTOOGTOAGCTGAGCTAAGCTGGGGGGGGGGGGGGGGGGG	1 nt identity
129x86 Sc3	722	GGAGACCTGGCTGGGGGGGGGGGGGGGGGGGGGGGGGGG	129xB6 Sg3	20	CTCOGOGGAGCTAGGTCGGAGCATGOGAAACAGGCTG	
MUSIGCD09	818	GOGGTGAGCTGAGCTGAGCTGGGGTGAGCTGAGCTGA	MUSIGCD09	851	CTAGGGTGAOCTGGGCTGGGGTGAGTGAGCTGGAGTGAGCTGGAGTGAGCTGGGCCTGGGCCTGGGCCTGGGGTGAGCTGGGGTGAGCTGGGGGTGAGCTGGGGGTGAGCTGGGGGTGAGCTGGGGGTGAGCTGGGGGGGG	
Mah4-2 -	1000		Meh4=40 -	1300		d at insect
129x86 543	750		129×86 Sr3	584	AGGCTGGGCAGCTCTGGAGGGAGGCTAGGCTAGCCTAG	- ur manifi,
	- 30		ugu		1	
MUSIGCD09	843	GAGCTGAGCTAGGGGGAGCTGGGGGGGGGGGGGGGGGGG	MUSIGCD09	420	CCTGAGCTGAGCTGAGCTGAGCTGAGCTGAGCTGAGCTG	
Msh4-3	-900	SAGCTGAGCTGGGGTGAGCTDCTTAGATGGAAATGTGAATAA 2 nt identity	Msh4-65 -	1200	GCTGAGCTGAGTTGGGTTGGGTGAAGGGAGCTAGGTAAGTGA	0 nt identity
129xB6 Sg3	820	GGCCAGCTACAGGTGAGCTGCCTTGGATGGAAATGTGAATAA	129xB6 5g3	577	GOGGACCAGGCTGOGCAGCTCTGGAGGGAGCTAGGGTAAGTG	

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 Ss MLH 30 129xB6 Sc	n 28) 28(13 9)	GAGCTGAGCTGAGCTGGGCTGACCTGGGCTGA GAGCTGAGCTGAGCTGGGCTGACAGGGGGGGGGGGG	0 nt identity	129хвб зв. 591 азотижетозоотижетърстозоотожестиже Mb 92 591 азотижетозоотажестърстозооксожаствоос 2 nt identify 129хвб 923 406 азоссместалодттикотърского сладиточности сладитозос
129XB6 Sm Mlb 37	251 251	GCTGGGGTGAGCTGAGCTGAGCTGAGCTAGGGTGAGC GCTGGGGTGAGCTGAGC	4 nt identity	19985 58 172 00022002700027000070000000000000000
Sg3-Balb/c	: 1146	GCTGGGTAGCTCTGGGG <u>EAGCT</u> CAGGAGAGGTAAGGGTGTG		129XB6 sm 216 GAGCTAGACTGAGCTGAGCTGAGCTGAGCTGGGTGAG
129XB6 Sm	552	GCTGAOCTOGGGTGAOCTGAGCTGAGCTGAGCTGAGCTGA		M1h 85 216 GAOCTAGACTGAGCTGAGCTAGGTGGGGGTTGTGGGGACCAGG 4 nt identity
Mih 53	552	GCTGAGCTG3GGTGAGCTGAGCTGTGTTAGGAGTGTAGGGACCAG	1 nt identity	129×B6 Sg3 65 ACCTCTGGGGGAGCTGGGGTAGGGGGTTGTGGGGACCAGG
Sg3-Balb/c	442	TGGGCAGCTCTGGGGCAGCTGGGGGTGTTAGGAGTGTAGGGACCAG		129хв6 sm 525 озосталосталосталостолосторозградствостаностородила
MUSIGCD09	550 0	TGAGCTGAGCTGAGCTGACTGACTGAGCTGAGCTGAGC		NDh 89 525 0000000000000000000000000000000000
M1h 56	773 (TGAGCTGAGCTCAGCTGAGCTGGGCAGCTCTGGAGGGAGCTAGOG	6 nt identity	125X80 303 433 1040640614000104061000040010100004
129×86 Sg3	567 0	TOGASCTOTOSSGACCAG <mark>ECTOSSC</mark> AGCTCTGGAGGGAGCTAGGG		129XB6 Sm 552 GAOCTGAGCTGAGCTGAGCTGAGCTGAGCTGAGCTGAGCT
129XB6 Sm	555	GAGCTOGOGTGAGCTGAGCTGAGCTGAGCTGAGCTGAGCT		S13 -Balb/c 732 GACCTGACCTGGGAAGAAGAAGATATGTGGGGTTGTGGGG S03-Balb/c 732 GGACACCTCTGGAAGAAGAAGATATGTGGGGTTGTGGGG
Mlh 58	555	GAGCTGGGGTGAGCTGAGCTGGGGTAGGTTGGAGTATGGG	14 nt identity	
129×B6 Sg3	103	CAGGCTGGGCAGCTA <mark>DAGGTGAGCT039GT</mark> AGGTF0GGAGTAT003		129XB6 Sm 206 GAGCTGGGCTGAGCTGAGCTGAGCTGAGCTGAGCTGA
129XB6 Sm	301	GGGTGAGCTGGGCTGAGCTGGGGTA		
Mlh 62	301	GGGTGAGCTGGGGCTGAGCTGGGGCTGGGGCAGCTACAGGTGAGCCA	2 nt identity	
129xB6 Sg3	660	1666766667TGTGAGGACCADSTGGGCAGCTACAGGTGAGCCA		HID 112 256 ACCTORECTARSCTORECTORECTORECTORECTORECTORECTORE
100-06 070	460			Balb/c Sg3 640 CCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC
#1569	286	AGCTCAGCTCACCCCAGCTCACECCAGCTCAGCCCAGCTCAG	1 nt identity Su inverted	
129xB6_8m3	502		at junction	MUSIGCD09 690 GCTGAGCTGAGCTGAGCTGAGCTGAGCTGAGCTGAGC
105000 095				x1h 124 31:228 GCTGAGCTGGGCTGGGCTGGGCTGGGGAGGGGGGGGGGG
129XB6 Sm	490	GAGCTGAGCTGOGGTGAGCTGGGGTGAGCT		129×B6 Sg3 490 AGCTGAGCAGCTACAGGTGAGCTGPGGTAGGAGGGAGTGTGG
M1h 70	196	GAGCTGAGCTGGAGTGAGCTGGGAAACAGGCTGGACAGCT	1 nt identity	129XB6 Sm 267 GCTGGGGTGAGCTGAGCTGAGCTGGGCTGAGCTAGGGTGAGC
129xB6 Sg3	14	SOGGAGCTAGGGTAGGTTGGACAGCTGGACAGCT		M1h 127 267 GCTGGGGTGAGCTGAGCTGAGCTGGGGTAGGAGGGAGTGTGG 3 nt identity
				129×86 Sg3 490 ACCTGAGCAGCTACAGGTGASCTGGGGTAGGAGGGAGTGTGG
129XB6 Sm	231	GAGCTAGGGTGAGCTGAGCTGAGCTGAGCTGAGCTGAG		129X86 Sm 174 AGTAGCTGAGAT0000GTGAGTGAGCTGAGCTGAGCTGGGGTG
M1576	231	GAGCTAGGGGGAGCTGAGCTGGGGGGGGCAGTTAGAGGTGAGTT	0 nt identity	H1h 130 174 AGTAGCTGAGATGGGGGTGAAAGGCTGGGCAGCTCTCAGGGAGC 1 nt identity
110400 803	86			129xB6 Sg3 517 TAGGAGGGAGTGTGGGGACCAGGCTGGGCAGCTCTCAGGGAGC
129XB6 Sm	439	TGAGCTGGGGTGAGCTGAGCTGGGGTGAGCTGAGCTG		129XB6 Sm 213 GCTGAGCTAGACTGAGCTGAGCTGAGCTGAGCTGGGGTG
M1h 79	439	TGAGCTGGGGTGAGCTGAGCTGAGCTGGGGTAGGTTGGAGG	0 nt identity	N1h 134 213 GCTGAGCTAGACTGAGCTGAGCTGAGGTCCCAGGTTATGCAGCTCCT 2 nt identity
129×B6 Sg3	101	ACCAGOCTGGGCAGCTAGAGCTGAGCTGGGGTAGGTTGGAGT		129xB6 Sg3 885 GCTGGGGGCTATCAGATCACAGGGTCCCCAGGTTATGCAGCTCCT
129XB6 Sm	201	TAGGGTGAGCTGAGCTGGGTGAGCTGAGCTAGCTGGGGTGA		129XB6 5m 532 GCTGAGCTGAGCTGGGGTGAGCTGGGGTGAGCTGAGCT
M1h 80	201	TAGGTGAGCTGAGCTGGCAGCTGGAGCGCAGCGCAGCGC	2 nt insert	M1h 135 512 GCTGAGCTGAGCTGGGGTGAACTAGGTGGAAGCATAGGATATT 1 nt identity
129xB6 Sg3	442	ActoggchgctctgggggagetAgggtggAagcAttagg		129xB6 Sg3 446 GOCAGCTCTGGGGGAGCTAGGGMAGGTGGAAGCATAGGATATT
129XB6 Sm	504	TGAGCTGOGGTGAGCT-SAGCTGOGGTSAGCTGAGCTGAGCTG		129XB6 Sm 92 GTTCTGAGCTGAGCTGAGCTGGGGTGAGCTCAGCTATGCTA
Pms 2	504	TGAGCTGGGGTGAGCTGGAGCTGGGGTAGGTTCGAGTATGGGG	10 nt identity	Pms 39 92 GTTCTGAGCTGAGCTGAGCTGGGCAGATTTGGGGCAGCTGA 5 nt identity
129xB6 Sg3	354	GCTGGGCAGCTCTGGGG <mark>CAGCTGGGGT</mark> AGGTTCGAGTATGGGG		129xB6 Sg3 377 TCGAGTATGGGGACTAACTTGGGCAGCTCGGGGCAGCTGA
129XB6 Sm	195	TOGOGTGAGCTGAGCTGGGCTGGGCTGAGCTGAGCTGAGC		129XB6 Sm 466 TGAGCTGAGCTGAGCTGGGGTGAGCTGAGCTGGGGGT
Pms 5	195	TGGGGTGAGCTGAGCTGGGCTATGCGGATATTAAGCTGAGCAG	0 nt identity	Pms 42 466 TGAGCTGAGCTGAGCTGGGGTGGGTGGGTGGAATGTGA 1 nt identity
129×B6 Sg3	735	COGRAGCTGAGGTAGGTGGAAGCATAGGATATTAAGCTGAGCAG		129×B6 5=3 817 GTTGGGCAGCTACAGGTGAGCTGGGTTAGATGGAAATGTGA
		······		
129XB6 Sm	211	GGGCTGAGCTAGACTGAGCTGAGCTGAGCTGGG		129XB6 Sm 260 GAGCTAAGCTGGGGTGAGCTGAGCTGAGCTGGGCTGGGC
VII6 16	211		2 nt identity	Pms 44 236 GAOCTAAGCTGGGGTGAGCTGADDACCAGOTTGGGCAGCTCT 1 nt identity
179XB0 203	862	GASCIGGGCTATCAGATCAGASSICCCASSITATGCASCIC		
129XB6 Sm	90	CTGTTCTGAGCTGAGATGAGCTDGGGTGAGCTCAGCTATGCTA		129XB6 Sm 384 GAGCTGAGCTGAGCTGGGGTGAGCTGGGGCTGAGCTGG
Pms 19	90	CTGTTCTGAGCTGAGATGAGCTAGGCTGGGCAGCTCTGGGGAG	0 nt identity	Pms 46 345 GAGCTGAGCTGAGCTGGGGTAGGAGGGAGTGTGGGGA 11 nt identity
Sg3-Balb/c	1031	GOGTGGGTGAGGTGTGGGGAACNGGCTGGGCAGCTCTGGGGAG		129×B6 Sg3 493 TGAGCAGCTACAGG <mark>FGAGCTGGGGT</mark> AGGAGGGAGTGTGGGGA
129XB6 Sm	280	GAGCTTOGCTGAGCTAGGGTGAGCTGGGGTGAGCTGGGGTGAG		129XB6 Sm 369 TGAGCTGAGCTGGAGTGAGCTGGGGCTGAGCTGGGGGTG
Pms 20	270	GAGCTTGGCTGAGCTAGGGTGA	5 nt insert	Pms 50 369 TGAGCTGAGCTGGAGTGAGCTAGGATATTAAGCTGAGCAGCT 1 nt identity
129xB6 Sg3	561	GGGGAGGTGGAGCTGTGGGGACCAGGCTGGGCAGCTCTGGAGG		129×B6 Sg3 459 AGCTAGGGTAGGTGGAAGCATAGGATATTAAGCTGAGCAGCT
130×06 Cm	122			
	100		0	Des 51 200 company acres and a set a
100-06 (-2)	135		o ni identity	
	4.74			
129XB6 Sm	226	GAGCTGAGCTAGGGTGAGCTGAGCTGAGCTGAGCTGAGC		129XB6 Sm 362 GGTGAGCTGAGCTGAGCTGGGCTGAGCTGAGCTGAGCTG
Pms 18	226	GAOCTGAOCTAGOCTGAOCTGAACTGGGDTAGGTTGGAGTA	11 nt identity	Pms 55 362 GGTGAGCTGAGCTGAGCTGGGAGGTAGGGTAGGTGGAAGCATAG 4 nt identity
129xB6 Sg3	103	CAGGCTGGGCAGCTA		129xB6 Sg3 440 AGACTGGGCAGCTCTGGGCGAGTTAGGGTAGGTGGAAGCATAG
129XB6 Sm	226	GAGCTGAGCTAGGGTGAGCTGAGCTGAGCTGAGCTAA		129XB Sm 437 GCTGAGCTGOGGTGAGCTGAGCTGAGCTGAGCTGAGCT
Pms 23	226	GAGCTGAGCTAGGGTGAGCTGCTGGGCAGCTCTCAGGGAGC	1 nt identity	Pms 56 332 GCTGAGCTGAGGTGAGCTGAGCTAACGAGAGGGTAA 1 nt identity
129xB6 Sg3	615	AGTGAGGGTATGGGGACCAGGCTGGGCAGCTCTCAGGGAGC		129xB6 Sg3 583 CAGGCTGGGCAGCTCTGGAGCTAGGGTAAGTGAGGGTAT
129xB6 Sm	418	CTCAGCTGGGCTGAGCTGGGCTGAGCTGGGTGAGCTGAG		
Pms 24	418	CCTGACCTOGGCTGAGCTGGGCAGCTCTGGGGCAG	0 nt identity	129XB6 5R 126 TATGCTACGCTGTGTGTGGGGCTGACTGAAATGAGATAC
129xB6 5g3	373	AGGTTCGAGTATGGGGACTACCTGGCCAGCTCGGGGCAG		PHE 58 126 TATECTATECTUTETTERUETEREGEGACTAGECTEGECAGET 4 nt identity
2-		· · · · · · · · · · · · · · · · · · ·		Ball/C 593 1023 GGGAGCTAGGETURGTURGTURGTUGGGAACAGGCTGGGGAGCT
129XB6 Sm	93	TTCTGAGCTGAGAIGAGCTGGGGGGGGGGGGGGGGCCAGCTATGCTAC		129XB6 Sm 374 TGAGCTGGAGTGAGCTGAGCTGGGGTGAGCTGGGGTGAGCTGG
Pms 26	93	TTCTGAGCTGAGATGAGCTGGGGTAGGTTCGAGTATGGGGA	10 nt identity	Pms 59 374 TGAGCTGGAGTGAGCTGAGCTGAGGTAGGTAGGTAGGTAG
129xB6 Sg3	349	GIGUAGUTCTGGGG <u>PAGUTGGGGT</u> RGGTTCGAGTATGGGGA		129xB6 Sg3 7 AGGCTGGACAGGTCTGGGGGGGGGGGCTAGGTTGGAGCATG
129XB6 Sm	226	GAGCTGAGCTAGGGTGAGCTGAGCTGAGCTGAGCTAA		129XB6 Sm 115 GTGAGCTCAGCTATGCTACGCTGTGTGGGGTGAGCTGATCTG
Pms 27	226	GAGCTGAGCTAGGGTGAGCTGCTGGGCAGCTCTCAGGGAGC	1 nt identity	Pms 60 115 GTGAGCTCAGCTATGCTACCCTGGGTAGCTCTGGGGGAGCCC 0 nt identity
129xB6 Sg3	615	AGTGAGGGTAT3GGGACCAGGTGGGCAGCTCTCAGGGAGC		Balb/c Sg3 1127 AGGTGGGGTATAGGAACCAGGCTGGGTAGCTCTGGGGGAGCCC
129xB6 Sm	173	GAGTAGCTGAGATGGGGTGAGCTGAGCTGGG		
Pms 29	173	GAGTAGCTGAGATGGGGTGAA	1 nt identity	RUBICLOV / // GUBICHUCIONUCIONUCIONUCIONUCIONUCIONUCIONUCION
129×86 Sg3	516	GTAGGAGGGAGTGTGGGGACCSGCCTGGGCAGCTCTCA09G	•	Pma 0.3 402 GOUTHAGETGAGETGAGETGAGETGAGAGGTGAAGGETAGGATAT 2 At Identity
	-			173XB0 5G3 429 AGACUTGRCIGGGAGUTGA <mark>SCI</mark> FAGITGGAAGCATAGGATAT
129XB6 Sm	351	GAGUTGAGCTGAGCTGGGCTGAGCTGGGCTGAGCTG	6 at ide-th-	
Pms 37	351	GAGCTGAGCTGAGCTGGAGGGACCAGGCTG	o nt identity	
TTAXR0 203	, 24g	LOGNOGONOC LNOGOL NOOTONODOL NI GORGACCAGOCTG		

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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Mouse	Percentage of junc	ctions with indicated				
	≥2 bp	≥5 bp	≥8 bp	P value ^a	Nbr of sequences	
	%					
Wild-type	44	3	0	0		32
Mlh1 ^{-/-}	41	22	13	9	0.035	23
Pms2 ^{-/-}	40	24	16	16	0.047	25
Msh ^{-/-}	19	0	0	0	0.004	32

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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