[Frontiers in Bioscience 12, 905-911, January 1, 2007]

MutS Homologues hMSH4 and hMSH5: Diverse Functional Implications in Humans

Chengtao Her, Nianxi Zhao, Xiling Wu, and Joshua D. Tompkins

School of Molecular Biosciences, PO Box 644660, Washington State University, Pullman, WA 99164-4660

TABLE OF CONTENTS

1. ABSTRACT

The DNA mismatch repair (MMR) pathway is one of the most critical genome surveillance systems for governing faithful transmission of genetic information during DNA replication. The functional necessity of this pathway in humans is partially reflected by the tight link between MMR gene mutations and the development of hereditary nonpolyposis colorectal cancer. Increasing evidence has suggested a broad involvement of MMR proteins in various aspects of DNA metabolism beyond the scope of DNA mismatch correction, such as in the processes of DNA damage response and homologous recombination. Though evidence is presently lacking for potential functional involvement of hMSH4 and hMSH5 in MMR, these two proteins are thought to play roles in meiotic and mitotic DNA double strand break (DSB) repair and DNA damage responses in human cells.

2. INTRODUCTION

The mammalian homologues of bacterial MutS protein represent key components of the DNA mismatch repair (MMR) pathway (for a recent review, see Ref. 1). The MMR system plays essential roles in the maintenance of genetic integrity during DNA replication, thus ensuring faithful transmission of genetic codes from parental cells to subsequent generations. Consistent with our current understanding of the functional roles of MMR in genome surveillance, mutations in a number of MMR genes have been causally linked to the predisposition of cancer developments in both humans and mice (2, 3). Hereditary nonpolyposis colorectal cancer (HNPCC) or Lynch syndrome is the most prominent form of disorders in human patients with MMR gene mutations. Most, if not all, MMR deficient tumors display a unique form of genomic alteration − microsatellite instability (MSI) (2, 3). In fact,

the identification of the first human MMR gene MutS homologue 2 (hMSH2) in 1993 and the realization that mutations in this gene were tightly linked to the pathogenesis of HNPCC have triggered and expedited extensive research in the field through the years (4-7). These studies not only produced ample evidence to substantiate the essential roles of the MMR pathway in the maintenance of genomic stability, but also revealed their involvement in an array of diverse cellular functions beyond the scope of DNA mismatch repair (1). For instance, it is presently known that many components of the MMR pathway are functionally required in the process of DNA damage signaling and meiotic homologous recombination (8-10).

Among all MMR components, members of MutS family of proteins (MSH) play critical conserved functions during the initiation phase of mismatch recognition and DNA recombination (1). To date, there are five MSH proteins identified in human cells, whose functional necessity in the process of MMR has been unambiguously established for MutS homologous proteins hMSH2, hMSH3, and hMSH6 (4, 5, 11, 12). The hMSH2-hMSH6 heterodimer recognizes both single-base mismatches and small insertion/deletion loops, whereas the second heterodimer composed of hMSH2 and hMSH3 recognizes small insertion/deletion loops (13-15). Most recently, the substrate specificities of these two heterodimers have also been recapitulated using purified human proteins in an *in vitro* reconstituted 5'-specific MMR assay system (16).

Despite also being MutS homologous proteins, experimental evidence has not been forthcoming to substantiate the potential involvement of the other two MutS homologous proteins hMSH4 and hMSH5 in the process of MMR in human cells. Rather, a number of studies performed in lower eukaryotes and mammals have established a meiotic requirement for MSH4 and MSH5 during prophase I (17-23). It is important to note that this meiotic requirement is hardly unique for MSH4 and MSH5; several other MMR proteins are also functionally required for the successful execution of meiosis (2). Thus, it is not surprising that the current emerging evidence, predominantly obtained from gene expression analysis, has leaned to support the idea that MSH5 and/or MSH4 might also be involved in mitotic processes beyond the scope of meiosis (24-32). The functional roles of MSH4 and MSH5 in meiotic processes of various organisms have been comprehensively reviewed in several recent articles (9, 10); therefore the following discussion will be mainly focused on the human hMSH4 and hMSH5 genes with a particular emphasis on the potential functional diversity of these two MutS family genes in humans.

3. HUMAN *hMSH4* AND *hMSH5* GENES AND THEIR VARIANTS

Retrospectively, the identification of six MutS homologues in *Saccharomyces cerevisiae* and the realization that a significant proportion of HNPCC families have no mutations in any known MMR genes have eminently set the stage in searching for additional MMR genes in humans. As a result, two additional human MutS-like genes, *hMSH4* and *hMSH5*, were subsequently cloned a few years later after the identification and characterization of *hMSH3*, *hMSH2*, and *hMSH6* – genes that are presently known to be involved in MMR in humans (4, 11, 12). The existence of highly conserved regions in the MutS family of proteins has warranted the use of a degenerate PCR approach to identify hMSH4 from human cDNA preparations (33). The human *hMSH4* gene contains 20 exons and spans approximately 116-kb on chromosome 1p31 (29, 33). With a 2808-bp putative open reading frame (ORF), the *hMSH4* gene encodes a protein that is 936 amino acids in length, with a predicted molecular mass of 104.8 kDa. The multiple alternatively spliced transcripts of the human *hMSH4* gene have been noted recently (29, 34), of which only one hMSH4 splicing variant (hMSH4sv), resulting from the skipping of the entire exon 19, has been functionally analyzed (29). The exon 19-skipping results in a frameshift of 7 codons followed by a stop codon in exon 20, leading to the production of an 850-amino acid-truncated protein that contains most of the highly conserved sequence motifs of MutS homologues except for the carboxyl terminal helix-turn-helix motif (29). Both hMSH4 and hMSH4sv transcripts can be readily detected in testis but are less prominent in the thymus, ovary, colon, pancreas, and brain, whereas only low levels of hMSH4 are expressed in the heart, liver, and placenta (29). Also of importance is another hMSH4 exon-skipping variant (ΔhMSH4), in which the entire exon 6 is omitted, leading to an in-frame deletion of hMSH4 aa273-330 (34). The potential functional implications of these two hMSH4 splicing variants will be discussed in the following section.

The rapid advance of the expressed sequence tag (EST) database has expedited the identification of the last MutS-like gene, *hMSH5*, in humans (26-28). The *hMSH5* gene contains 26 exons, including two alternative overlapping initial exons, and spans approximately 25-kb within the MHC class III region on human chromosome 6p21.3 (26). The *hMSH5* gene harbors a 2505-bp ORF and encodes an 834-amino acid protein with a predicted molecular mass of 92.9 kDa (26-28). In contrast to the limited expression profile of hMSH4 in humans, the full-length hMSH5 transcripts are virtually detectable in all tissues examined with the most abundant expression in testis. Other organs that display noticeable hMSH5 expression include the thymus, skeletal muscle, bone marrow, spinal cord, brain, trachea, ovary, and lymph node (26-28). The wide tissue distribution pattern of hMSH5 has also been noted through RT-PCR analysis and human EST database mining (30; UniGene EST Profile Viewer). The seemingly unparalleled expression profiles of these two genes have raised a likely scenario that hMSH5 could possess certain functions that may be independent of hMSH4. In fact, roles of hMSH5 in the generation of immunoglobulin and T-cell receptor as well as in the process of DNA damage response and repair have already been postulated (27, 28, 31). Though the extent of concordance between the levels of mRNAs and proteins is largely undetermined in human tissues, both hMSH4 and hMSH5 proteins can be detected from immunoprecipitates of 293T and HeLa cell extracts (F. Zhu and C. Her, unpublished observation).

The *hMSH5* gene also produces multiple alternative transcripts, of which four hMSH5 variants that maintain the reading frame have been archived in NCBI sequence database (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 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 (30), whereas hMSH5b harbors one extra amino acid residue in between codons 654 and 655 (apparently due to the retention of the last 3 nucleotides of hMSH5 intron 20; 26). hMSH5d represents the shortest hMSH5 variant. Although it contains both 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.

Although the existence of different hMSH5 variant transcripts keenly supports the possibility that *hMSH5* may encode multiple products, a full appreciation of the functional aspects of these potential protein isoforms requires a further experimental examination of each of the hMSH5 variants; to this end it is necessary to determine whether hMSH5 variants are the result of sequence variations within corresponding introns and/or regulated by a yet-to-be-defined mechanism. In essence, the biological necessity of these hMSH5 variants in human cells has to be explored through a better understanding of their tissue distribution patterns and a thorough deciphering of hMSH5 functional domains, particularly those encoded by exons involved in alternative splicing of the gene. Ultimately, that information should make it possible to correlate the different splicing patterns (exon inclusion or exclusion) with the potentially diverse functional properties associated with the corresponding protein variants. However, at present, only hMSH5a (hMSH5sv) has been experimentally analyzed (30). The expression profile of hMSH5a appears to be distinguishable from that of hMSH5; in particular the expression of this splicing variant, but not hMSH5, is absent or below the detection limit in the brain, heart, and skeletal muscle (30). Furthermore, the expression of hMSH5a displays a large variation in tumor cell lines with breast and lung carcinomas showing the most abundant expression (30). Though experimental evidence is presently lacking to suggest any potential tissue-specific regulation for the other two hMSH5 splicing variants, the apparently distinct tissue distribution patterns of hMSH5 and hMSH5a have underscored the need to perform similar analysis for all hMSH5 splicing variants to gain a better understanding of the functions associated with the human *hMSH5* gene.

In addition to the existence of multiple alternatively spliced transcripts, the potentially diverse functions involved with the *hMSH4* and *hMSH5* genes are also 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 these two genes – their corresponding single amino acid changes include hMSH4 A60V, A90T, A97T, E162K, I365V, Y589C, S914N; hMSH5 P29S, L85F, Y202C, V206F, R351G, L377F, P786S (NCBI SNP database). However, the allele frequencies, haplotypes, and functional implications of most, if not all, SNPs are largely undetermined; in fact only one non-synonymous SNP, *hMSH5* C85T, has been recently characterized as a common genetic polymorphism with an allele frequency of 11.6% in an American Caucasian population of 99 individuals (30) and 17% in a Chinese population of 279 individuals (D. Lin and C. Her, unpublished observations). It is conceivable that some of the non-synonymous SNPs could result in subtle changes of protein functions, for which a brief perspective is provided in the next section.

4. BIOCHEMICAL PROPERTIES AND PROTEIN INTERACTING PARTNERS

Being MutS family members, hMSH4 and hMSH5 proteins contain the conserved sequence motifs that have been described for all MutS homologous proteins, which include an ATP binding domain and a helix-turn-helix structural motif located at the carboxyl terminal half of MutS homologous proteins (26, 33). However, neither hMSH4 nor hMSH5 interacts with the other human MutS homologous proteins that are known to function in DNA mismatch repair (28). Rather the mammalian MSH4 and MSH5 interact with each other leading to the formation of a unique hetero-complex (27, 28, 30, 35, 36). Similar to other MutS family of proteins, the hMSH4-hMSH5 hetero-complex is capable of catalyzing ATP hydrolysis, of which the ATPase activity was significantly stimulated in the presence of DNA molecules containing a branch-immobile Holliday junction structure (37). The observation that purified recombinant hMSH4-hMSH5 hetero-complex can specifically bind to the core of the Holliday junction structure has implicated a direct role of hMSH4-hMSH5 in the processing of recombination intermediate structures during repair of DNA DSBs (37).

It is interesting to note that both amino and carboxyl terminal regions (the first 109 and the last 103 amino acid residues) of hMSH5 are involved in protein interaction through the formation of a composite hMSH4-interacting domain, whereas only the last 93 amino acid residues of hMSH4 is required for mediating protein interaction with hMSH5 (30), suggesting an asymmetric structural partition of hMSH4 and hMSH5 in the hetero-complex formation. In addition, several lines of evidence have shown that MSH4 could mediate homotypic interactions (32, 35, 38), in which the homodimerization domain of hMSH4 has been recently mapped to the N-terminal region composed of amino acid residues 148-387 (32). The physical separation of hMSH4 interaction domains that are responsible for mediating homotypic and heterotypic protein interactions has suggested the feasibility of forming a multimeric hMSH4-hMSH5 protein complex in human cells. Obviously, a more complete understanding of the functional roles of hMSH4-hMSH5 during homologous recombination requires a detailed structural characterization of relevant protein complexes together with DNA recombination intermediate structures.

The relatively broad expression patterns of hMSH5, and to a lesser extent hMSH4, have long argued for their potential mitotic functions; the seemingly differential expression patterns of these two genes in various human tissues also raise a possibility that hMSH4 and hMSH5 might also function independently in humans (26-29, 33). One plausible scenario is that, through interaction with different protein partners, hMSH4 and hMSH5 might be involved with multiple cellular processes, of which the formation of hMSH4-hMSH5 hetero-complex may be only required for a limited array of functions. It has been demonstrated previously that hMSH4 physically interacts with hMLH1 as well as its binding partner hMLH3 (39-41), in which the N-terminal region of hMSH4 has been implicated in mediating interaction with an hMLH1 proteolytic degradation product but not the full-length hMLH1 (39). Yeast two-hybrid and biochemical studies have also suggested that hMSH4 interacts with VBP1, hRad51, and hDMC1 (29, 42). It has been shown most recently that hMSH5 interacts with the non-receptor tyrosine kinase c-Abl, which leads to hMSH5 tyrosine phosphorylation in response to ionizing radiation induced DNA DSBs (31). Interestingly, hRad51 can also be found in the purified protein complex containing both hMSH5 and c-Abl (31; W. Yi and C. Her, unpublished observations). However, it is presently unknown whether these proteins are parts of the same and/or several different protein complexes in human cells. In this context, it would be of great interest to know whether these interacting protein partners have any effects on the interaction between hMSH4 and hMSH5.

Recent studies have demonstrated that the formation of hMSH4-hMSH5 protein complex dictates the subsequent recruitment of GPS2 (32); the latter is initially identified as an intracellular signaling protein that has also been implicated in mediating cellular DNA damage responses (43-45). The fact that GPS2 only interacts with the interaction interface of hMSH4-hMSH5 hetero-complex suggests that the GPS2 associated cellular pathway could be involved in coupling the downstream molecular events to the specific functions of hMSH4-hMSH5 hetero-complex. Of particular pertinence to this association is the coexistence of GPS2 and histone deacetylase 3 (HDAC3) in the same protein complex (46). A growing body of evidence in recent years has underscored the functional requirement of histone acetylases and deacetylases in DSB repair (47-51), in which the dynamic interplay between DSB-induced histone acetylation and deacetylation may control the way that a DSB could be sensed and repaired (52). It is interesting to note that in mammalian cells the deacetylation of histone H4 (K5, K8, K12, K16) requires HDAC3 (53), and histone H4 becomes significantly deacetylated at K5 and K8 lysine residues during HR repair of DNA DSB (50). These observations are compatible with the hypothesis that hMSH4-hMSH5 plays an important role in the repair of DNA DSBs, in which the localized histone H4 modifications coordinate with the repair processes.

It has been shown that the formation of hMSH4-hMSH5 hetero-complex can be regulated through competitive protein interaction and post-translational modification (29, 31). It has been noted that the interaction between hMSH4 and VBP1 could negatively regulate the formation of hMSH4-hMSH5 hetero-complex (29), which is in agreement with the observation that during early stages of mouse testis development GPS2 up-regulation accompanies the down-regulation of VBP1 immediately prior to, or at the onset of, the first meiotic wave (32). Although the precise functional role of hMSH4-VBP1 interaction is currently unknown, the involvement of VBP1 in the process of microtubule assembly has led to a postulated role for hMSH4 in chromosome segregation through microtubule manipulation (29). It has been recognized recently that, in response to ionizing radiation, hMSH5 undergoes c-Abl dependent tyrosine phosphorylation, and consequently this posttranslational modification leads to the dissociation of hMSH4-hMSH5 hetero-complex (31). Since the formation of hMSH4-hMSH5 hetero-complex is required for interaction with GPS2 – possibly in complex with HDAC3, it is conceivable that tyrosine phosphorylation of hMSH5 could result in a dynamic transformation of the hMSH4-hMSH5 associated protein complex, which might be functionally required during the progression of DNA DSB repair.

Apparently, more work is needed for a better understanding of the mechanistic basis underlying the interplay between various hMSH4-hMSH5 interacting protein partners and the functional effects of dynamic reconfiguration of the hMSH4-hMSH5 complex on DNA DBS repair. In this regard, the existence of multiple hMSH4 and hMSH5 splicing variants and various non-synonymous SNPs will certainly pose a daunting task in the endeavor to gain an in-depth appreciation of their functions. For instance, the protein encoded by the splicing variant hMSH4sv is unable to interact with hMSH5, but it is capable of mediating interaction with VBP1 (29). Furthermore, the other known hMSH4 splicing variant, ΔhMSH4, is expected to be "defective" in homotypic interaction since it lacks the entire exon 6 that encodes part of the hMSH4 homodimerization domain (32, 34). On the contrary, the hMSH5 splicing variant (hMSH5sv) displayed equivalent capacity as hMSH5 to interact with hMSH4 (30). In addition, subtle effects of hMSH5 P29S on hMSH4 interaction and on c-Abl tyrosine kinase activation have also been explored recently (30, 31). Situated within the interacting domain for both hMSH4 and c-Abl, the P29S alteration causes a moderate reduction of protein interaction with hMSH4, and to some extent enhances c-Abl kinase activation (30, 31). Given the essential role of Msh5 in ovarian development in mice (21, 22), it is worthy of note that the *hMSH5* C85T (P29S) allele was enriched in a small ovarian cancer patient population (30). 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. Indeed, a recent study has suggested the co-segregation of two hMSH5 SNPs (L85F and P786S) on the same allele in humans (T. Behrens, personal

communication), and hMSH5 protein containing these two single amino acid alterations displays significantly compromised ability to interact with hMSH4 (C. Her and T. Behrens, unpublished observations), which is most likely due to the fact that these two alterations are located within the composite hMSH4-interacting domain on hMSH5 (30). It is expected that a highly coordinated action of hMSH4 and hMSH5 is pivotal for their function in the processes of both meiotic and mitotic DNA DSB repair and damage response. Therefore, a detailed understanding of diverse functional effects of hMSH4 and hMSH5 variants will provide a necessary foundation for the establishment of functional links between gene mutations, genetic instability, and subsequent cancer predisposition.

5. SUMMARY

Although the precise molecular mechanisms involved with MSH4 and MSH5 in the process of meiosis are completely undefined, these two MutS homologues are frequently referred to as meiosis-specific proteins based on the fact that there is no evidence to support their involvement in DNA mismatch repair. However, emerging experimental evidence in recent years has supported the idea that the human hMSH4 and hMSH5 proteins have evolved to possess additional functions beyond the scope of meiosis. This view is particularly supported by the observation that both hMSH4 and hMSH5 are expressed in non-meiotic tissues, in which hMSH5 displays a much broader tissue distribution pattern. In addition, most, if not all, of the identified hMSH4-hMSH5 interacting protein partners possess mitotic functions including DNA DSB repair and DNA damage response.

The functional disparity of various interacting proteins is consistent with the belief that hMSH4 and hMSH5 might be involved with multiple cellular processes through interactions with different protein partners. The seemingly ubiquitous expression profile of hMSH5 has also suggested that hMSH5 could function in the absence of hMSH4. Taken together, in addition to their important roles in meiotic homologous recombination, the current available experimental evidence has also implicated the involvement of hMSH4 and hMSH5 in the process of DNA DSB repair and cellular response to DNA damage. Having identified the major pieces of a puzzle, we are facing a great challenge in our attempt to define the exact temporal and spatial relationships of hMSH4-hMSH5 associated proteins and their corresponding meiotic and mitotic functions.

6. ACKNOWLEDGEMENTS

We want to acknowledge the support of the School of Molecular Biosciences of Washington State University, and to thank the editor and anonymous reviewers for valuable comments.

7. REFERENCES

1. Iyer R. R, A. Pluciennik, V. Burdett & P. L. Modrich: DNA mismatch repair: functions and mechanisms. *Chem Rev* 106, 302-323 (2006)

2. Buermeyer A. B, S. M. Deschenes, S. M. Baker & R. M. Liskay: Mammalian DNA mismatch repair. *Annu Rev Genet* 33, 533-564 (1999)

3. Harfe B. D & S. Jinks-Robertson: DNA mismatch repair and genetic instability. *Annu Rev Genet* 34, 359-399 (2000)

4. Fishel R, M. K. Lescoe, M. R. Rao, N. G. Copeland, N. A. Jenkins, J. Garber, M. Kane & R. Kolodner: The human mutator gene homolog MSH2 and its association with hereditary nonpolyposis colon cancer. *Cell* 75, 1027-1038 (1993) Erratum in: *Cell* 77:167 (1994)

5. Leach F. S, N. C. Nicolaides, N. Papadopoulos, B. Liu, J. Jen, R. Parsons, P. Peltomaki, P. Sistonen, L. A. Aaltonen, M. Nystrom-Lahti, X.-Y. Guan, J. Zhang, P. S. Meltzer, J.-W. Yu, F.-T. Kao, D. J. Chen, K. M. Cerosaletti, R. E. K. Fournier, S. Todd, T. Lewis, R. J. Leach, S. L. Naylor, J. Weissenbach, J.-P. Mecklin, H. Jarvinen, G. M. Petersen, S. R. Hamilton, J. Green, J. Jass, P. Watson, H. T. Lynch, J. M. Trent, A. de la Chapelle, K. W. Kinsler & B. Vogelstein: Mutations of a mutS homolog in hereditary nonpolyposis colorectal cancer. *Cell* 75, 1215-1225 (1993)

6. Peltomaki P, L. A. Aaltonen, P. Sistonen, L. Pylkkanen, J. P. Mecklin, H. Jarvinen, J. S. Green, J. R. Jass, J. L. Weber, F. S. Leach, G. M. Petersen, S. R. Hamilton, A. de la Chapelle & B. Vogelstein: Genetic mapping of a locus predisposing to human colorectal cancer. *Science* 260, 810-812 (1993)

7. Liu B, R. E. Parsons, S. R. Hamilton, G. M. Petersen, H. T. Lynch, P. Watson, S. Markowitz, J. K. Willson, J. Green, A. de la Chapelle, K. W. Kinzler & B. Vogelstein: hMSH2 mutations in hereditary nonpolyposis colorectal cancer kindreds. *Cancer Res* 54, 4590-4594 (1994)

8. Stojic L, R. Brun & J. Jiricny: Mismatch repair and DNA damage signalling. *DNA Repair* (Amst) 3, 1091-1101 (2004)

9. Kolas N. K & P. E. Cohen: Novel and diverse functions of the DNA mismatch repair family in mammalian meiosis and recombination. *Cytogenet Genome Res* 107, 216-231 (2004)

10. Hoffmann E. R & R. H. Borts: Meiotic recombination intermediates and mismatch repair proteins. *Cytogenet Genome Res* 107, 232-248 (2004)

11. Fujii H & T. Shimada: Isolation and characterization of cDNA clones derived from the divergently transcribed gene in the region upstream from the human dihydrofolate reductase gene. *J Biol Chem* 264, 10057-10064 (1989)

12. Palombo F, P. Gallinari, I. Iaccarino, T. Lettieri, M. Hughes, A. D'Arrigo, O. Truong, J. J. Hsuan & J. Jiricny: GTBP, a 160-kilodalton protein essential for mismatch-binding activity in human cells. *Science* 268, 1912-1914 (1995)

13. Palombo F, I. Iaccarino, E. Nakajima, M. Ikejima, T. Shimada & J. Jiricny: hMutSß, a heterodimer of hMSH2 and hMSH3, binds to insertion/deletion loops in DNA. *Curr Biol* 6, 1181-1184 (1996)

14. Acharya S, T. Wilson, S. Gradia, M. F. Kane, S. Guerrette, G. T. Marsischky, R. Kolodner & R. Fishel: hMSH2 forms specific mispair-binding complexes with hMSH3 and hMSH6. *Proc Natl Acad Sci U S A* 93, 13629-13634 (1996)

15. Genschel J, S. J. Littman, J. T. Drummond & P. Modrich: Isolation of MutSbeta from human cells and comparison of the mismatch repair specificities of MutSß and MutSα. *J Biol Chem* 273, 19895-19901 (1998) Erratum in: *J Biol Chem* 273, 27034 (1998)

16. Zhang Y, F. Yuan, S. R. Presnell, K. Tian, Y. Gao, A. E. Tomkinson, L. Gu & G.-M. Li: Reconstitution of 5'-directed human mismatch repair in a purified system. *Cell* 122, 693-705 (2005)

17. Ross-Macdonald P & G. S. Roeder: Mutation of a meiosis-specific MutS homolog decreases crossing over but not mismatch correction. *Cell* 79, 1069-1080 (1994)

18. Hollingsworth N. M, L. Ponte & C. Halsey: MSH5, a novel MutS homolog, facilitates meiotic reciprocal recombination between homologs in Saccharomyces cerevisiae but not mismatch repair. *Genes Dev* 9, 1728-1739 (1995)

19. Zalevsky J, A. J. MacQueen, J. B. Duffy, K. J. Kemphues & A. M. Villeneuve: Crossing over during Caenorhabditis elegans meiosis requires a conserved MutS-based pathway that is partially dispensable in budding yeast. *Genetics* 153, 1271-1283 (1999)

20. Kelly K. O, A. F. Dernburg, G. M. Stanfield & A. M. Villeneuve: Caenorhabditis elegans msh-5 is required for both normal and radiation-induced meiotic crossing over but not for completion of meiosis. *Genetics* 156, 617-630 (2000)

21. Edelmann W, P. E. Cohen, B. Kneitz, N. Winand, M. Lia, J. Heyer, R. Kolodner, J. W. Pollard & R. Kucherlapati: Mammalian MutS homologue 5 is required for chromosome pairing in meiosis. *Nat Genet* 21, 123-127 (1999)

22. de Vries S. S, E. B. Baart, M. Dekker, A. Siezen, D. G. de Rooij, P. de Boer & H. te Riele: Mouse MutS-like protein Msh5 is required for proper chromosome synapsis in male and female meiosis. *Genes Dev* 13, 523-531 (1999)

23. Kneitz B, P. E. Cohen, E. Avdievich, L. Zhu, M. F. Kane, H. Hou Jr, R. D. Kolodner, R. Kucherlapati, J. W. Pollard & W. Edelmann: MutS homolog 4 localization to meiotic chromosomes is required for chromosome pairing during meiosis in male and female mice. *Genes Dev* 14, 1085-1097 (2000)

24. Bawa S & W. Xiao: A mutation in the MSH5 gene results in alkylation tolerance. *Cancer Res* 57, 2715-2720 (1997)

25. Bawa S & W. Xiao: A single amino acid substitution in MSH5 results in DNA alkylation tolerance. *Gene* 315, 177-182 (2003)

26. Her C & N. A. Doggett: Cloning, structural characterization, and chromosomal localization of the human orthologue of Saccharomyces cerevisiae MSH5 gene. *Genomics* 52, 50-61 (1998)

27. Winand N. J, J. A. Panzer & R. D. Kolodner: Cloning and characterization of the human and Caenorhabditis elegans homologs of the Saccharomyces cerevisiae MSH5 gene. *Genomics* 53, 69-80 (1998)

28. Bocker T, A. Barusevicius, T. Snowden, D. Rasio, S. Guerrette, D. Robbins, C. Schmidt, J. Burczak, C. M. Croce, T. Copeland, A. J. Kovatich & R. Fishel: hMSH5: a human MutS homologue that forms a novel heterodimer with hMSH4 and is expressed during spermatogenesis. *Cancer Res* 59, 816-822 (1999)

29. Her C, X. Wu, M. D. Griswold & F. Zhou: Human MutS homologue MSH4 physically interacts with von Hippel-Lindau tumor suppressor-binding protein 1. *Cancer Res* 63, 865-872 (2003)

30. Yi W, X. Wu, T.-H. Lee, N. A. Doggett & C. Her: Two variants of MutS homolog hMSH5: prevalence in humans and effects on protein interaction. *Biochem Biophys Res Commun* 332, 524-532 (2005) Erratum in: *Biochem Biophys Res Commun* 340, 1018 (2005)

31. Yi W, T.-H. Lee, J. D. Tompkins, F. Zhu, X. Wu & C. Her: Physical and functional interaction between hMSH5 and c-Abl. *Cancer Res* 66, 151-158 (2006)

32. Lee T. H, W. Yi, M. D. Griswold, F. Zhu & C. Her: Formation of hMSH4-hMSH5 heterocomplex is a prerequisite for subsequent GPS2 recruitment. *DNA Repair* (Amst) 5, 32-42 (2006)

33. Paquis-Flucklinger V, S. Santucci-Darmanin, R. Paul, A. Saunieres, C. Turc-Carel & C. Desnuelle: Cloning and expression analysis of a meiosis-specific MutS homolog: the human MSH4 gene. *Genomics* 44, 188-194 (1997)

34. Santucci-Darmanin S, R. Paul, J. F. Michiels, A. Saunieres, C. Desnuelle & V. Paquis-Flucklinger: Alternative splicing of hMSH4: two isoforms in testis and abnormal transcripts in somatic tissues. *Mamm Genome* 10, 423-427 (1999)

35. Her C, X. Wu, W. Wan & N. A. Doggett: Identification and characterization of the mouse MutS homolog 5: Msh5. *Mamm Genome* 10, 1054-1061 (1999)

36. Her C, X. Wu, S. M. Bailey & N. A. Doggett: Mouse MutS homolog 4 is predominantly expressed in testis and

interacts with MutS homolog 5. *Mamm Genome* 12, 73-76 (2001)

37. Snowden T, S. Acharya, C. Butz, M. Berardini & R. Fishel: hMSH4-hMSH5 recognizes Holliday Junctions and forms a meiosis-specific sliding clamp that embraces homologous chromosomes. *Mol Cell* 15, 437-451 (2004)

38. Pochart P, Woltering D, Hollingsworth NM. Conserved properties between functionally distinct MutS homologs in yeast. J Biol Chem 272, 30345-30349 (1997)

39. Santucci-Darmanin S, D. Walpita, F. Lespinasse, C. Desnuelle, T. Ashley & V. Paquis-Flucklinger: MSH4 acts in conjunction with MLH1 during mammalian meiosis. *FASEB J* 14, 1539-1547 (2000)

40. Santucci-Darmanin S, S. Neyton, F. Lespinasse, A. Saunieres, P. Gaudray & V. Paquis-Flucklinger: The DNA mismatch-repair MLH3 protein interacts with MSH4 in meiotic cells, supporting a role for this MutL homolog in mammalian meiotic recombination. *Hum Mol Genet* 11, 1697-1706 (2002)

41. Lipkin S. M, V. Wang, R. Jacoby, S. Banerjee-Basu, A. D. Baxevanis, H. T. Lynch, R. M. Elliott & F. S. Collins: MLH3: a DNA mismatch repair gene associated with mammalian microsatellite instability. *Nat Genet* 24, 27-35 (2000)

42. Neyton S, F. Lespinasse, P. B. Moens, R. Paul, P. Gaudray, V. Paquis-Flucklinger & S. Santucci-Darmanin: Association between MSH4 (MutS homologue 4) and the DNA strand-exchange RAD51 and DMC1 proteins during mammalian meiosis. *Mol Hum Reprod* 10, 917-924 (2004)

43. Spain B. H, K. S. Bowdish, A. R. Pacal, S. F. Staub, D. Koo, C. Y. Chang, W. Xie & J. Colicelli: Two human cDNAs, including a homolog of Arabidopsis FUS6 (COP11), suppress G-protein- and mitogen-activated protein kinase-mediated signal transduction in yeast and mammalian cells. *Mol Cell Biol* 16, 6698-6706 (1996)

44. Jin D. Y, H. Teramoto, C. Z. Giam, R. F. Chun, J. S. Gutkind & K. T. Jeang: A human suppressor of c-Jun N-terminal kinase 1 activation by tumor necrosis factor alpha. *J Biol Chem* 272, 25816-25823 (1997)

45. Peng Y. C, F. Kuo, D. E. Breiding, Y. F. Wang, C. P. Mansur & E. J. Androphy: AMF1 (GPS2) modulates p53 transactivation. *Mol Cell Biol* 21, 5913-5924 (2001)

46. Zhang J, M. Kalkum, B. T. Chait & R. G. Roeder: The N-CoR-HDAC3 nuclear receptor corepressor complex inhibits the JNK pathway through the integral subunit GPS2. *Mol Cell* 9, 611-623 (2002)

47. Bird A. W, D. Y. Yu, M. G. Pray-Grant, Q. Qiu, K. E. Harmon, P. C. Megee, P. A. Grant, M. M. Smith & M. F. Christman: Acetylation of histone H4 by Esa1 is required for DNA double-strand break repair. *Nature* 419, 411-415 (2002)

48. Jazayeri A, A. D. McAinsh & S. P. Jackson: Saccharomyces cerevisiae Sin3p facilitates DNA double-strand break repair. *Proc Natl Acad Sci U S A* 101, 1644-1649 (2004)

49. Downs J. A, S. Allard, O. Jobin-Robitaille, A. Javaheri, A. Auger, N. Bouchard, S. J. Kron, S. P. Jackson, & J. Cote: Binding of chromatin-modifying activities to phosphorylated histone H2A at DNA damage sites. *Mol Cell* 16, 979-990 (2004)

50. Tamburini B. A & J. K. Tyler: Localized histone acetylation and deacetylation triggered by the homologous recombination pathway of double-strand DNA repair. *Mol Cell Biol* 25, 4903-4913 (2005)

51. Munshi A, J. F. Kurland, T. Nishikawa, T. Tanaka, M. L. Hobbs, S. L. Tucker, S. Ismail, C. Stevens & R. E. Meyn: Histone deacetylase inhibitors radiosensitize human melanoma cells by suppressing DNA repair activity. *Clin Cancer Res* 11, 4912-4922 (2005)

52. Moore J. D & J. E. Krebs: Histone modifications and DNA double-strand break repair. *Biochem Cell Biol* 82, 446-452 (2004)

53. Hartman H. B, J. Yu, T. Alenghat, T. Ishizuka & M. A. Lazar: The histone-binding code of nuclear receptor co-repressors matches the substrate specificity of histone deacetylase 3. *EMBO Rep* 6, 445-451 (2005)

Key Words: MSH4, MSH5, c-Abl, GPS2, HDAC3, recombination, DSB repair, MMR, Review

Send correspondence to: Dr Chengtao Her, School of Molecular Biosciences, PO Box 644660, Washington State University, Pullman, WA 99164-4660, Tel: 509 335-7537, Fax: 509 335-9688, E-mail: cher@wsu.edu

http://www.bioscience.org/current/vol12.htm