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The Role of MutS Homologues MSH4 and MSH5 in DNA Metabolism and Damage Response

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1. Introduction

The DNA mismatch repair (MMR) pathway is one of the most important genome surveillance systems involved in governing faithful transmission of genetic information during DNA replication and homologous recombination (Jiricny 2006). MMR deficiency attributes to a phenotype known as microsatellite instability (MSI), a condition that predisposes individuals to a heightened risk of cancer development (Harfe & Jinks-Robertson 2000; Iyer *et al.* 2006; Jiricny 2006; Kunkel & Erie 2005). Notably, hereditary nonpolyposis colorectal cancer (HNPCC) or Lynch syndrome is the most common malignancy identified in individuals with MMR gene mutations. The MMR pathway relies on the coordinated functions of a family of proteins that recognize mismatched nucleotides and initiate subsequent repair actions ranging from excision of a fragment of DNA containing the mismatched nucleotide to DNA repair synthesis. The MMR system is well conserved evolutionarily from bacteria to humans, of which the eukaryotic MMR pathway is evolved to possess multiple homologous genes that carry out conserved and diverse functions corresponding to their bacterial counterparts (Modrich 1991). Genes encoding homologues of the bacterial MMR proteins such as MutS and MutL have been identified in a variety of eukaryotes including yeast, plants, nematodes, and mammals. However, the bacterial MutH appears to be an exception—this methylation-sensitive endonuclease, exclusively functioning in gram-negative bacteria, directs the action of MMR to hemimethylated newly synthesized bacterial DNA (Iyer *et al.* 2006).

All eukaryotic organisms possess multiple MutS homologues—collectively known as MSH proteins—which may number as high as seven (MSH1 to MSH7), although the mitochondrial-localizing MSH1 of *Saccharomyces cerevisiae* (Reenan & Kolodner 1992) and the *Arabadopsis thalia* MSH7 (Culligan & Hays 1997) do not appear to be fully conserved (Fishel & Wilson 1997). The functionality of these proteins is similar to that of their counterparts in bacteria such as *Eschericia coli*. Prokaryotic MutS exists as a homodimer, while eukaryotic MSH proteins form heterodimers in the forms of MSH2-MSH3, MSH2-MSH6 and MSH4-MSH5. Two of these eukaryotic heterodimers (MSH2-MSH3 and MSH2-

MSH6) play fundamental roles in mitotic post-replicative MMR (Fishel & Wilson 1997; Kolodner 1996), in which incorrectly matched bases are replaced with proper partners.
In spite of the high levels of sequence homology to the other MSH proteins, what role, if any, of the MutS homologues MSH4 and MSH5 in the process of MMR has yet to be experimentally determined, and the biochemical function(s) of the heterocomplex formed by MSH4 and MSH5 also awaits to be further delineated (Her *et al.* 2007). Nevertheless, high levels of expression of both genes in the testes and ovaries implicate a direct role for hMSH4 and hMSH5 in development and meiosis (Bocker *et al.* 1999; Her & Doggett 1998; Moens *et al.* 2002). Genetic studies in *S. cerevisiae*, *Caenorhabditis elegans*, and *Mus musculus* highlight an important role for MSH4 and MSH5 in meiotic development of viable gametes, but provide no evidence to substantiate their potential role in the repair of mismatched bases like the other MutS homologues (de Vries *et al.* 1999; Edelmann *et al.* 1999; Hollingsworth *et al.* 1995; Kelly *et al.* 2000; Kneitz *et al.* 2000; Ross-Macdonald & Roeder 1994; Zalevsky *et al.* 1999). The observation that purified recombinant hMSH4-hMSH5 heterocomplex can specifically bind to recombination intermediate structures such as the Holliday junction (Snowden *et al.* 2004) has implicated a direct role for hMSH4-hMSH5 in the process of meiotic and mitotic recombinational double-strand break (DSB) repair. Gene knockout of *Msh4* and/or *Msh5* in mice results in defective chromosome synapsis in meiotic prophase I, and therefore sterility—likely attributed to defective homologous recombination (de Vries *et al.* 1999; Edelmann *et al.* 1999; Kneitz *et al.* 2000). Although the expression patterns of MSH4 and MSH5 mRNA in testis support their functional role in meiosis, low levels of MSH4 and MSH5 expression have been identified in many other, non-meiotic organs, and these two genes are not necessarily expressed in concert (de Vries *et al.* 1999; Edelmann *et al.* 1999; Her *et al.* 2001; Her *et al.* 2003; Her *et al.* 1999; Kneitz *et al.* 2000; Paquis-Flucklinger *et al.* 1997; Winand *et al.* 1998), implicating MSH4 and/or MSH5 can function in other pathways beyond the scope of meiosis and may function separately as well (Her *et al.* 2007). In fact, recent evidence supports the notion that MSH4 and MSH5 are involved in the process of mitotic DNA DSB repair, and may be also involved in other aspects of DNA damage repair and response (Tompkins et al. 2009; Sekine et al. 2007).

2. Structural and functional features of MSH4 and MSH5

2.1 Gene structure and expression of MSH4 and MSH5

2.1.1 *S. cerevisiae* MSH4 and MSH5

The identification of *MSH4* and *MSH5* was first performed in the budding yeast *S. cerevisiae,* and that led to the classification of these proteins as meiosis-specific members of the MutS homologue family. *MSH4* was isolated by a transposon insertion screen resulting in the generation of *lacZ* fusion genes expressed specifically in meiotic cells (Burns *et al.* 1994). Following electrophoretic separation of yeast chromosomes, the *MSH4* gene was mapped to chromosome VI by Southern blot analysis, located 2 cM from *SEC4*. Sequence analysis of an *MSH4*-complementing subclone identified an open reading frame (ORF) of 2634 bp encoding the 878-amino acid MSH4 protein. As a member of the MutS family, MSH4 shows 35% identity to *E. coli* MutS and 31-36% identity to the other yeast MutS homologues. The similarity is most pronounced in the C-terminal region, which contains the putative ATP-binding domain and helix-turn-helix DNA-binding motif. Despite its extensive homology to bacterial MutS, which functions in the initiation of MMR through direct binding of mispaired bases, MSH4 is meiosis specific. Expression of mutant MSH4 results in defective

reciprocal recombination and nondisjunction of homologous chromosomes at meiosis I and consequently spore inviability. The MSH4 protein is specifically expressed in meiotic cells where it localizes to discrete chromosomal locations with no apparent involvement in MMR (Ross-Macdonald & Roeder 1994).

The *MSH5* gene is mapped to chromosome IV, located 1.9 cM from *CDC36*. The *MSH5* ORF is composed of 2703 nucleotides and encodes a protein of 901 amino acids with a predicted molecular mass of 102 kDa (Hollingsworth *et al.* 1995). Although MSH5 exhibits strong homology to the MutS family of proteins, like MSH4, it is not involved in MMR. Diploids lacking *MSH5* display decreased spore viability, increased chromosomal nondisjunction during meiosis I, with a concomitant decrease in reciprocal exchange between—but not within—homologous chromosomes. However, lack of *MSH5* expression does not correlate to a decrease in gene conversion. Mutants lacking *MSH4* or *MSH5* are phenotypically comparable, indicating that these two yeast genes are in the same epistasis group and are likely to serve similar functions (Hollingsworth *et al.* 1995).

2.1.2 Mouse MSH4 and MSH5

Sequence analysis revealed that the mouse *Msh4* ORF is 2874 bp in length with a 196 bp 3'-UTR followed by a poly(A) tract. A polyadenylation signal (AATAAA) is located 24 nucleotides 5' upstream from the poly(A) tract. The 958-amino acid protein encoded by this ORF contains the highly conserved sequence motifs present in all MutS homologues. The cDNA shares 84.1% and 89.3% sequence identities with its human orthologue in nucleotide and amino acid sequences, respectively. The amino terminus of Msh4, on the other hand, is the most divergent and shares no sequence homology with that of the human hMSH4 (Her *et al.* 2001; Kneitz *et al.* 2000). Northern blot analysis has indicated that *Msh4* is predominantly expressed in the testis, but low levels of expression are also present in the heart, brain, and liver, whereas dot-blot analysis, besides confirming the Northern results, has also revealed low levels of Msh4 expression in several other non-meiotic tissues (Her *et al.* 2001).

The mouse *Msh5* gene is located on chromosome 17 in a region that is syntenic to the locus on human chromosome 6 harboring the human gene. The gene for Msh5 contains 24 exons and spans approximately 18 kb with exon length varying from 36 bp for exon 7 to 392 bp for exon 24; intron lengths range from 79 bp for intron 17 to 4687 bp for intron 11. The first 248 nucleotides of exon 1 and the last 133 nucleotides of exon 24 are noncoding. Comparison between the human and mouse homologues reveals that the mouse *Msh5* gene shares a high degree of structural homology with that of the human *hMSH5* gene. The locations of most exon-intron splicing junctions as well as the lengths of all internal coding exons of *Msh5* are identical to that of *hMSH5*. The mouse Msh5 ORF is 2502 bp in length and encodes an 833-amino acid polypeptide with a predicted molecular weight of 92.6 kDa and an isoelectric point of 6.33. Msh5 contains the same set of highly conserved sequence motifs that present in all other MutS proteins (de Vries *et al.* 1999; Edelmann *et al.* 1999; Her *et al.* 1999). Like Msh4, high levels of Msh5 expression are largely confined to the testis while relatively low levels of Msh5 expression are detectable from heart, spleen, liver, and lung (Her *et al.* 1999).

Both male and female mice lacking *Msh4* or *Msh5* are infertile as a result of meiotic arrest (de Vries *et al.* 1999; Edelmann *et al.* 1999; Kneitz *et al.* 2000). Spermatocyte chromosomes of *Msh4* or *Msh5* deficient mice do not synapse properly in late zygonema and early pachynema despite DSB formation. Chromosome pairing, normally triggered by meiotic

DSB, involves mainly nonhomologous chromosomes in *Msh4* or *Msh5* deficient mice, of which only a fraction of nuclei in *Msh5*-null males show partial pairing; while in *Msh4*-null spermatocyte pairing is typically higher at 70% of all nuclei. As a consequence, germ cells in *Msh4* or *Msh5* deficient mice fail to enter pachynema and die by apoptosis, leading to testes devoid of post-leptotene spermatocytes. In comparison, female mice deficient in *Msh4* or *Msh5* suffer similar consequences as in males experiencing pre-pachytene meiotic catastrophe, particularly oocytes become apoptototic prior to birth. In female mice lacking both *Msh4* and *Msh5*, the oocyte pool is completely lost and is accompanied by ovarian degeneration during the first eight to ten weeks of postnatal period (Kneitz *et al*. 2000).

2.1.3 Human hMSH4 and hMSH5

The human *hMSH4* gene is composed of 20 exons and spans 116 Kb on chromosome 1p31. With a 2808 bp ORF, the *hMSH4* gene encodes a protein of 936 amino acids with a predicted molecular mass of 104.8 kDa (Paquis-Flucklinger *et al.* 1997). In contrast, the *hMSH5* gene contains 26 exons and spans approximately 25 Kb within the MHC class III region on human chromosome 6p21.3. The *hMSH5* gene harbors a 2501 bp ORF encoding an 834-amino acid protein with a predicted molecular mass of 92.9 kDa (Her & Doggett 1998; Winand *et al.* 1998). Moderate levels of hMSH4 transcripts are present in the testis and low levels of hMSH4 transcripts are also detectable in several non-meiotic tissues including the thymus, ovary, colon, pancreas, brain, heart, liver, and placenta (Her *et al.* 2003; Paquis-Flucklinger *et al.* 1997). In contrast, the full-length hMSH5 transcripts are detectable in virtually all tissues examined with the most abundant expression in the testis. Northern blot analysis shows the presence of distinct hMSH5 hybridization signals in a variety of tissues such as the thymus, skeletal muscle, bone marrow, spinal cord, brain, trachea, ovary, and lymph node (Bocker *et al.* 1999; Her and Doggett 1998; Winand *et al.* 1998). This wide hMSH5 tissue distribution pattern has been also recapitulated in RT-PCR analysis of various human tissues and is well reflected in the human EST database. The difference in the expression patterns of these two genes raises the possibility that hMSH4 and hMSH5 may function independently of one another in certain tissue types beyond the meiotic arena (Her *et al.* 2007).

2.1.4 Splicing variants and nonsynonymous polymorphisms of hMSH4 and hMSH5 in humans

Recent evidence substantiates the existence of multiple alternatively spliced transcripts for *hMSH4* and *hMSH5* in human cells (Table 1). Although the functional significance of these alternatively spliced transcripts is presently unknown, the expression profiles and some properties of the proteins encoded by the splicing variants are being characterized. For instance, one hMSH4 splicing variant, hMSH4sv, resulting from exon 19-skipping, has been analyzed. hMSH4sv harbors a frameshift of 7 amino acids followed by a stop codon in exon 20, thus producing an 850-amino acid polypeptide. Truncated at the carboxyl terminal, the hMSH4sv contains most of the conserved sequence motifs presented in all MutS homologues except for the carboxyl terminal helix-turn-helix motif (Her *et al.* 2003). The tissue distribution profile of hMSH4sv is similar but not identical to that of hMSH4, suggesting that hMSH4sv might be subjected to differential regulation in some tissue types including the heart, liver, placenta, and ovary (Her *et al.* 2003). Intriguingly, the protein encoded by *hMSH4sv* is incapable of interacting with hMSH5, but it does interact with von

Hippel-Lindau tumor suppressor-binding protein 1(VBP1) (Her *et al.* 2003). Also of note is another hMSH4 exon-skipping variant (ΔhMSH4) that lacks the entire exon 6 leading to an in-frame deletion of hMSH4 amino acid residues 273 to 330 (Her *et al.* 2003; Santucci-Darmanin *et al.* 1999). The hMSH4 homodimerization domain, residing in between amino acid residues 148 and 387 (Her *et al.* 2003), significantly overlaps with the region encoded by *hMSH4* exon 6, thus ΔhMSH4 is expected to be defective in homotypic interaction.

The *hMSH5* gene produces multiple alternative transcripts, of which four hMSH5 variants that maintain the reading frame have been identified (hMSH5a, hMSH5b, hMSH5c, and hMSH5d; UniGene database) and hMSH5c appears to be identical to that of the originally described human hMSH5. Referenced by the deduced amino acid sequence of hMSH5, it is evident that hMSH5a (hMSH5sv) encodes an 851-amino acid protein containing a 17-amino acid insertion between codons 179 and 180, owing to the retention of the last 51-bp of hMSH5 intron 6 (Yi *et al.* 2005), whereas hMSH5b harbors one extra amino acid residue between codons 654 and 655—due to the retention of the last 3 nucleotides of *hMSH5* intron 20 (Her & Doggett 1998). hMSH5d represents the shortest hMSH5 variant. Although it contains the 17-amino acid insertion and the one extra amino acid residue described above, hMSH5d lacks 30 amino acid residues corresponding to codons 744 to 773. The existence of different hMSH5 variant transcripts keenly supports the possibility that *hMSH5* may encode multiple products; a thorough understanding of the functional aspects of these potential protein isoforms requires detailed experimental examination of these variants. In particular, it is necessary to determine whether these hMSH5 variants are resulted from sequence variations within the corresponding introns, or they are created by a yet-to-be-defined mechanism. Presently, besides hMSH5, only one splicing variant, hMSH5sv, has been experimentally analyzed (Yi *et al.* 2005). The expression profile of hMSH5sv appears to be distinguishable from that of hMSH5; for example, the expression of hMSH5sv, but not hMSH5, is absent or below the detection limit in the brain, heart, and skeletal muscle. In addition, the expression of hMSH5sv displays a large variation in tumor cell lines with breast and lung carcinomas showing the most abundant expression. In contrast to hMSH4sv, displaying impaired interaction with hMSH5, hMSH5sv has maintained its capacity to interact with hMSH4 (Yi *et al.* 2005).

In addition to the existence of multiple alternatively spliced transcripts, potential diverse functions involved with the *hMSH4* and *hMSH5* genes are also being reflected by the fact that both genes are associated with many coding region single nucleotide polymorphisms (SNPs), of which many are non-synonymous. There are at least seven non-synonymous SNPs that have been identified for each of the genes (Table 1). For hMSH4 and hMSH5, the corresponding single amino acid changes caused by these SNPs are hMSH4 A60V, A90T, A97T, E162K, I365V, Y589C, S914N, and hMSH5 P29S, L85F, Y202C, V206F, R351G, L377F, P786S. However, the allele frequencies, haplotypes, and functional implications of most, if not all, SNPs are largely undetermined; in fact only one non-synonymous SNP (rs2075789), hMSH5 C85T ($hMSH5^{P29S}$), has been characterized experimentally as a common genetic polymorphism with an allele frequency of 11.6% in an American Caucasian population of 99 individuals and 17% in a Chinese population of 279 individuals (Her *et al.* 2007; Yi *et al.* 2005). Located within the hMSH5 amino terminal proline-rich interacting domain for hMSH4 and c-Abl, the Pro^{29} to Ser alteration causes a moderate reduction of protein interaction with hMSH4, whereas this alteration promotes the activation of c-Abl kinase activity and therefore enhances ionizing radiation (IR) induced p73-dependent apoptosis

	Variants and nonsynonymous polymorphisms	Changes in amino acid (aa) residues
hMSH4	hMSH4sv	Exon 19 skipping leading to the production of a 850 aa polypeptide, of which the last 7 aa are frame-shifted
	ΔhMSH4	Exon 6 skipping leading to the deletion of aa 273 to 330
	A60V	Ala^{60} to Val
	A90T	Ala^{90} to Thr
	A97T	Ala^{97} to Thr
	E162K	Glu^{162} to Lys
	I365V	Ile^{365} to Val
	Y589C	Tyr^{589} to Cys
	S914N	Ser^{914} to Asn
hMSH5	hMSH5a (hMSH5sv)	17 aa insertion between aa position 179 and 180
	hMSH5b	1 aa insertion between aa position 654 and 655
	hMSH5d	17 aa insertion between aa position 179 and 180 1 aa inertion between aa position 654 and 655 and deletion of aa 744 to 773
	P29S	Pro^{29} to Ser
	L85F	Leu^{85} to Phe
	Y202C	Tyr^{202} to Cys
	V206F	Val^{206} to Phe
	R351G	Arg^{351} to Gly
	L377F	Leu^{377} to Phe
	P786S	Pro^{786} to Ser

Table 1. hMSH4 and hMSH5 splicing variants and nonsynonymous polymorphisms.

(Tompkins *et al.* 2009; Yi *et al.* 2006; Yi *et al.* 2005). Given the essential role of Msh5 in ovarian and testicular development in mice (de Vries *et al.* 1999; Edelmann *et al.* 1999), it is interesting to note that the hMSH5 C85T SNP is relatively enriched in ovarian cancer patients and is associated with a higher risk for azoospermia or severe oligozoospermia in humans (Xu *et al.* 2010; Yi *et al.* 2005). Another noteworthy hMSH5 variant is $hMSH5^{L85F/P786S}$—encoded by an *hMSH5* allele harboring two co-segregating SNPs (C253T and C2356T)—that displays compromised ability to interact with hMSH4. Intriguingly, the allele for $hMSH5^{L85F/P786S}$ has been associated with the occurrence of human immunoglobulin deficiency syndromes, *i.e.* IgA deficiency (IgAD) and common variable immune deficiency (CVID) (Sekine *et al.* 2007).

It is also important to note that two hMSH5 noncoding SNPs have been linked to conditions in humans as well. A recent genome-wide association study (GWAS) of 511,919 SNPs in populations with Caucasian origin has identified a high risk factor for lung cancer development within the *hMSH5* gene locus at 6p21.33 (Wang *et al.* 2008). Specifically, a

significant increase in lung cancer susceptibility is associated with rs3131379, a SNP located within intron 10 of the *hMSH5* gene. Another study, designed to identify genetic markers for the adverse reaction associated with the use of Allopurinol—a common medication for gout and hyperuricemia, has revealed a tight link with the *hMSH5* locus (Hung *et al*. 2005). The evidence from this study demonstrates a significant association of a separate hMSH5 SNP (rs1150793) with the risk of developing severe cutaneous adverse reactions (SCAR) in Han Chinese patients treated with Allopurinol.

Although the existence of multiple hMSH4 and hMSH5 splicing variants and various SNPs poses a daunting task for a thorough appreciation of their functions, close analysis of their properties at molecular and cellular levels, especially for those with clinical significance, would be necessary for delineating the mechanistic basis underlying their potential link to disease conditions in humans. It is highly plausible that functional effects similar to those observed for hMSH5 P29S could also be conferred by other hMSH5 and/or hMSH4 non-synonymous SNPs. It should not be a surprise that different combinations of these SNPs might associate with an array of subtle functional alterations; that, to a certain extent, could also affect the dynamic interplay among hMSH4-hMSH5 associated proteins and subsequent downstream events.

2.2 Structural properties of MSH4 and MSH5 proteins

2.2.1 General molecular structure of MutS homologues

A common characteristic of MutS homologous proteins is their essential role in binding and recognizing mismatched base pairs—a function facilitated by their ability to act as DNA-binding ATPases. Whilst structures of eukaryotic MSH proteins remains undetermined, insights towards the mechanistic aspects of mismatch recognition may be inferred from the crystal structure of bacterial MutS protein. Crystallographic studies of homologues in *T. aquaticus* and *E. coli* suggest that the MutS homodimer binds to heteroduplex DNA during mismatch recognition as a "structural heterodimer" (Junop *et al.* 2001; Lamers *et al.* 2000; Obmolova *et al.* 2000). The homodimerization of MutS protein is mediated through the region harboring MutS ATPase activity. The dimerization domain is far apart from the DNA binding domain, but these regions coordinate through conformational changes triggered by MutS binding to heteroduplex DNA or ATP (Lamers *et al.* 2004; Lamers *et al.* 2003). In essence, these studies indicate that MutS is a modular protein with separate domains which, when dimerized at their carboxyl termini, act to encircle mismatch-containing DNA during the initiation stages of repair process. Deletion analysis of MutS protein confirms that the carboxyl terminus, which also includes a P-loop motif for nucleoside triphosphate binding, is involved in homodimerization. The amino terminal end is necessary for binding to mismatch-containing DNA, and through the binding and hydrolysis of ATP in the carboxyl terminal, MutS may dissociate from mismatched DNA once repair is underway. Although no crystallographic analysis of eukaryotic MSH proteins is presently available, noting the high degree of homology between MutS homologues and their bacterial counterparts, it is not difficult to conjecture that the MSH proteins found in yeast, mouse, and humans may contain similar structural features to those of prokaryotic MutS proteins.

2.2.2 Eukaryotic MSH4-MSH5 complex

Whereas functional bacterial MutS protein exists as homodimers, the eukaryotic MSH family is far more complex and contains multiple heterodimers composed of different MSH

proteins. MSH heterodimers carry out diverse cellular functions including MMR and DNA damage response. However, MSH4 and MSH5 are unique in their intracellular roles. Although they contain the conserved sequence motifs found in all MutS homologues—such as the ATP binding domain and a helix-turn-helix structural motif located at the carboxyl terminal half of the protein (Burns *et al.* 1994; Her & Doggett 1998; Her *et al.* 2001; Her *et al.* 1999; Hollingsworth *et al.* 1995; Paquis-Flucklinger *et al.* 1997)—neither MSH4 nor MSH5 interact with the other MSH proteins known to function in MMR. MSH4 and MSH5 interact with each other exclusively, forming a distinctive heterocomplex (Bocker *et al.* 1999; Her *et al.* 2001; Her *et al.* 1999; Winand *et al.* 1998; Yi *et al.* 2005). Unlike other MSH proteins, which contain specific amino acid residues for recognition and binding of mismatched base pairs, MSH4 and MSH5, either individually or as a heterocomplex, are unable to detect or respond to mismatches as they lack structural motifs for binding of mismatched base pairs (Obmolova *et al.* 2000). It is postulated that the MSH4-MSH5 heterocomplex may configure in a way that can accommodate large recombination intermediate DNA structures such as Holliday junction intermediates (Obmolova *et al.* 2000). Evidence obtained with the purified recombinant hMSH4-hMSH5 heterocomplex points to their role in the recognition and binding of artificial DNA structures resembling four-way junctions (Snowden *et al.* 2004), suggesting that the hMSH4-hMSH5 heterocomplex is uniquely equipped for processing recombinational intermediates.

The human hMSH4 and hMSH5 interact with one another via the carboxyl terminal region of hMSH4 and both the amino and carboxyl terminal regions of hMSH5. The first 109 and the last 103 amino acid residues of hMSH5 are necessary for the formation of a composite hMSH4-interacting domain; however, only the carboxyl terminal 93 amino acid residues of hMSH4 are required to interact with hMSH5 (Yi *et al.* 2005), suggesting an asymmetric structural partition of hMSH4 and hMSH5 in the heterocomplex. The hMSH4-hMSH5 heterocomplex is suggested to form a sliding clamp structure that stabilizes and preserves Holliday junctions during prophase of meiosis I or in the repair of DSBs. A model for hMSH4-hMSH5 in meiotic recombination has been proposed (Snowden *et al.* 2004), implicating their role in linking DSB repair to the regulation of crossover (CO) formation. It is known that the amino terminal region of hMSH4, composed of amino acid residues 148-387, is involved in mediating homotypic interaction (Her *et al.* 1999; Lee *et al.* 2006). Due to the physical separation of the hetero- and homo-interacting domains on hMSH4, it has been suggested that hMSH4 and hMSH5 may form a multimeric protein complex such as a tetramer. In addition, it has been demonstrated that the interface of hMSH4-hMSH5 heterocomplex forms a composite interaction domain for GPS2; the latter is a protein factor involved in intracellular signaling and DNA damage response (Jin *et al.* 1997; Lee *et al.* 2006; Peng *et al.* 2001; Spain *et al.* 1996). The interplay of GPS2 with the hMSH4-hMSH5 heterocomplex may provide a link to downstream molecular events required for Holliday junction processing and subsequent resolution.

2.2.3 Protein interacting partners of MSH4 and MSH5

The MSH4-MSH5 heterocomplex is thought to participate in a limited array of functions, leaving the individual proteins to coordinate specific cellular processes independent of one another throughout various mammalian tissues. It has been shown that hMSH4 physically interacts with hMLH1 as well as its binding partner hMLH3, in which the amino terminal region of hMSH4 interacts with hMLH1 proteolytic degradation products, rather than the full-length hMLH1 (Lipkin *et al.* 2000; Santucci-Darmanin *et al.* 2002; Santucci-Darmanin *et*

al. 2000). Furthermore, hMSH4 interacts with VBP1, Rad51, and DMC1 (Her *et al.* 2003; Her *et al.* 2007; Neyton *et al.* 2004). In addition to its interacting partner hMSH4, hMSH5 has been shown to interact with the non-receptor tyrosine kinase c-Abl, hMRE11, and histone demethylase SMCY (Akimoto *et al.* 2008; Kato *et al.* 2007; Yi *et al.* 2006), of which the interaction with c-Abl mediates hMSH5 tyrosine phosphorylation in response to IR-induced DSBs (Yi *et al.* 2006). Coherent with this observation, the ubiquitous DNA damage repair protein hRad51 coexists in the protein complex containing both hMSH5 and c-Abl (Her *et al.* 2007).

Interaction with different protein partners may provide a foundation for hMSH4 and hMSH5 to act independently in specific cellular processes, during which protein interactions can also modulate the functions of hMSH4 and hMSH5. For example, the interaction of VBP1 with hMSH4 negatively regulates the formation of hMSH4-hMSH5 heterocomplex (Her *et al.* 2003). The biological relevance of this observation in mitotic cells is presently not known. It is reported that, during early stages of mouse testis development, the up-regulation of GPS2 coincides with the down-regulation of VBP1 immediately prior to, or at the onset of, the first meiotic wave, presumably facilitating the formation of Msh4-Msh5 heterocomplex (Lee *et al.* 2006). The hMSH4-hMSH5 interaction is also subjected to regulation by c-Abl-mediated hMSH5 tyrosine phosphorylation. In particular, hMSH5 is shown to undergo IR-induced c-Abl-dependent tyrosine phosphorylation, and consequently this posttranslational modification leads to the dissociation of hMSH4-hMSH5 heterocomplex (Tompkins *et al.* 2009; Yi *et al.* 2006). Since the formation of hMSH4-hMSH5 heterocomplex is required for the interaction with GPS2 (likely in a complex with HDAC3), it is expected that hMSH5 tyrosine phosphorylation will result in a dynamic transformation of the hMSH4-hMSH5 associated protein complex, which might be functionally required during recombinational DSB repair.

Recent evidence suggests that factors influence the hMSH4-hMSH5 interaction will also affect their subcellular localization. It appears that hMSH4-hMSH5 dimerization enhances their nuclear localization—possibly facilitated by a nuclear localization signal (NLS) located in the middle of hMSH5 protein, or by masking of a CRM1-dependent nuclear export signal (NES) on the carboxyl terminal region of hMSH5 within the hMSH4-interacting domain (Lahaye *et al.* 2010; Neyton *et al.* 2007).

2.3 Functions of MSH4 and MSH5

2.3.1 Role in meiotic recombination

Meiotic recombination occurs in meiotic prophase starting with the formation of programmed DSBs induced by the expression of SPO11, a protein highly conserved and enriched in germ cells at recombination hotspots (Baudat & de Massy 2007; Keeney *et al.* 1997). After 5′ end resection at the break, the emerging 3′ single-stranded overhang invades undamaged homologous chromosome or sister chromatid, leading to the formation of meiotic recombination intermediate structures including the Holliday junction and thus facilitating DSB repair (Szostak *et al.* 1983). The completion of meiotic homologous recombination is achieved through the resolution of Holliday junction structures by cleavage and rejoining to re-form two separate DNA molecules. The two major outcomes of meiotic homologous recombinational DSB repair are chromosomal reciprocal exchange or CO and gene conversion or non-crossover (Mahadevaiah *et al.* 2001). While non-crossover is important for conserving genetic identities, chromosomal CO is crucial for creating genetic diversity and therefore promoting survival along the way of evolution. Furthermore, COs

are critical for successful meiosis, of which both the frequency and the distribution of COs are well controlled in a way that governs the fidelity of chromosome segregation (Youds & Boulton 2011).

Studies carried out in budding yeast have provided evidence to indicate that both MSH4 and MSH5 are involved in meiotic recombination but not in MMR (Hollingsworth *et al.* 1995; Ross-Macdonald & Roeder 1994). *MSH4*-null mutation in *S. cerevisiae* is not associated with elevated rate in mismatches of reporter genes Can^R (Reenan & Kolodner 1992) and Thr+ (Kramer *et al.* 1989) or defective gene conversion, instead the *MSH4*-null strain displays 2 to 3-fold reduction in CO and an increase in homologous chromosome nondisjunction, leading to reduced spore viability (Ross-Macdonald & Roeder 1994). Similar effects are evident in *S. cerevisiae* with mutant *MSH5*, in which MMR is proficient in the processes of both meiosis and mitosis, and meiotic gene conversion frequency is not significantly different from the wild type. However, CO is reduced 2 to 3-fold and homologous chromosome nondisjunction is elevated in meiosis I. As a consequence, spore viability is reduced to 37% from 72% observed in the wild type (Hollingsworth *et al.* 1995). *S. cerevisiae* strain lacking both *MSH4* and *MSH5* does not show a synergistic effect on CO frequency in meiosis I, and the spore viability is compatible with either mutants, indicating *MSH4* and *MSH5* act in the same pathway that facilitate meiotic crossing over recombination (Hollingsworth *et al.* 1995). Decreased crossover interference and delayed or incomplete chromosome synapsis are also observed in *MSH4* mutant yeast, suggesting a role for MSH4 in the regulation of crossover distribution (Novak *et al.* 2001).

Like budding yeast, *C. elegans MSH4* and *MSH5* are functionally conserved with respect to their role in promoting meiotic CO. With a null mutation in *him-14*, the *C. elegans* ortholog of *MSH4*, CO is abolished during meiosis I in both oocytes and spermatocytes. As a result, formation of chiasmata is absent and chromosome segregation is severely defective. Most of the *him-14* embryos, although produced in normal amounts, fail to hatch; and and among the hatched that reached to adulthood, 45% are males (Zalevsky *et al.* 1999; Zetka & Rose 1995). This is consistent with an increase in non-disjunction, because males are normally arisen through spontaneous non-disjunction with a frequency of about 0.1% in the hermaphrodite germ line. The *him-14* mutants, however, do not show any abnormality in meiotic chromosome pairing and synapsis (Zalevsky *et al.* 1999). Similar to their budding yeast counterparts, the mutant worms do not display increased "mutator" phenotype, assayed by the means of levamisole resistance, indicating that Him-14 is not required for MMR in *C. elegans* (Zalevsky *et al.* 1999). In spite of high degree of conservation, *C. elegans* and *S. cerevisiae MSH4* mutants differ significantly in their effects on CO. Without *MSH4*, CO is completely abrogated in *C. elegans* (Zalevsky *et al.* 1999), yet about 30-50% is retained in the budding yeast (Ross-Macdonald & Roeder 1994). This difference has helped to fashion the current view that *C. elegans* has a single or a dominant pathway to create meiotic CO for which *MSH4* is indispensible, and budding yeast has alternative *MSH4*-independent pathway(s) for CO generation. While MSH4 is crucial for proper chromosome segregation, in contrast to yeast, *C. elegans* Him-14 does not appear to act on chromosome pairing and synapsis.

Likewise germ cells from *C. elegans msh-5* mutants are able to progress through meiosis with normal chromosome pairing and synapsis. *Msh-5* mutants lay eggs at a same rate as the wild type, and they produce a normal number of embryos, however, more than 97% of the embryos fail to hatch and 42% of those survived to adulthood are males, owing to defective segregation of homologous chromosomes (Kelly *et al.* 2000). Similar to the *him-14* mutants, CO frequency in *Msh-5* mutants is reduced to about 1% of the wild type, and these mutants

lack chiasmata formation (Kelly *et al.* 2000). On the basis of their nearly identical effect on meiotic recombination, it is conceivable that both MSH4 and MSH5 are indispensible for *C. elegans* meiotic CO, and these two MutS homologues act in the same process through apparently the only pathway in this species.

In mice, meiotic recombination is also initiated by Spo11-dependent DSBs (Mahadevaiah *et al.* 2001). *Msh5* knockout mice are generally healthy and undergo normal development, however their reproductive organs do not develop properly. For example, testes in *Msh5*-null animals are significantly smaller in size and ovaries quickly become rudimentary after birth (de Vries *et al.* 1999; Edelmann *et al.* 1999). Although their mating behavior appears normal, *Msh5*-null mice are sterile. Histological analysis demonstrates that the testes of *Msh5*-null mice are completely devoid of epididymal spermatozoa, presumably due to apoptosis before pachytene—the stage of meiosis when crossover structures between homologous chromosomes become experimentally visible (de Vries *et al.* 1999; Edelmann *et al.* 1999). Closer examination of *Msh5*-null spermatocytes and oocytes reveals disrupted chromosome pairing and impaired synaptonemal complex formation, indicating *Msh5* is essential for homologous chromosome pairing and synapsis during meiosis I in mice (de Vries *et al.* 1999; Edelmann *et al.* 1999). Since the single strand DNA binding protein Rad51 typically represents a marker for recombination initiation (Bishop 1994; Moens *et al.* 2002), the observed Rad51 foci formation on unsynapsed chromosomes in *Msh5*-null mice tends to suggest that Msh5 is not involved in the initiation of meiotic recombination (Edelmann *et al.* 1999).

Similarly, *Msh4*-null mice develop normally but are infertile. These mutant mice display severe abnormality in chromosome pairing in early prophase I (Kneitz *et al.* 2000), indicating Msh4 is also required for meiotic homologous chromosome pairing. However, *Msh4* deficiency appears to cause less severe defect in chromosome pairing in comparison to *Msh5*-null animals. Approximately 69% of nuclei in *Msh4*-null spermatocytes have some degree of chromosome pairing, but less than 10% of the nuclei contain very few paired chromosomes in *Msh5*-null mice (Kneitz *et al.* 2000). Meiotic phenotypes of *Msh4* and *Msh5* double-knockout mice are similar to the *Msh5*-null mice, suggesting that Msh4 and Msh5 act together in promoting meiotic chromosome pairing and synapsis in the early phase of meiotic recombination, possibly with Msh5 upstream of Msh4 (Kneitz *et al.* 2000).

In human oocytes, both hMSH4 and hMSH5 proteins are localized on meiotic chromosomes throughout meiotic prophase I and become co-localized at zygonema with SYCP3, a key component of synaptonemal complex, thus supporting the idea that human hMSH4 and hMSH5 may possess meiotic properties similar to their mouse counterparts (Lenzi *et al.* 2005). This view is further supported by the physical interaction observed between hMSH4 and hRad51 as well as the co-localization of their counterparts in mouse spermatocytes (Neyton *et al.* 2004)—indicative of an early role for MSH4, presumably for MSH5 as well, in meiotic recombination. The presence of hMSH4 and hMSH5 foci on meiotic chromosomes after synaptonemal complex formation is highly suggestive of an additional role for these proteins in the late stages of meiotic recombination (Lenzi *et al.* 2005). Consistent with this speculation is the observed *in vitro* physical interaction between hMSH4 and hMLH1, the later is commonly regarded as a marker for crossing over recombination (Lynn *et al.* 2004). Together with the co-localization of these two proteins in mouse spermatocytes (Santucci-Darmanin *et al.* 2000), it is suggested that MSH4 and MSH5 play at least two separate roles in meiosis I—an early role in homology searching that leads to proper chromosome pairing and a late role in the processing of recombination intermediate structures. Binding of

purified hMSH4-hMSH5 heterocomplex to the core of the Holliday junction intermediate structures has provided *in vitro* evidence to support the late role of these two proteins. It is hypothesized that the binding of progenitor Holliday junction DNA provokes ATP binding to both hMSH4 and hMSH5 in the heterocomplex, and the loading of hMSH4-hMSH5 is projected to stabilize and preserve meiotic recombination intermediates prior to proper resolution (Snowden *et al.* 2004).

In short, both MSH4 and MSH5 play important roles in meiotic recombination. The similarity in phenotypes among *MSH4*-null, *MSH5*-null, and *MSH4*-null/*MSH5*-null organisms suggests that these two MutS proteins act in the same pathways and likely function as a heterocomplex at least during certain phases of the recombination process. Although *MSH4* and *MSH5* homologues in lower eukaryotes and mammals appear to share conserved properties supporting their common function in meiosis, their precise effects diverge in different species. For instance, the major action of MSH4 and MSH5 in lower eukaryotes is on the generation and control of COs, whereas in mammals these two MutS homologues have evolved to exert an early role in governing homologous chromosome pairing and synapsis.

2.3.2 Role in mitotic recombination and DNA damage response

The most described function of MMR proteins is the repair of base-pairing errors arising from DNA replication or recombination. Defects in MMR proteins lead to mutations and MSI. Mutations in MMR genes are known to cause Lynch syndrome (or HNPCC) and to increase the risk and progression of a wide-variety of sporadic cancers. During MMR, replication errors are recognized either by MutSα, a heterodimer of MSH2 and MSH6 that binds to single base-base mismatches or small insertion-deletion loops, or by MutSβ, a heterodimer of MSH2 and MSH3 that binds to larger insertion-deletion loops (Jiricny 2006). The repair process then proceeds with the recruitment of a MutL activity (*i.e.* one of these three complexes: MLH1-PMS2, MLH1-PMS1 or MLH1-MLH3) and exonucleases to facilitate subsequent excision followed by DNA repair synthesis.

The MMR proteins are frequently recognized as major players in mediating cellular responses to DNA damage. As components of the BRCA-1-associated genome surveillance complex (Wang *et al.* 2000), MMR proteins are thought to mediate DNA damage response through either direct sensing of various DNA distortions or functioning as transducers to couple damage detection and the control of cell cycle progression (Jiricny 2006). For instance, MMR proteins are involved in provoking a G2/M phase cell cycle arrest that is vital for both DNA repair and cell death by apoptosis, especially for cells carrying excessive DNA lesions. To this end, it is important to note that MMR deficiency has been linked to DNA damage tolerance, and MMR deficient cells are found to be frequently resistant to killing by various chemotherapeutics including alkylating agents and antimetabolites. Given that resistance to chemotherapeutic agents is of a great concern in cancer treatment, a thorough understanding of the molecular mechanisms involved with MMR in cellular DNA damage response will undoubtedly pave a way for devising more effective therapeutic strategies.

Although MSH4 and MSH5 have not been indicated experimentally to function in the process of MMR, cumulating evidence has pointed to their potential involvement in mitotic DNA repair and damage response in addition to their role in meiosis. The discrepant expression patterns of MSH4 and MSH5 in mammalian tissues have suggested that these two proteins may function independently of one another and may operate beyond meiosis

(Her *et al.* 2007; Yi *et al.* 2005). Studies in yeast have raised the possibility that MSH5 may be involved in cellular response to DNA damage induced by alkylating agents. Specifically, a mutant *MSH5* allele (*i.e. msh5-14*, encoding MSH5^{Y823H}) but not *MSH5*-null mutant in the yeast strain XS-14 (lacking O^6-methylguanine methyltransferase) confers cellular tolerance to alkylating compound N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) (Bawa and Xiao 1997; 2003). Although the exact mechanism of action remains to be explored, these studies do indicate that this gain-of-function MSH5 mutant is involved in mediating mitotic cellular response to DNA damage.

It is evident that many of the proteins involved in meiotic recombination are also important players in mitotic recombinational DSB repair. Thus, the involvement of MSH5 in meiotic recombination is suggestive of a potential role for MSH5 in mitotic recombinational DSB repair. DSBs are considered to be the most lethal form of DNA lesion that may arise from replication fork collapse, exposure to DNA damaging agents, or programmed cellular processes including the initiation of meiotic recombination in germ cells, class-switch recombination (CSR), and V(D)J recombination in lymphocytes (Ataian & Krebs 2006). In response to DSB formation, dividing cells usually undergo G2/M phase arrest, then either repair the lesion and resume cell cycle or enter the path of apoptosis depending on the extent of damage. Cells lacking proper G2/M cell cycle arrest, apoptosis, or damage repair are often at a higher risk for malignant transformation. The repair of DSBs requires either the non-homologous end joining (NHEJ) or the homologous recombination pathways. While NHEJ is a rapid means utilized by somatic cells to repair DSBs, it is error-prone and can result in alteration of DNA sequences for non-compatible breaks (Dery & Masson 2007; Helleday *et al.* 2007). On the other hand, homologous recombination is a more accurate repair pathway during S/G2 phases as it utilizes the homologous template usually provided by a sister chromatid or homologous chromosome (Saleh-Gohari & Helleday 2004). Similar to meiotic homologous recombination, this homology-directed DSB repair will also produce two alternative outcomes, CO or non-crossover/gene conversion. Loss of essential homologous recombination gene products often results in chromosome instability, by which cells exhibit increased sensitivities to a variety of DNA damage agents such as IR, cisplatin, and gemcitabine. In addition, homologous recombination deficient cells may also display DNA damage tolerance and resistance to killing by DNA damaging agents as well (Crul *et al.* 2003; Khanna & Jackson 2001; Takata *et al.* 2001; van Waardenburg *et al.* 2004; Zdraveski *et al.* 2000).

As supported by the observed interactions with hRad51, c-Abl, and hMRE11 as well as structures resembling Holliday junctions (Her *et al.* 2007; Kato *et al.* 2007; Neyton *et al.* 2004; Snowden *et al.* 2004; Yi *et al.* 2006), hMSH4 and hMSH5 are expected to play functional roles in mitotic DNA damage response. In *C. elegans* oocytes, silencing of *RAD51* on a *MSH5* deficient background results in chromosome fragmentation, while a comparable defect is also noted when *MSH4* and *BRCA1* homologues are concomitantly silenced, indicating the existence of functional interplay of these proteins in the maintenance of chromosome integrity (Adamo *et al.* 2008; Rinaldo *et al.* 2002). The interaction between hMSH5 and c-Abl can provoke two different cellular actions depending on the severity of DNA damage (Tompkins *et al.* 2009). Endogenous hMSH5 protein undergoes IR dose and time dependent induction, and it appears that increased levels of hMSH5 can promote IR-triggered apoptosis. However, this effect is more prominent in cells treated with a relatively high dose of IR (> 2 Gy). This is reminiscent of a previous observation that the linear correlation between DSB repair and the number of γH2Ax foci only exists in cells irradiated with IR at

doses below 2 Gy (Bouquet *et al.* 2006), suggesting the existence of a dynamic regulatory mechanism controlling DSB repair and apoptosis. Present evidence is compatible with the view that the expression of hMSH5 is normally maintained at a low level in unperturbed cells, and DNA damage-triggered hMSH5 induction promotes c-Abl activation and subsequent initiation of a p73-mediated caspase 3-dependent apoptotic response (Tompkins *et al.* 2009). Coherently, the peculiar interaction between $hMSH5^{P29S}$ and c-Abl is capable of over-activating c-Abl, leading to increased cellular radiosensitivity. In contrast, moderate hMSH5 induction is expected to facilitate recombinational DSB repair. Recent experimental results have demonstrated a functional requirement for c-Abl-mediated hMSH5 phosphorylation in DSB repair (Her *et al.* 2007; Tompkins *et al.* 2009). It is demonstrated, by the use of a chromosomally integrated recombination reporter, that hMSH5 tyrosine phosphorylation is an essential early event for non-crossover DSB repair. In fact, cells harboring a phosphorylation deficient hMSH5 mutant are more sensitive to DSB-inducing anticancer drug cisplatin. Collectively, these studies tend to suggest a dual role for the induction of hMSH5 protein in recombinational DSB repair and DNA damage-triggered apoptotic response.

2.3.3 Role in immunoglobulin diversification

Genomic rearrangement that occurs during B-cell development in the form of V(D)J recombination is essential for the generation of antibody repertoire, and additional diversity is achieved by elevated mutation rate and gene sequence shuffling in corresponding processes known as somatic hypermutation (SHM) and CSR (Li *et al.* 2004b; Schroeder & Cavacini 2010). While SHM targets the "hot spots" in variable (V) region of immunoglobulin (Ig) to enhance antigen recognition, CSR is responsible for Ig isotype switching by way of recombining the switch (S) regions upstream of each functional genes (C_H) in the constant (C) region of Ig heavy (H) chain, *i.e.* switching from IgM or IgD to other isotypes that mediate diverse effector functions in the daughter cells (Li *et al.* 2004b; Schroeder & Cavacini 2010). Both SHM and CSR start from deamination of dC to dU by activation-induced cytidine deaminase, a centroblast B-cell specific protein (Muramatsu *et al.* 2000; Muramatsu *et al.* 1999). Deamination of dCs leads to G-U mismatches and may subsequently generate C to T or G to A mutations. In addition, uracil N-glycosylase, apurinic/apyrimidinic endonucleases, and MMR proteins are required to generate extended single base mutations and single-stranded DNA nicks (Di Noia & Neuberger 2002; Ehrenstein & Neuberger 1999; Ehrenstein *et al.* 2001; Guikema *et al.* 2007; Imai *et al.* 2003; Martin & Scharff 2002; Rada *et al.* 2002; Schrader *et al.* 1999). During CSR, DSBs have to be generated in the S regions, possibly by way of single-strand breaks, to allow intrachromosomal deletion via recombination between the two S regions (Wuerffel *et al.* 1997), in which DSBs are primarily repaired by NHEJ. The tandem repeats of short consensus sequences in the S regions are too short for homologous recombination, however they are enough for microhomology-mediated end joining (MMEJ), a specified type of NHEJ using small homologous regions to anneal the two overhangs at the break site. It is generally accepted that MMEJ represents a prominent process of NHEJ in CSR (Kenter 1999), and the length of microhomology at S-S junctions may change due to impaired MMEJ (Stavnezer 2000).

It is well documented that proteins involved in MMR and DSB repair are also involved in the process of CSR. In fact, *Msh2*, *Msh6*, *Mlh1*, and *Pms2* knockout mice all exhibit impaired CSR and SHM (Ehrenstein & Neuberger 1999; Martin & Scharff 2002; Schrader *et al.* 1999). Mice deficient for early DSB response factors ATM or H2AX, and NHEJ proteins Ku70, Ku80, or DNA-PKcs, show reduced CSR activities but with normal SHM (Bemark *et al.* 2000; Casellas *et*

al. 1998; Manis *et al.* 2002; Manis *et al.* 1998; Reina-San-Martin *et al.* 2004; Reina-San-Martin *et al.* 2003). MutS and MutL homologues are not only required to assist MMEJ, but they also play overlapping and distinct roles in the process of switch recombination (Ehrenstein *et al.* 2001; Li *et al.* 2004a; Schrader *et al.* 1999; Schrader *et al.* 2002). This assessment is mainly obtained through analyzing microhomology at the S-S junctions as well as the distribution of breakpoints in the absence of each protein (Ehrenstein *et al.* 2001; Li *et al.* 2004a; Schrader *et al.* 1999; Wu *et al.* 2006). Collectively, deficiency of MutS and MutL homologues has been associated with three different phenotypes at the S-S junctions—*Msh2* or *Mlh3* deficiency leads to a decrease in the length of microhomology (Schrader *et al.* 2002; Wu *et al.* 2006), *Msh6*-null B cells show no change of microhomology (Li *et al.* 2004a), and an increase in the length of microhomology is evident in *Mlh1*- or *Pms2*-null B cells (Ehrenstein *et al.* 2001; Schrader *et al.* 2002). MLH1-PMS2 acts downstream of MSH2-MSH6 during MMR, similar effect would be anticipated if the process of MMR played a predominant role. The presence of different microhomology phenotypes indicates that these MMR proteins are involved, at least in part, in different sub-pathways during MMEJ. Indeed, PMS2 has been speculated to interact and stabilize MMEJ intermediates, whereas MSH2 might participate in DNA end processing (Ehrenstein *et al.* 2001; Schrader *et al.* 1999; Schrader *et al.* 2002).

Present evidence suggests that MSH4 and MSH5 are additional players in CSR. These two MutS homologues are known to function in the early and late steps of meiotic recombination as well as in mitotic DNA damage response—events that share some similarities to crucial steps in the complex CSR process. Transcripts of *hMSH5* are present in human spleen and peripheral blood B cells (Her & Doggett 1998; Sekine *et al.* 2007). Association studies have linked one of the non-synonymous alleles of *hMSH5* to IgAD and CVID, two syndromes that are attributable to abnormal CSR. The allele encoding the $hMSH5^{L85F/P786S}$ variant is significantly more frequent in IgAD patients and with borderline significance in CVID patients. One *hMSH5* SNP (rs3131378), located within intron 12, is also tightly associated with IgAD and CVID. Furthermore, CVID patients carrying the $hMSH5^{L85F/P786S}$ allele display increased microhomology at Sμ-Sα1 joints, which commonly associate with far fewer mutations (Sekine *et al.* 2007). The L85F and P786S alterations, located within the hMSH4-interacting domain, significantly compromise the protein interaction between hMSH5 and hMSH4, suggesting both hMSH5 and hMSH4 are required for efficient CSR (Sekine *et al.* 2007).

In mice, however, the effects of Msh5 on CSR seem to be strain-specific. In an earlier study, most of the congenic H-$2^{b/b}$ MRL/*Ipr* mice (introgression of H-2^{b} MHC haplotype from129/Sv onto a MRL/*Ipr* background) show undetectable serum IgG3, reduced levels of serum IgA and elevated levels of serum IgM and IgG2a in older mice, suggesting impaired CSR in these animals. Microarray analysis of gene expression reveals a hypomorphic allele of *Msh5* on the H-2^{b} haplotype. Comparing to controls, H-$2^{b/b}$ MRL/*Ipr* mice without serum IgG3 ($IgG3^{neg}$) exhibit significantly increased microhomology at Sμ-Sγ3 (*i.e.* IgM to IgG3 switch) and Sμ-Sα (*i.e.* IgM to IgA switch) joints in splenic B cells (Sekine *et al.* 2007). This phenotype of increased microhomology is also observed in B cells from *Msh5*-null FVB mice (de Vries *et al.* 1999) and *Msh4* knockout mice on C57BL/6 background (Sekine *et al.* 2007). These studies indicate that both MSH5 and MSH4 are involved in CSR and may function as a heterodimer. Interestingly, studies performed with *Msh5*-null C57BL/6 mice show no detectable alterations in the length of microhomology (Guikema *et al.* 2008). It is conceivable that the different effects of Msh5 deficiency on CSR in these two mouse strains might be attributable to different levels of *Msh5* expression, of which MRL/lpr is a high *Msh5*

expresser, whereas C57BL/6 expresses very low levels of Msh5, and the difference between them is about 100-fold (Sekine *et al.* 2007; Sekine *et al.* 2009). It is also important to note that these two *Msh5*-null mouse lines have been reported to display different degrees of meiotic chromosome pairing defects (de Vries *et al.* 1999; Edelmann *et al.* 1999), suggesting that the role of *Msh5* might be influenced by its associated genetic backgrounds.

3. Conclusion

The MMR system has received a considerable amount of attention over the last decade. It is now clear that the MMR system is multifaceted and participates in several different pathways of DNA metabolism. Among all MMR components, the MutS family of proteins plays critical and conserved functions during the initiation phase of mismatch recognition. In contrast to those MutS homologues involved in MMR, the functions of MSH4 and MSH5 are still not fully understood. These two MutS homologues share similar structure and sequence features with the other members of the MutS family. Present evidence suggests that MSH4 and MSH5 have evolved to function in recombinational DSB repair, DNA damage signaling, and immunoglobulin class switch recombination. Although the mechanistic details of their involvement in these processes have yet to be elucidated, it is conceivable that the actions of hMSH5 in recombinational DSB repair is both hMSH4-independent and hMSH4-dependent, presumably coordinating with their functions in homology searching and the resolution of recombination intermediate structures. Available evidence supports a scenario that the expression of hMSH5 is maintained at a low level under normal conditions, whereas DNA damage-elicited hMSH5 induction can promote c-Abl activation and the initiation of a p73-mediated caspase 3-dependent apoptotic response. On the contrary, moderate hMSH5 induction, caused by less severe DNA damage, is expected to facilitate recombinational DSB repair. It is plausible that hMSH4 and hMSH5 may also play a role in CSR, in particular, through manipulating the process of MMEJ.

The current and emerging evidence has lent support to the idea that MSH4 and MSH5 are involved in diverse functions by engaging different pathways through various interactions with different proteins. Undoubtedly, the current knowledge about these two MutS homologues has created a solid steppingstone for future exploration of their biological functions and potential association with disease conditions in humans.

4. Acknowledgment

We would like to take this opportunity to thank all previous and current members of the Her laboratory at Washington State University, particularly, for their contributions to our current understanding of the role of hMSH4 and hMSH5 proteins in various cellular processes in human cells. The work conducted in the Her laboratory is generously supported by research grants from the National Institutes of Health, U.S. Department of Health and Human Services.

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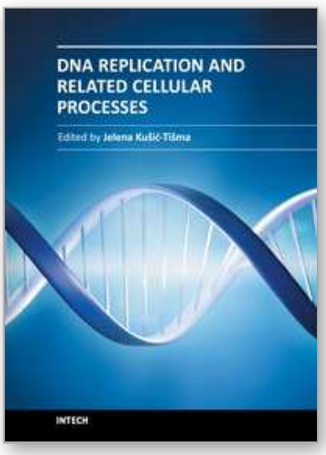

DNA Replication and Related Cellular Processes

Edited by Dr. Jelena Kusic-Tisma

ISBN 978-953-307-775-8

Hard cover, 300 pages

Publisher InTech

Published online 26, September, 2011

Published in print edition September, 2011

Since the discovery of the DNA structure researchers have been highly interested in the molecular basis of genome inheritance. This book covers a wide range of aspects and issues related to the field of DNA replication. The association between genome replication, repair and recombination is also addressed, as well as summaries of recent work of the replication cycles of prokaryotic and eukaryotic viruses. The reader will gain an overview of our current understanding of DNA replication and related cellular processes, and useful resources for further reading.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Xiling Wu, Keqian Xu and Chengtao Her (2011). The Role of MutS Homologues MSH4 and MSH5 in DNA Metabolism and Damage Response, DNA Replication and Related Cellular Processes, Dr. Jelena Kusic-Tisma (Ed.), ISBN: 978-953-307-775-8, InTech, Available from: http://www.intechopen.com/books/dna-replication-and-related-cellular-processes/the-role-of-muts-homologues-msh4-and-msh5-in-dna-metabolism-and-damage-response

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