

Novel and diverse functions of the DNA mismatch repair family in mammalian meiosis and recombination

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Abstract. The mismatch repair (MMR) family is a highly conserved group of proteins that function in genome stabilization and mutation avoidance. Their role has been particularly well studied in the context of DNA repair following replication errors, and disruption of these processes results in characteristic microsatellite instability, repair defects and, in mammals, susceptibility to cancer. An additional role in meiotic recombination has been described for several family members, as revealed by extensive studies in yeast. More recently, the role of the mammalian MMR family in meiotic progression has been elucidated by the phenotypic analysis of mice harboring tar-

geted mutations in the genes encoding several MMR family members. This review will discuss the phenotypes of the various mutant mouse lines and, drawing from our knowledge of MMR function in yeast meiosis and in somatic cell repair, will attempt to elucidate the significance of MMR activity in mouse germ cells. These studies highlight the importance of comparative analysis of MMR orthologs across species, and also underscore distinct sexually dimorphic characteristics of mammalian recombination and meiosis.

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Meiotic recombination – reciprocal and non-reciprocal

In sexually reproducing organisms meiosis is the cellular event during which one replicative S-phase is followed by two successive divisions to produce gametes with haploid numbers of chromosomes. The success of the meiotic program is dependent on prophase I events, characterized in most cases by homologous chromosome pairing and synapsis to form a proteinaceous synaptonemal complex (SC), and recombination between homologous, non-sister, chromatids (Champion and Hawley, 2002).

Each recombination event is initiated by the formation of a double strand break (DSB) in one sister chromatid (Keeney et

al., 1997). Meiotic DNA double-strand breaks occur early in prophase I and are unlike those occurring due to accidental damage or replication machinery slippage in mitotic cells. Instead, meiotic breaks are induced and propagated by expression of key genes, and can only be repaired/resolved once they effect homolog recognition, synapsis and, under appropriate circumstances, homologous recombination (Szostak et al., 1983; Baudat et al., 2000; Champion and Hawley, 2002). Two types of homologous recombination occur at meiosis; one is reciprocal recombination, characterized by an exchange of markers flanking a section of hybrid DNA, resulting in a crossover between the two non-sister strands involved; the second is non-reciprocal, whereby only hybrid DNA results (Fogel and Hurst, 1967; Hurst et al., 1972; Szostak et al., 1983).

In humans the level of divergence between coding regions of homologous chromosomes is approximately 0.05 % (<1 bp in every 1000 bp), while congenic mouse strains are approximately 0.02% diverged (Cargill et al., 1999). Hybrid (or heteroduplex) DNA in meiotic recombination intermediates can span more than 1 kb, and any divergent sequences or mismatches present in these spans are substrates for mismatch repair (MMR). At the same time, MMR processes also serve to limit

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recombination between sequences that are too highly (>1%) divergent, so-called homeologous recombination. At recombination intermediates, MMR can result in gene conversion-type or restoration-type mismatch repair of hybrid DNA, presumably with equal probability, such that 50% of reciprocal events are accompanied by a gene conversion (Bishop et al., 1987; Willis and Klein, 1987). However, it may be that a strand-selection mechanism exists that favors the use of the continuous (non-broken) strand as template (Foss et al., 1999). When repair of these mismatches is defective the products of meiosis have an increase in post-meiotic segregation (PMS), a term defined by fungal mutants to describe the observation of sectorized spore colonies during tetrad analysis. In this review "crossover" and "reciprocal recombination" will be used to discuss those outcomes that have reciprocal exchange with hybrid DNA, and "gene conversion" will be used to refer to non-reciprocal recombination (hybrid DNA without a reciprocal exchange event).

The canonical mismatch repair system in E. coli

The mismatch repair (MMR) machinery recognizes and repairs disruptions in the Watson-Crick basepairing of a DNA double helix. The *Escherichia coli* MMR system is the paradigm, and mutants (MutS, MutL, MutH and MutU) were named for a so-called "mutator" phenotype. MutS protein acts as a homodimeric ATPase that binds the DNA phosphate-sugar backbone directly. This mismatch, or "kinked" backbone, detection stimulates ADP to ATP exchange and induces a molecular switch, resulting in conformational changes in both the protein and the DNA (Obmolova et al., 2000). Recent evidence from Acharya et al. (2003) has demonstrated that this change also involves the formation of a stable sliding clamp that is capable of motion along as much as 1 kb of DNA adjacent to the mismatch, a process which then allows for the subsequent loading of multiple MutS sliding clamps (Acharya et al., 2003). Exchange of ADP for ATP on MutS also signals and recruits a MutL homodimer, and the MutS/MutL complex undergoes hydrolysis-dependent translocation similar to that seen with the MutS sliding clamp alone (Acharya et al., 2003), possibly toward the MutH endonuclease which is stimulated by MutL to cut the unmethylated newly synthesized strand. MutL appears to facilitate the unloading of MutS sliding clamps, and might additionally induce loading of MutU (Helicase II/UvrD) at the site of the nick to induce unwinding of the nascent strand (Yamaguchi et al., 1998). Four single-strand DNA exonucleases, RecJ, ExoVII, ExoI or ExoX, are able to remove the mispair in either a 5' to 3' or 3' to 5' direction, depending on the side of the MutH-induced nick. Their functions appear to be redundant, or they may compensate for one another, as MMR is only disrupted when all four are missing (Feschenko et al., 2003). Excised DNA is subsequently resynthesized by DNA polymerase III and religated via an as yet unidentified ligase (Modrich and Lahue, 1996; Buermeier et al., 1999).

Typically, MMR targets mismatches that occur as a result of replication errors in mitotic cells, but MMR also prevents homeologous recombination (recombination between sequences that are diverged by more than 10–20%) by way of base-pair mismatch rejection. Interestingly, small insertion/deletion

loops (IDLs) between mispaired bases are not the only targets recognized by MMR proteins. Others include cisplatin-induced cross-links, and damage from oxidation and alkylating agents. Moreover, eukaryotic homologs of MutS and MutL interact with other repair pathways such as transcription coupled base-excision repair and nucleotide excision repair (Tsutakawa and Cooper, 2000). Thus, while MutS and MutL have essential roles in the subsequent removal and repair synthesis of DNA, they function primarily in recognition of strand anomalies, DNA binding, conformational changes, and signaling to downstream effectors. As such, the generalized term "mismatch repair" can be considered somewhat of a misnomer.

MMR family of proteins are highly conserved

Homologs of the bacterial MutS and MutL proteins have been conserved through evolution, being identified in fungi, mice, humans, plants, worms and flies (Table 1). The importance of this gene family in maintaining genomic stability has been underscored by the observation that mutations in several family members are associated with human non-polyposis colorectal cancer (HNPCC) (Lynch, 1999; Pedroni et al., 2001; Wei et al., 2002). In addition, spontaneous mutations in these genes are found in 2–13% of spontaneously arising colorectal cancer cases. In most cases, such tumors are characterized by microsatellite instability, which results from an inability to repair replication errors at sites of long simple mono-, di-, and trinucleotide repeats.

Both MutS and MutL homolog proteins act primarily as heterodimers and have DNA binding domains, though MutL heterodimers are thought to bind MutS heterodimers which are bound to DNA. Mice and humans have five MutS homologs, MSH2-6, and four MutL homologs, MLH1, MLH3, PMS2 (for post-meiotic segregation, orthologous to yeast PMS1) and PMS1 (orthologous to yeast MLH2). The MutS homolog complexes that are most recognized to act in mammalian somatic cell repair are MSH2-MSH6 (MutS α) and MSH2-MSH3 (MutS β), while a third MutS heterodimer (MutS γ) of MSH4-MSH5 has a predominant function in meiotic cells (Paquis-Flucklinger et al., 1997; Her and Doggett, 1998; Bocker et al., 1999; Edelmann et al., 1999; Her et al., 1999, 2001; Kneitz et al., 2000; Cohen and Pollard, 2001). Interestingly, recent evidence indicates that MSH2 can form a damage signaling module with ATR and, via MSH6, they regulate phosphorylation of CHK1 and SMC1 in the face of MNNG chemical damage (Wang and Qin, 2003). Furthermore, MMR family members affect somatic cell hypermutation and class switch recombination of immunoglobulin genes (Martin et al., 2003; Bardwell et al., 2004). MLH1 is the primarily utilized MutL homolog, heterodimerizing with PMS2 (MutL α), PMS1 (MutL β) or MLH3 (MutL γ), this last heterodimer functioning primarily in meiotic events (Fig. 1). MutL α appears to be the primary MutL heterodimer effecting MMR, both in mice and in yeast. Thus, the majority of HNPCC cases are a result of mutations in MSH2 and MLH1, while non-classical (those that do not conform to the Amsterdam criteria for establishing hereditary colorectal cancer risk) HNPCC cases have been associated with mutations in MSH6 (Lucci-Cordisco et al., 2001; Pedroni et al., 2001).

Table 1. MUTS, MUTL and EXO1 homologs in eukaryotic species

<i>Escherichia coli</i> (4.6 MB) ^a	<i>Homo sapiens</i> (3000 MB) ^a	<i>Mus musculus</i> (2500 MB) ^a	<i>Drosophila melanogaster</i> (180 MB) ^a	<i>Arabidopsis thaliana</i> (125 MB) ^a	<i>Gallus gallus</i> (120 MB) ^a	<i>Caenorhabditis elegans</i> (100 MB) ^a	<i>Coprinus cinereus</i> (37.5 MB) ^a	<i>Saccharomyces cerevisiae</i> (15 MB) ^a	<i>Schizosaccharomyces pombe</i> (13.8 MB) ^a
MutS	NID ^b Msh2 Ref 3 Msh3 Ref 10 Msh4 Ref 15 Msh5 Ref 19 Msh6 NID	NID Msh2 Ref 4 Msh3 Ref 11 Msh4 Ref 16 Msh5 Ref 20 Msh6 Ref 23 NID	NID Spel1 Ref 5 NID NID NID Msh6 Ref 24 NID	Msh1 Ref 1 ^d Msh2 Ref 6 Msh3 Ref 12 NID NID Msh6 Ref 25 Msh7 Ref 29	NID Msh2 (AB104853) NID NID NID Msh6 (AI981357) NID	NID Msh2 Ref 7 NID Him-14 Ref 17 Msh5 Ref 21 Msh6 Ref 26 NID	NID NID NID Msh5 (AY334360) NID NID	Msh1 Ref 2 Msh2 Ref 8 Msh3 Ref 13 Msh4 Ref 18 Msh5 Ref 22 Msh6 Ref 27 NID	Msh1 (NP594677) ^c Msh2 Ref 9 Swi4 Ref 14 NID NID Msh6 Ref 28 NID
MutL	Pms2 Ref 30 Mlh1 Ref 34 Pms1 Ref 39 Mlh3 Ref 41	Pms2 Ref 31 Mlh1 Ref 35 Pms1 Ref 40 Mlh3 Ref 42	NID NID NID NID	Pms2 (NP_567236) Mlh1 Ref 35 NID Mlh3 Ref 43	Pms2 (CK61433) Mlh1 Ref 36 Pms1 (CB018436) NID	Pms2 (CAA18355) Mlh1 (CAB07283) NID Ced H12 Ref 43	NID NID NID NID	Pms1 Ref 32 Mlh1 Ref 37 Mlh2 Mlh3 Ref 44	Pms1 Ref 33 Mlh1 Ref 38 NID NID
MMR exo-nucleases	Exo1 Ref 45	Exo1 Ref 46	Tos Ref 47	NID	NID	NID	NID	Exo1 Ref 48	Exo1 Ref 49

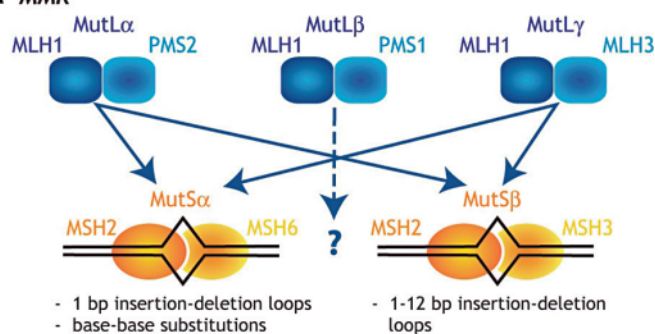
^a Haploid genome size (MB = megabasepairs).

^b NID: Not identified.

^c Numbers in parentheses represent NCBI accession numbers.

^d References: 1 Abdelnoor et al. 2003; 2 Hunter and Borts 1997; 3 Martin et al. 2000; 4 de Wind et al. 1995; Reitmair et al. 1995; 5 Flores and Engels 1999; 6 Ade et al. 1999, 2001; Leonard et al. 2003; 7 Degtyareva et al. 2002; Tijsterman et al. 2002; 8 Chambers et al. 1996; Pochart et al. 1997; 9 Rudolph et al. 1999; 10 Watanabe et al. 1996; 11 Wei et al. 2002; 12 Ade et al. 1999; 13 New et al. 1993; 14 Tornier et al. 2001; 15 Paquis-Flucklinger et al. 1997; 16 Kneitz et al. 2000; 17 Zalevsky et al. 1999; Zetka and Rose 1995; 18 Ross-Macdonald and Roeder 1994; 19 Her and Doggett 1998; 20 Edelmann et al. 1999; 21 Kelly et al. 2000; 22 Hollingsworth et al. 1995; 23 Edelmann et al. 1997; 24 Harfe and Jinks-Robertson 2000; 25 Ade et al. 1999; 26 Tijsterman et al. 2002; 27 Iaccarino et al. 1996; 28 Tornier et al. 2001; 29 Ade et al. 1999; Culligan and Hays 2000; Dong et al. 2002; 30 Horii et al. 1994; 31 Baker et al. 1995; 32 Prolla et al. 1994; Schar et al. 1997; 33 Schar et al. 1997; 34 Tomer et al. 2002; 35 Baker et al. 1996; Edelmann et al. 1996; 35 Jean et al. 1999; 36 Pigozzi 2001; 37 Hoffmann et al. 2003; Hunter and Borts 1997; Prolla et al. 1994; Wang et al. 1999; 38 Marti et al. 2003; 39 Leung et al. 2000; 40 Prolla et al. 1998; 41 Lipkin et al. 2001; 42 Lipkin et al. 2002; 43 Harfe and Jinks-Robertson 2000; 44 Wang and Kung 2002; 45 Qiu et al. 1999; Schmutte et al. 2001; 46 Wei et al. 2003; 47 Digilio et al. 1996; 48 Kirkpatrick et al. 2000; Tsubouchi and Ogawa 2000; 49 Rudolph et al. 1998; Szankasi and Smith 1995.

A MMR



B Meiosis

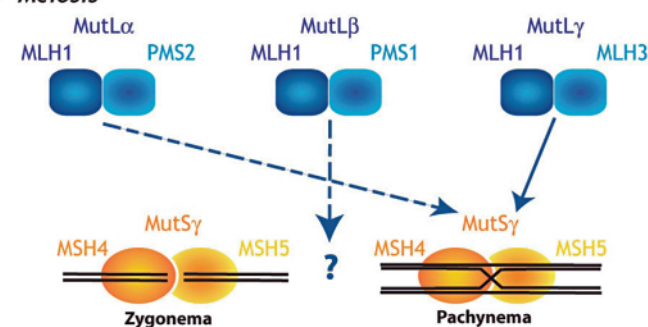


Fig. 1. Cartoon of mammalian mismatch repair complexes in repair (A) and meiosis (B). (A) Mammalian somatic cell mismatch repair. Current biochemical and genetic evidence indicates that MLH1 is the pivotal MutL homolog. MLH1 functions as a heterodimer, binding with MutL homologs PMS1, PMS2, and MLH3, which subsequently bind to MutS heterodimers attached to aberrant DNA. This MutS/MutL complex signals downstream effectors to remove and repair lesions such as 1-bp insertion-deletion loops, base-base substitutions and 1–12 bp insertion-deletion loops (MutLα and MutLγ). The function of MutLβ complex is still poorly understood. (B) MutL and MutS homologs involved in mammalian meiosis. MSH4 and MSH5 appear to be predominantly employed during meiosis, possibly recruiting MLH1-MLH3 heterodimers. During meiosis, DNA joint molecules are formed by the induction of double-strand DNA breaks and their subsequent repair. At this time heteroduplex DNA is created, which may contain mismatched DNA that are substrates for repair via the MMR pathway. However, substrates for MutS homolog DNA binding, such as double Holliday junctions, may result in ADP-ATP exchange, thereby signaling downstream events in recombination.

Table 1 (continued)

<i>E. coli</i> (4.6 MB)		Meiotically active heterodimers	Putative meiotic function
MutS	(Msh1)	NID	Maintenance of mitochondrial genome integrity.
	(Msh2)	MSH2-MSH3 and MSH2-MSH6	No known role in mammalian meiosis, both sexes of mutant mice are fertile, and have apparently normal meioses (de Wind et al. 1995). However MSH2 may act to suppress homeologous recombination (Chambers et al. 1996). MMR of meiotic heteroduplex DNA in <i>S. cerevisiae</i> . MSH2 and MSH2-MSH6 heterodimer bind Holliday junctions in vitro (Alani et al. 1997; Marsischky et al. 1999). Heterodimer of MSH2/MSH3 recognizes heteroduplex loops, mismatched bp and branched DNA structures with a free 3' tail, and acts to recruit RAD1/RAD10 in <i>S. cerevisiae</i> (Sugawara et al. 1997). MSH2 supresses homeologous recombination during meiosis.
	(Msh3)	MSH2-MSH3	see Msh2. <i>Msh3</i> ^{-/-} mice are fertile with no apparent meiosis defects (Wei et al. 2002).
	(Msh4)	MSH4-MSH5	Heterodimerizes with Msh5 but has no repair function. Male and female mice with targeted mutation of Msh4 are sterile (Kneitz et al. 2000). MSH4 colocalizes with RPA (Moens et al. 2002) and MLH1 (Santucci-Darmanin et al. 2000). May function in an interference regulated meiotic recombination pathway. In <i>S. cerevisiae</i> MSH4 affects the distribution and frequency of homologous crossing-over (Khazanehdari and Borts, 2000; Novak et al. 2001; Pochart et al. 1997; Ross-Macdonald and Roeder 1994).
	(Msh5)	MSH4-MSH5	MSH5 facilitates crossing over with Msh4 in <i>S. cerevisiae</i> (Hollingsworth et al. 1995). Male and female mice are sterile due to a block prior to, or due to inhibition of, synapsis prior to pachytene (de Vries et al. 1999; Edelman et al. 1999).
	(Msh6)	MSH2-MSH6	Though MSH2-MSH6 binds Holliday junctions in vitro, MSH6 appears to have no meiotic phenotype and mice have apparently normal fertility (Wei et al. 2002).
	(Msh7)	NID	No apparent meiotic role.
MutL	(Pms1/2)	MLH1-PMS2 (MLH1- PMS1 in yeast)	Corrects mismatches occurring in meiotic heteroduplex DNA in <i>S. cerevisiae</i> (Wang et al. 1999). Female mice are fertile and males are infertile. A portion of male SCs exhibit mispairing, sperm head formation is defective and no mature sperm are competent for fertilization (Baker et al. 1995). Mice with PMS2 and MSH2 deficiency have normal levels of recombination, indicating that homeologous recombination is not increased, or not affected, in these backgrounds (Qin et al. 2002).
	(Mlh1)	MLH1-PMS2 (PMS1 yeast) MLH1- MLH3 MLH1- MLH2	Heterodimerizes with Mlh3 and localizes to sites of reciprocal recombination (Lipkin et al. 2002) and promotes crossing over in <i>S. cerevisiae</i> (Hunter and Borts 1997). In <i>S. cerevisiae</i> MLH1-PMS1 corrects heteroduplex mismatches (Prolla et al. 1994) and MLH1-MSH2 promotes Mendelian segregation but does not act in mismatch correction, nor crossing over (Wang et al. 1999).
	(Pms2/Mlh2)	NID	Mice with a targeted mutation of Pms1 have apparently normal meiosis (Prolla et al. 1998).
	(Mlh3)	MLH1- MLH3	Mice with an Mlh3 targeted mutation are infertile, and mice completely lack chiasmata (Lipkin et al. 2002). Thus, MLH3 affects reciprocal recombination but has weak, if any, repair activity. MLH3 interacts with MSH4 biochemically (Santucci-Darmanin et al. 2002) and with MLH1 (Lipkin et al. 2002).
MMR Exonucleases (Exo1)	NID	5' to 3' exonuclease, also affects 3' to 5' repair in mice, and both male and female mice are sterile (Wei et al. 2003). Exo1 <i>S. cerevisiae</i> mutants have increased MI non-disjunction, indicating that Exo1 may promote crossing over (Kirkpatrick et al. 2000). In <i>S. pombe</i> Exo1 acts to repair heteroduplex DNA with MSH2 and PMS1.	

Like the *E. coli* MutL homodimer, the eukaryotic MutL homolog heterodimers are thought to act as an adaptor complex, linking the MMR system to downstream effectors. In the case of true MMR events, these downstream events involve the DNA excision and repair enzymes (exonucleases, polymerases, ligases, etc.) responsible for removing and replacing the aberrant nucleotide tract. Although MMR specific endonucleases, helicases, and ligases have not been identified, one MMR 5' to 3' exonuclease, EXO1, has been found to affect both 5' and 3' repair in mice (Wei et al., 2003) and DNA polymerase δ appears to be involved in repair synthesis. In addition, the MutL heterodimer also links MMR events to the cell cycle and

checkpoint machinery and is known to induce the expression of pro-apoptotic factors in the face of irreparable damage (Zhang et al., 1999; Schofield and Hsieh, 2003). Thus, MutS and MutL homolog family members have been repeatedly employed as DNA-binding and signaling molecules outside of the typical mismatch repair pathway.

In budding yeast, *Saccharomyces cerevisiae*, MMR family members have been found to be necessary to repair mismatches in meiotic hybrid DNA. Yeast *msh2* and *pms1* mutants exhibit increased post-meiotic segregation without apparent defects in reciprocal recombination. Conversely, MMR family members such as MLH3, MSH4 and MSH5 do not repair hybrid DNA at

meiosis, and instead affect reciprocal recombination and/or interference (Borts et al., 2000; Schofield and Hsieh, 2003).

Although much is known about the meiotic function of MMR protein family members in yeast, studies in mammals are confounded by greater genome complexity and size, more DSBs than eventual recombination products, and difficulty in isolating recombination intermediates and all products of a single meiosis. However, immunocytology and molecular genetic approaches in mice have provided much insight into the roles of these proteins in mammals. This review will outline some of the recent advances in mammalian meiotic MMR studies using various targeted mutations made through homologous recombination. Together with the more complete biochemical evidence from yeast and other organisms, we hope to add insight to the mechanisms involved in meiotic progression in mammals. In particular, this review seeks to address the complexity of MMR interactions within the context of recombination, with comparison to the canonical role of MMR proteins in repair of mismatches. In addition, the review will compare and contrast MMR-driven events across species as a means to identify the roles of the MMR proteins in adapting to increased genome size in mammalian species.

Mammalian MMR proteins and homologous recombination

MSH4 and MSH5

Much of our knowledge of the biochemical function of MSH4-MSH5 heterodimers has arisen as a result of studies in budding yeast. *S. cerevisiae* Msh4 and Msh5 proteins have been shown to form a hetero-oligomeric complex (Pochart et al., 1997) and both *msh4* and *msh5* mutants have wild-type levels of gene conversion and postmeiotic segregation, indicating that MutS γ does not normally participate in meiotic MMR per se. Yeast recombination intermediates can be physically observed, and strand exchange occurs at wild-type levels yet crossing over is reduced by about 40 to 50% in *msh4/5* mutants, with a resultant increase in non-disjunction and decreased spore viability (Ross-Macdonald and Roeder, 1994; Hollingsworth et al., 1995). Although deleting yeast *Msh4* does not prevent meiotic progression at zygotene, as it does in mice, synapsis and SC formation are delayed, and interference is disrupted for a subset of crossovers, in a manner epistatic with the synaptonemal complex-encoding genes, *Zip1* and *Zip2* (Novak et al., 2001). The term interference refers to the process by which the distance between neighboring reciprocal recombination events is regulated, such that for positive interference the formation of one crossover site will reduce the probability of a second forming in its vicinity. In this way, crossovers are distributed according to SC and chromosome length, rather than at random, with each SC having at least one. The defect in interference in *msh4* yeast could be due to delayed synapsis and SC formation, or a failure to load Msh4 (*zip1* and *zip2* mutants have delayed Msh4 localization). Interestingly, *Schizosaccharomyces pombe* does not have a synaptonemal complex structure nor Msh4/5 orthologs, and subsequently no crossover interference (De Los Santos et al., 2003). There is indication that the

S. cerevisiae Msh4-Msh5 heterodimer biases the resolution of Holliday junctions towards reciprocal recombination, and that they mediate interference. However, as yet, neither yeast Msh4 nor Msh5 have been shown to bind Holliday junctions in vitro, while yeast Msh2-Msh6 have (Alani et al., 1997; Marsischky et al., 1999), leading to the suggestion that Msh4-Msh5 heterodimers function prior to the appearance of these structures (Schofield and Hsieh, 2003). Recent studies have demonstrated that while human MSH4-MSH5 does not bind other types of DNA structures, it does bind Holliday junctions in vitro. This binding stimulates ATPase-dependent sliding clamp activity. Such activation does not occur when MSH2-MSH6 binds to these structures (R. Fishel, personal communication).

Interestingly, a point mutation in yeast Msh5 results in DNA alkylation tolerance (Bawa and Xiao, 2003). Msh4 and Msh5 are considered meiosis-specific MutS homologs, partly since they do not function in mismatch repair, as detected by microsatellite instability, but also their mRNA expression level is low in tissues other than testis (Paquis-Flucklinger et al., 1997). However, there is some indication that Msh4 is expressed at low levels in many other mouse tissues (Her et al., 2001). Whether this mRNA is degraded or translated into protein is unknown. These findings suggest that MSH4 and MSH5 may have a role in detection of DNA damage and cell death in somatic cells, a pathway that may be facilitated by numerous DSBs, induced by the mismatch repair system, that subsequently lead to cell death. Similar to the meiotic requirement for these gene products in mice and yeast, the nematode MSH4 ortholog, encoded by the *him-14* gene, appears to be essential for crossing-over. However, like yeast, pairing and synapsis still proceeds in *him-14* mutants (Zalevsky et al., 1999). Likewise, *Caenorhabditis elegans* MSH5 is essential for crossing-over and chiasma formation and/or maintenance (Kelly et al., 2000).

Mammalian homologs of Msh4 and Msh5 have been identified in mice and humans, and both are most highly expressed in meiotic cells (Wijnen et al., 1996; Paquis-Flucklinger et al., 1997; Her and Doggett, 1998; Her et al., 1999). Both MSH4 and MSH5 proteins have the conserved MutS C-terminal ADP binding motif and protein-protein interaction domain, yet there is some divergence of N-terminal DNA binding residues that may confer alternate binding specificity or function. Amino acids known to be required for mismatch recognition are largely absent in both MSH4 and MSH5, which correlates with these proteins being functionally inactive in post-replicative mismatch repair. Interestingly, structure prediction indicates that an MSH4/5 heterodimer likely contains a hole approximately 30 by 70 Å that would be large enough for a recombination intermediate to slide through as an MSH4/5 sliding clamp proceeds (Obmolova et al., 2000).

Protein-protein interaction studies of mouse and human proteins expressed in cultured cells indicate that MSH4 and MSH5 dimerize via their carboxy-termini (Winand et al., 1998; Bocker et al., 1999; Her et al., 1999, 2001). Furthermore, MSH4 and MSH5 both co-immunoprecipitate with RAD51 in mouse spermatocyte extracts, and also with MLH1, while RAD51 and MLH1 fail to interact biochemically (P.E. Cohen, unpublished observations). These studies, along with immunolocalization data, indicate that MSH4-MSH5 appear on inter-

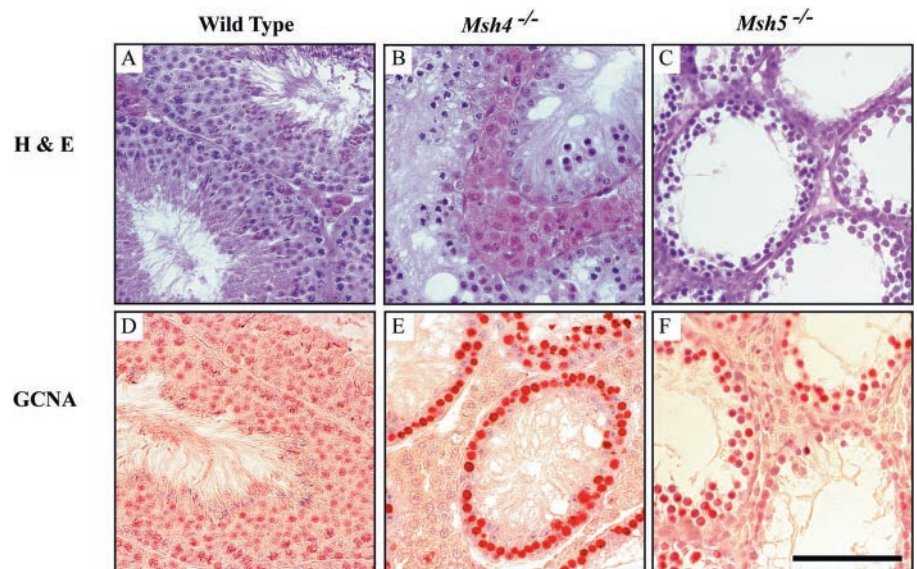


Fig. 2. Immunohistochemistry of post-pubertal testes from wild-type mice (**A, D**), and from mice with targeted deletions of the MutS homologs, *Msh4* (**B, E**) and *Msh5* (**C, F**). (**A**) Hematoxylin and Eosin (H&E) staining of a testis section from a wild-type C57BL/6J mouse. The testis-tubules have an outer layer of Sertoli cells, types A and B spermatogonia, primary and secondary spermatocytes and developing spermatozoa and spermatids in the center. (**B**) H&E staining of testis sections from a mouse with targeted deletion of *Msh4*. These mice exhibit abnormal chromosomal synapsis and germ cell development arrests during prophase I. As such, these mice lack secondary spermatocytes and later developing cells, and are infertile. The tubules contain vacuous spaces that are outgrowths of Sertoli cells (Sertoli-cell bodies). Leydig cell hypertrophy is evident in both mutant mouse strains. (**C**) H&E staining of *Msh5* deleted mouse testis. Testicular morphology and

spermatocyte development are identical to *Msh4* mice, and these animals are also infertile. (**D**) Germ cell nuclear antigen-1 (GCNA1) labeling of testis sections from a wild-type C57BL/6 mouse. GCNA1 recognizes spermatogonial cells and primary spermatocytes, until the diplotene stage, and gradually depletes until there is none detected in elongating spermatids. (**E**) GCNA1 staining of testis from a mouse with a targeted *Msh4* deletion. GCNA1 indicates that testes from these mice have apparently normal numbers of Sertoli cells and spermatogonia, however apoptosis increases during prophase I and there is a shortage of primary spermatocytes. The testes are devoid of spermatids and spermatozoa, and both males and females are sterile. (**F**) GCNA1 staining of testis from a mouse with a targeted deletion of *Msh5*. The meiotic phenotype and testicular morphology is identical to that for *Msh4*, and both males and females are sterile. Bar = 100 μ m.

mediate meiotic nodules (MN) in zygonema of prophase I, and persist through until late pachynema or early diplonema. Specifically, the number of MSH4 foci on spermatocyte chromosome cores during zygonema is approximately 142 per nucleus (Kneitz et al., 2000), roughly half that seen for RAD51 foci at the same stage (Plug et al., 1996), and these MSH4-positive foci steadily decline through late zygonema and into pachynema (Kneitz et al., 2000; Moens et al., 2002). By the mid-pachytene stage, the number of MSH4 foci is approximately 47 per nucleus, roughly double the number of MLH1 foci that are seen at this stage (Anderson et al., 1999; Kneitz et al., 2000).

Immunolocalization of MSH4 indicates that it colocalizes with the single-strand binding protein, replication protein A (Moens et al., 2002). RPA is thought to mediate homology search and strand exchange by binding single stranded DNA with RAD51 and DMC1 (Ashley et al., 1995; Moens et al., 2002). However, RPA single-strand binding activity has also been implicated with a role in the MMR pathway (Lin et al., 1998; Ramilo et al., 2002), and it is unclear if all RPA foci at prophase I mediate the same recombination/resolution pathway at all MNs with which they associate. RPA localizes as discrete foci to the SC, starting at mid-leptotene, that increase to more than 200 foci until mid-zygotene, and decline to zero by mid to late pachytene, and associates with MSH4 at this time. However, unlike RPA, MSH4 localization persists into the

beginning of the desynaptic diplotene stage (Kneitz et al., 2000; Santucci-Darmanin et al., 2000), and the functional implication of its interaction with RPA remains uncertain.

Male and female mice bearing targeted deletions of either *Msh4* or *Msh5* are sterile due to a lack of germ cells caused by defective meiosis (Figs. 2 and 3). In males, meiotic disruption occurs prior to, or prevents, complete synapsis (Fig. 4). However, these mice do not have a microsatellite instability phenotype as expected for a mismatch repair deficiency, there is no apparent increase in cancer incidence, and they are proficient in mismatch repair (de Vries et al., 1999; Edelman et al., 1999; Kneitz et al., 2000). The similar phenotypes that result from deletion of either protein indicate that they interact together or in the same pathway during recombination and/or synapsis, and is supported by biochemical evidence of their interactions in mammalian germ cell extracts (P.E. Cohen, unpublished observations). During meiosis, spermatocytes from *Msh4*^{-/-} or *Msh5*^{-/-} male mice show apparent complete accumulation of axial element structures along homologous chromosomes, but this does not ensure successful pairing and synapsis. In some spermatocytes of these mutant animals RAD51 hyperlocalizes to chromosome cores, indicating either a failure of DSB resolution, or increased DSB formation that leads to apoptosis. By pachynema, when WT chromosomes show complete synapsis of

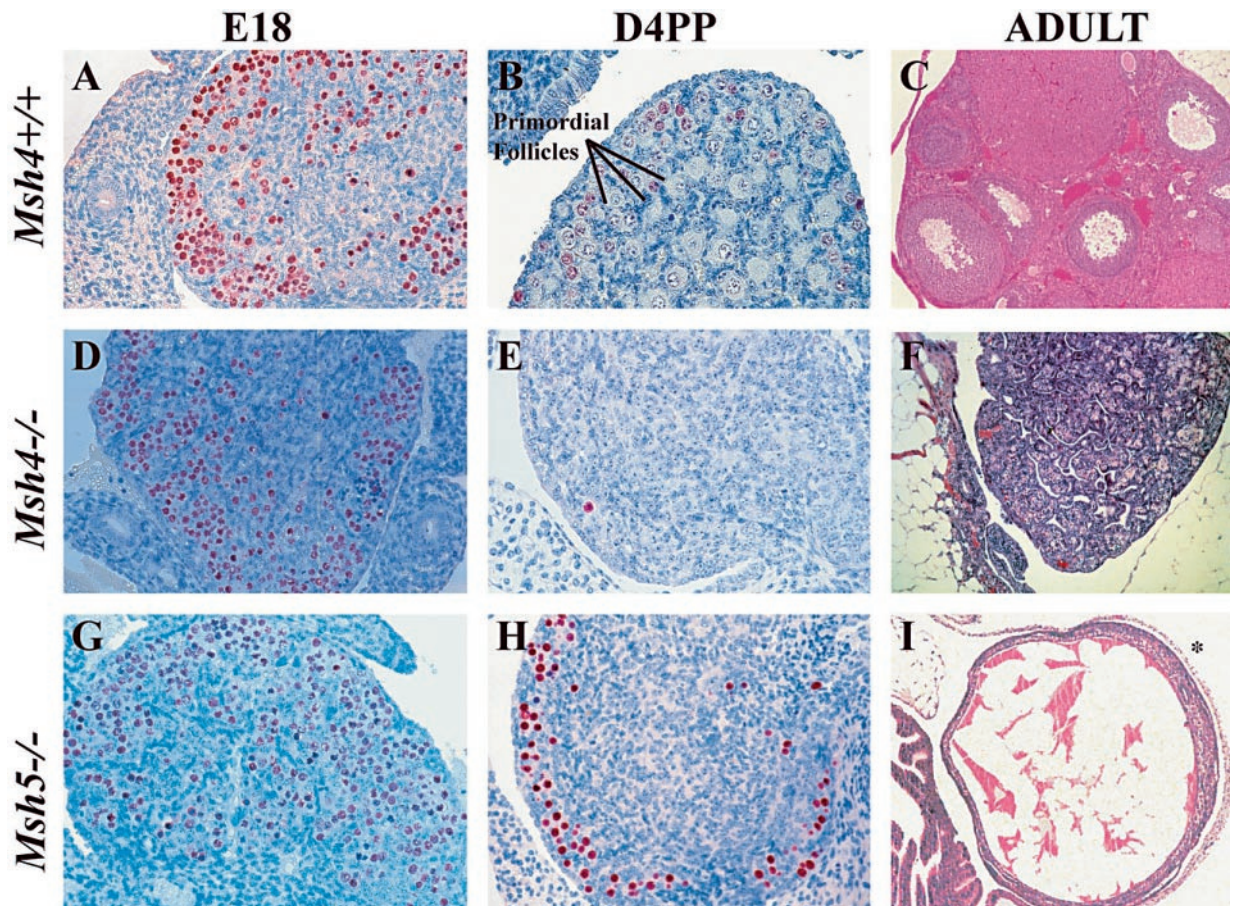


Fig. 3. Immunohistochemistry of ovaries from mice at day 18 of gestation (E18), day 4 post-partum (D4PP) and post-puberty (ADULT) from wild-type C57BL/6J mice (**A, B, C**), and from mice with targeted deletions of the MutS homologs, *Msh4* (**D, E, F**) and *Msh5* (**G, H, I**). (**A**) GCNA1 staining of ovary sections from wild-type *Msh4*^{+/+} mice on a C57BL/6J background indicates that at day 18 of gestation the ovaries have large numbers of prophase I and interstitial cells. (**B**) GCNA1 staining of D4PP ovary sections indicates that the number of prophase I cells declines as oocytes progress into dictyate arrest, and primordial follicles start to form. (**C**) H&E staining of adult ovary sections showing normally developing pre-antral and antral follicles. (**D**) GCNA1 staining of E18 ovary sections from an *Msh4*^{-/-} fetus. At this early stage of development these ovaries have no noticeably lower numbers of prophase I stage cells. (**E**) GCNA1 of D4PP ovaries from *Msh4*^{-/-} females shows that by this stage there are markedly fewer prophase I cells compared with wild type, and there are no primordial follicles, due to cells

arresting prior to full synapsis in pachynema. (**F**) H&E of *Msh4*^{-/-} post-pubertal, adult, ovaries indicates a heterogeneous phenotype, a proportion of ovaries contain fibrous tissue devoid of oocytes, as in **F**, and others are devoid of all tissue including oocytes, as in the *Msh5*^{-/-} ovary in **I**. Interestingly, some fetuses have one ovary that looks like **F** and the other looks like **I**. (**G**) GCNA1 staining of ovary sections from E18 *Msh5*^{-/-} fetuses. Like *Msh4*^{-/-} ovaries these mice do not have a noticeably lower number of prophase I oocytes compared with wild type. (**H**) GCNA1 staining of ovary sections from D4PP *Msh5*^{-/-} mice indicates that, like *Msh4*^{-/-} ovaries, there are no primary follicles forming and there are fewer prophase I cells than wild type, due to disruption of meiosis prior to full pachytene synapsis. (**I**) H&E staining of adult ovary sections from *Msh5*^{-/-} mice. As with *Msh4*^{-/-} mice, the ovarian phenotype is heterogeneous, with some appearing as in **F** and some as in **I**. * = Ovarian bursa.

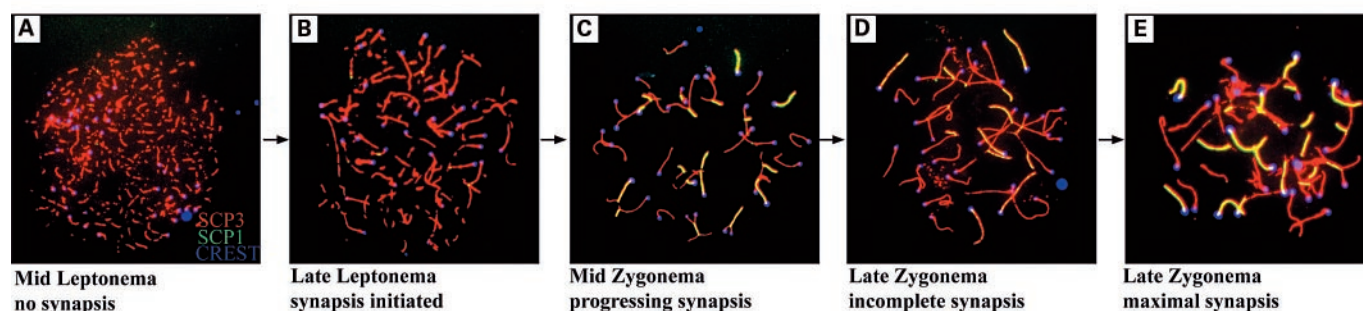
autosomes and of the XY pseudoautosomal region (PAR), spermatocytes from *Msh4*⁻ and *Msh5*⁻ deficient mice exhibit massive failure of synapsis, with almost no fully/normally synapsed bivalents being observed (Fig. 4).

Prophase I occurs during gestation in female mammals, and ovaries from *Msh4*^{-/-} and *Msh5*^{-/-} females at mouse embryonic day (E)18.5 show normal numbers of oocytes compared to wildtype ovaries of the same gestational age (Fig. 3). However, shortly after birth when oocytes are normally still progressing toward dictyate arrest, the number of pre-meiotic and prophase I germ cells, as detected by germ cell nuclear antigen 1 (GCNA1) immunoreactivity (Wang and Enders, 1996), is significantly diminished in *Msh4*^{-/-} and *Msh5*^{-/-} females com-

pared to wild type (Edelmann et al., 1999; Kneitz et al., 2000). By 6 days post-partum (pp) the ovaries of *Msh4*^{-/-} and *Msh5*^{-/-} females are almost completely devoid of GCNA1 signal, whereas wild-type mice still have significant levels of immunoreactivity (Edelmann et al., 1999; Kneitz et al., 2000), indicating the complete loss of oocytes by one week of age (Fig. 3).

Chromosomal analysis of meiotic progression in oocytes from MSH4⁻ and MSH5⁻ deficient mice reveals similar meiotic disruption to that seen in males of the same genotype. Oocytes from *Msh4*^{-/-} females show significant disruption of synapsis at zygonema, though more regions of synapsis are evident in the female germ cells than seen in the male germ cells, perhaps reflecting differences in regulation and/or meiotic checkpoint stringency

Msh4^{-/-} and *Msh5*^{-/-} spermatocytes



Wild-type, *Mlh1*^{-/-} and *Mlh3*^{-/-} spermatocytes

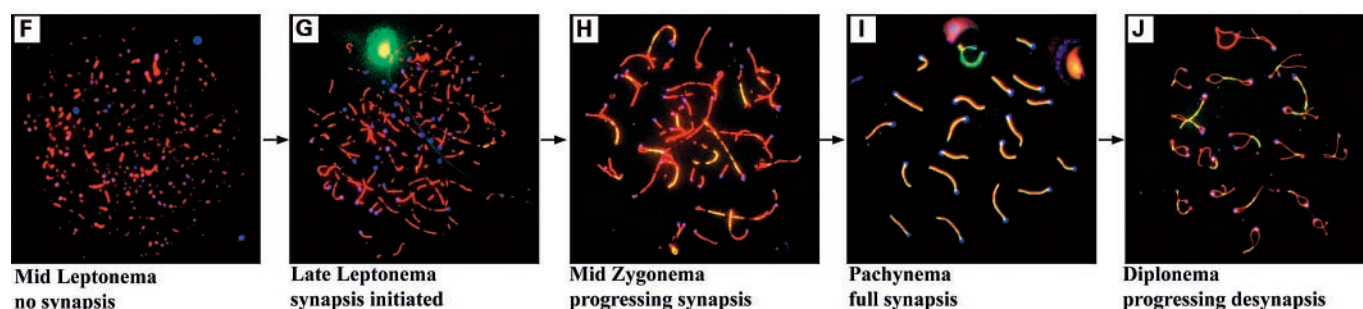


Fig. 4. Immunofluorescent representation of synaptic progression as determined by SCP3 (red), SCP1 (green) and centromere, CREST (blue) labeling, through meiotic prophase I in male mice deficient for MutS homologs (**A, B, C, D, E**) and wild type (similar to MutL homologs; **F, G, H, I, J**). (**A**) *Msh4*^{-/-} spermatocyte during early prophase I (leptonema stage). The chromosome cores, as indicated by SCP3 (red), are just beginning to form, there are 40 unpaired centromeres, and there is no synapsis (as indicated by SCP1, green). (**B**) Chromosome core formation progresses and some synapsis begins (as indicated by the green areas). (**C**) Synapsis, and SCP1 localization, increases as zygonema progresses. (**D**) In *Msh4*^{-/-} mouse spermatocytes (shown) and *Msh5*^{-/-} mouse spermatocytes (not shown) synapsis is never completed. Here the chromosome cores are compacted but there are 35 centromeres (a mixture of paired and unpaired). (**E**) This figure shows the maxi-

mal amount of synapsis found in both *Msh4*^{-/-} and *Msh5*^{-/-} mice, here there are 28 centromeres (a mixture of paired and unpaired) and SCP1 only localizes to a few synapsed regions, compared to 20 paired centromeres and complete localization of SCP1 between all homologs (and the X-Y pseudoautosomal region) in wild type. (**F**) Wild-type (shown), *Mlh1*^{-/-} (not shown), and *Mlh3*^{-/-} (not shown) mouse spermatocytes, chromosome cores begin to form during early prophase I (leptonema) and there are 40 unpaired centromeres. (**G**) As leptonema progresses, some synapsis is initiated. (**H**) During zygonema synapsis and centromere pairing progresses. (**I**) During pachynema synapsis is complete, SCs compact, and there are 20 paired centromeres. (**J**) During late prophase I (diplonema) the SCP1 synaptic component is gradually lost from the SCs and the homologs repel one another. An overlap of green and red fluorescence results in yellow coloring.

between the sexes (Edelmann et al., 1999; Kneitz et al., 2000). Thus, in both male and female mice, the consequence of MSH4 or MSH5 loss is more severe than in yeast and worms, with failure of pairing and synapsis and non-homologous associations in the former, and apparently normal synapsis in the latter.

MLH1

In *S. cerevisiae*, Mlh1 is essential for ensuring appropriate levels of crossing over during meiosis, while analysis of *mlh1msh4* double mutant strains indicates that the two act in the same pathway to maintain recombination rates (Hunter and Borts, 1997). Mutants for *mlh1* display increased non-disjunction, coupled with higher rates of post-meiotic segregation (Borts et al., 2000).

In mice, electron microscope (EM) defined meiotic nodules localize to the SC throughout prophase I. Nodules that occur late in prophase I correspond in number and distribution with chiasmata, the sites of reciprocal exchange, and as such are referred to as recombination nodules (Carpenter, 1975). MLH1

immunofluorescence shows that localization to SCs begins during mid-pachynema, and the number and distribution pattern of MLH1 foci correspond to that of chiasmata, both in mice and in humans (Tease, 1978; Baker et al., 1996; Barlow and Hulten, 1998; Anderson et al., 1999; Woods et al., 1999; Tease et al., 2002). EM analysis of the protein components of meiotic nodules throughout prophase I indicates that MLH1 is a component of only the recombination nodules and thus marks the sites of crossing over in mice and humans (Moens et al., 2002). When prophase I is precociously induced with the phosphatase inhibitor okadaic acid, MLH1 colocalizes with chiasmata and recombination nodules (Moens et al., 2002; Marcon and Moens, 2003). Variability in the total number of MLH1 foci at pachynema is consistent with sex-specific differences in genetic exchange, that is higher in females than in males, confirming that MLH1 marks the sites of reciprocal exchange (Anderson et al., 1999; Woods et al., 1999; Koehler et al., 2002; Lynn et al., 2002). The number of MLH1 foci per bivalent correlates with the length of the SC, in that longer SCs have more foci than do

shorter ones, consistent with non-random, interference regulation of crossing over. However, the relationship between length and number only holds after each bivalent has at least one focus, meaning that short (less than 6 μ m) SCs have more than the expected number of foci, as predicted by length (Froenicke et al., 2002; Kleckner et al., 2003).

Mlh1^{-/-} male and female mice are sterile as a result of meiotic failure during prophase I. In the case of *Mlh1* null males, chromosomes undergo normal synapsis and initiation of recombination, as demonstrated by the accumulation of SC proteins (Baker et al., 1996; Cohen and Pollard, 2001) and the appearance of phosphorylated histone H2AX and RAD51 at leptotema and zygotema (P.E. Cohen, unpublished observations). By pachynema, chromosome cores from *Mlh1*^{-/-} mouse spermatocytes are fully synapsed and progress normally through to diplotema (Baker et al., 1996). However, at least 10-fold fewer chiasmata are formed, or stabilized, such that only a few residual meiotic nodules are observed at pachytene (N.K. Kolas, A. Svetlanov, P.E. Cohen, manuscript in preparation). As a result most homologs cannot remain associated after desynapsis, resulting in the appearance of univalents during diplotene, and testis that are completely devoid of mature sperm (Fig. 5).

In adult *Mlh1*^{-/-} females, oocytes look remarkably healthy and ovarian follicles are observed at all stages of follicular growth (Edelmann et al., 1996). However, these oocytes fail to fertilize properly and are not viable (Edelmann et al., 1996). Thus, chiasma loss results in both male and female sterility at, or after, diplotene (Baker et al., 1996; Edelmann et al., 1996). Oocyte chromosomes from *Mlh1*^{-/-} females do not align properly at metaphase and cannot form a proper spindle (Woods et al., 1999). These oocytes show multiple abnormalities in spindle formation and polar body extrusion, and the few that make it past MI cannot proceed past the two-cell zygote stage (Edelmann et al., 1996; Woods et al., 1999; Eaker et al., 2002). By contrast, spermatocyte spindles are apparently undisrupted by scattered chromosomes (Eaker et al., 2002), but the lack of appropriate spindle tension results in apoptosis at or before metaphase I (Eaker et al., 2002). Thus, while the deficiency in chiasma maintenance is similar for male and female *Mlh*^{-/-} animals, the apoptotic response is quite different, suggesting distinct checkpoint mechanisms in males and females. In addition, the phenotypic consequence to oocyte meiosis in *Mlh1* null females is quite different to that seen in mice lacking either MSH4 or MSH5, since the meiotic failure occurs later in the absence of MLH1, and thus the oocytes may escape elimination at a pachytene checkpoint. Instead, oocytes from *Mlh1*^{-/-} females enter diplotema and then dictyate arrest after birth, as in wild type, and it is not until meiosis resumes at ovulation that the meiotic defects are manifested.

MLH3

All evidence points to MLH1 as a molecular marker of reciprocal exchange raising the question as to its MutL-binding partner at these sites. Immunolocalization and immunoprecipitation studies indicate that this partner is MLH3 (Fig. 6). Indeed, analysis of yeast mutants for *mlh3* indicates similar reductions in crossing over to that seen in *mlh1* mutant strains, while crossing over appears unaffected in *mlh2* or *pms1* mutant

strains (Wang et al., 1999). Interestingly, however, in mice MLH3 binds to SCs as early as late zygotene/early pachytene, prior to MLH1 (Lipkin et al., 2002). Furthermore, once MLH1 does bind to these sites, there are consistently two or three MLH3 foci that do not coincide with an MLH1 focus raising the possibility that those MLH3 lone foci (a) still have yet to bind MLH1, (b) are present alone or as a homodimer, or (c) may be interacting with another MutL homolog binding partner (Lipkin et al., 2002), though biochemical evidence is lacking for a functional interaction between MLH3 and PMS2, the most likely candidate. Interestingly, the development of recombination nodules, and thus crossovers, is dependent on MLH3. In *Mlh3*^{-/-} spermatocytes and oocytes, no meiotic nodules can be found at the EM level after zygotene, and there are no MLH1 foci identified both at the EM and LM level (Lipkin et al., 2002). On the other hand, when *Mlh1* is deleted, several MLH3 foci remain at the late EM-defined nodules (N.K. Kolas, A. Svetlanov and P.E. Cohen, manuscript in preparation), but the status of these nodules (recombinational or otherwise) is uncertain. The persistence of several chiasmata at metaphase I in male *Mlh1*^{-/-} mice suggests that the MLH3 foci localize to sites of exchange and MLH1 may be required for maintenance (Baker et al., 1996; Edelmann et al., 1996).

With the exception of the residual crossovers observed in spermatocytes from *Mlh1* null males, the meiotic phenotype of *Mlh3*^{-/-} mice is similar to that seen for the *Mlh1* null animals (Fig. 5). Male mice are sterile as a result of prophase I defects occurring at pachynema and into diplotema (Lipkin et al., 2002) such that air-dried chromosome preparations at metaphase I reveal mostly achiasmate univalent chromosomes. Interestingly, MLH1 fails to localize to these chromosomes at pachynema in the absence of MLH3 suggesting, as mentioned above, that MLH3 is recruited to meiotic nodules first, followed by MLH1. Thus, by mid-pachynema, spermatocytes from *Mlh3*^{-/-} fail to accumulate both MLH1 and MLH3 on their chromosome cores, appear to have no identifiable recombination nodules, and have no chiasmata. As a consequence, chromosomes fail to remain synapsed after breakdown of the central element of the SC at diplotema. Similar to *Mlh1*^{-/-} mice, *Mlh3*^{-/-} females have normal-sized ovaries containing an apparently full complement of follicles at all stages of development (Lipkin et al., 2002). However, oocytes from these ovaries fail to undergo proper fertilization due to their severe reduction in reciprocal recombination events.

Like MLH1, MLH3 has also been found to colocalize with replication protein A (RPA) in mouse spermatocyte preparations (Lipkin et al., 2002). RPA, a homolog of *E. coli* single-strand binding protein, localizes to the SCs just downstream of the RecA homologs RAD51 and DMC1. RPA is thought to play a role in homolog recognition by binding single-stranded intermediates (Ashley et al., 1995; Moens et al., 1997, 2002; Tarsounas et al., 1999). EM analysis of mouse SCs shows that RPA colocalizes with MSH4 and MLH3 (Lipkin et al., 2002; Moens et al., 2002). Whether MLH3, MSH4 and MSH5 colocalize on the SC still remains to be seen but in vitro experiments indicate that MLH3 and MSH4 can interact (Santucci-Darmanin et al., 2002) and MLH1 and MSH4 colocalize at MNs (Santucci-Darmanin et al., 2000).

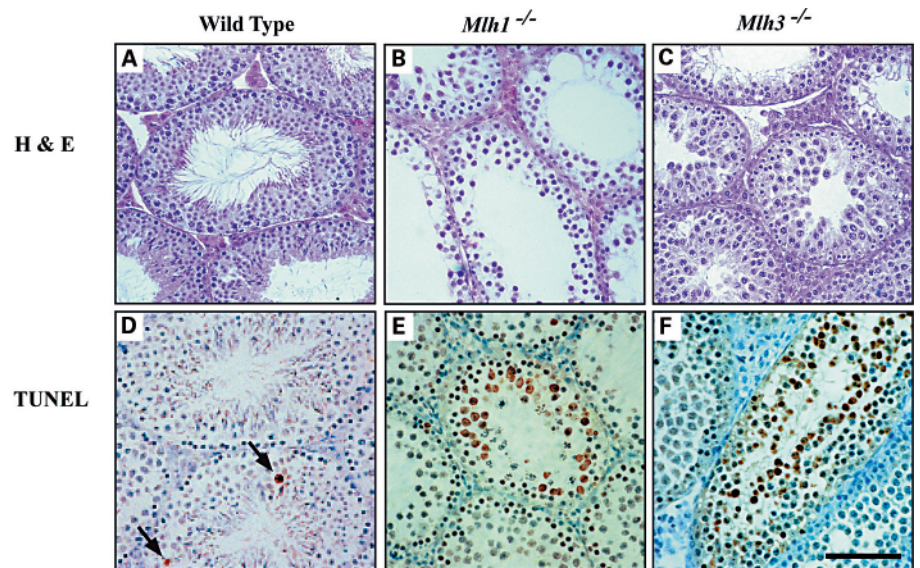


Fig. 5. Immunohistochemistry of post-pubertal testes from wild-type mice (**A, D**) and from mice with targeted deletions of the MutL homologs, *Mlh1* (**B, E**) and *Mlh3* (**C, F**). (**A**) H&E staining of testis sections from a wild-type C57BL/6J mouse. The testicular seminiferous tubules contain a base layer of Sertoli cells and spermatogonia, with progressing stages of spermatogenic cells radiating into the lumen of the tubule. (**B**) H&E-stained testis sections from an *Mlh1*^{-/-} mouse. (**C**) H&E-stained testis sections from an *Mlh3*^{-/-} mouse. Notice that in B and C the testes have apparently normal numbers of Sertoli cells and spermatogonia but there are fewer primary spermatocytes and the tubules are devoid of secondary spermatocytes and later

cells, including mature spermatids, and the mice are subsequently sterile. Testes from *Mlh1*^{-/-} males appear more devoid of spermatogenic cells than do their *Mlh3*^{-/-} counterparts. In both cases, Leydig cells (outside the seminiferous tubules) appear normal. (**D**) TUNEL labeling for apoptotic cells in a testis section from a wild-type mouse. The arrows indicate the brown, positively stained, apoptotic cells. (**E**) TUNEL of *Mlh1*^{-/-} testes. (**F**) TUNEL of *Mlh3*^{-/-} mouse testes. Notice the increased proportion of TUNEL positive cells in **E** and **F**, due to a loss of chiasmata and subsequent metaphase I checkpoint activation, in the absence of either *Mlh1* or *Mlh3*. Bar = 100 μ m.

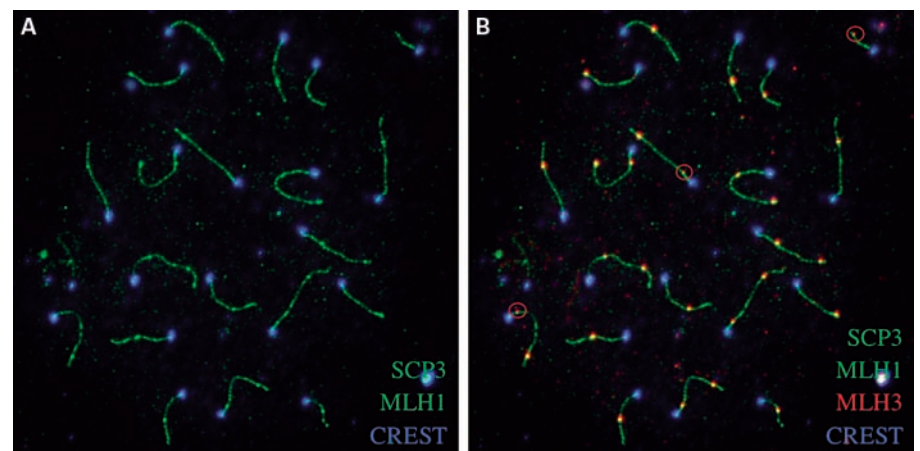


Fig. 6. Immunolocalization of MutL homologs, MLH1 and MLH3, to the synaptonemal complexes of mouse pachytene-stage spermatocytes. (**A**) Immunolocalization of MLH1 (bright green foci), the SC component SCP3 (green) and the centromeres recognized by human CREST antibody (blue). Both of these MLH1 and SCP3 antibodies are raised in mouse and are secondarily recognized by an antibody conjugated to FITC. The SCP3 is titrated to a minimal amount, that still recognizes cores, to allow the MLH1 foci to

stand out. (**B**) Immunolocalization of MLH1, SCP3, CREST and MLH3. The same field of view as in A, with green MLH1 and SCP3, showing MLH3 (red) localization. The majority of the MLH3 foci overlap with MLH1 foci, resulting in a yellow color. Three MLH3 foci (circled) in this picture do not coincide with MLH1 foci. Either these MLH3 lone foci have yet to acquire MLH1, or they are a separate subset of MLH3 foci, of unknown function, that never acquire MLH1.

PMS2

The mammalian protein PMS2 is orthologous to yeast Post Meiotic Segregation 1 (Pms1). Yeast, *pms1* mutants exhibit a pronounced mutator phenotype characteristic of genes encoding MMR proteins. Moreover, as its name implies, these mutants exhibit deviations from the expected Mendelian segregation of markers (4:4, 6:2 and 2:6) during tetrad analysis, instead resulting in the recovery of allelic ratios of 5:3 or 3:5. This increased post-meiotic segregation phenotype is proposed to arise from a failure to repair mismatches in heteroduplex DNA (Wang et al., 1999). Human individuals bearing heterozygous mutations in PMS2 show an increased predisposition to colorectal and other cancers that are characterized by microsatellite instability (Kolodner and Alani, 1994; Lynch and Smyrk, 1996). Mutations in mouse *Pms2* also result in MMR-related genomic instability phenotypes, including microsatellite instability and increased cancer predisposition (Baker et al., 1995).

Male *Pms2*^{-/-} mouse mutants are sterile as a result of meiosis I disruption, but unlike the phenotypes of *Mlh1* and *Mlh3* null males, the meiotic disruption is less tractable. Instead, spermatocytes are lost progressively through prophase I, with increased non-homologous chromosomal interactions being reported along with defects in chromosome synapsis (Baker et al., 1995). Interestingly, however, much of the meiotic defect appears to be strain-dependent (A. Svetlanov, M. Lenzi, N. Kolas, and P. Cohen, manuscript in preparation), though in all cases the males are sterile, producing few sperm that are non-viable (Baker et al., 1995). These data suggest a role for PMS2 in mammalian meiosis, but one that remains unclear at the current time.

While *Pms2* null males are sterile, the null females remain fertile, suggesting that PMS2 is required only for male meiotic progression. Furthermore, studies in our laboratory have indicated that MLH1 and MLH3 localize normally to late meiotic nodules in *Pms2*^{-/-} males (A. Svetlanov, M. Lenzi, N. Kolas, and P. Cohen, manuscript in preparation), suggesting that reciprocal recombination events occur normally in the absence of PMS2. In view of the yeast data indicating a role for Pms1 in gene conversion, these data indicate that mammalian PMS2 may play a role in heteroduplex directed meiotic mismatch repair, rather than a role in reciprocal exchange events per se.

Other MMR proteins

Msh2 is transcribed in mouse testis (Richardson et al., 2000); however, protein immunolocalization is still evasive. Similar information concerning other MutS homologs remains limited. However, *Msh2*, *Msh3* and *Msh6* null mice are all fertile and give rise to apparently normal litter sizes (de Wind et al., 1995, 1999; Reitmair et al., 1995; Edelmann et al., 1997, 2000), suggesting that their roles in meiotic progression and recombination are minimal, or perhaps redundant. *S. cerevisiae* *Msh2* and *Msh6* have been shown to bind Holliday junctions in vitro, while *Msh4* and *Msh5* have not (Alani et al., 1997). In yeast, there may be a role for MMR in heteroduplex rejection (i.e. unwinding of heteroduplex), and MSH2 and PMS2 play anti-homeologous recombination roles during mitotic MMR in both yeast and mice. However, studies to date

indicate that homeologous recombination is not increased during meiosis in *Msh2* and *Pms2* null mice (Qin et al., 2002).

Downstream of mismatch recognition in the MMR pathway are the 3'-5' and 5'-3' exonucleases that serve to remove mismatched bases, and thus they initiate mismatch correction. In eukaryotes, only the 5'-3' exonuclease, EXO1, has thus far been identified and characterized. Exo1 belongs to the Rad2 gene family and was originally identified in *S. pombe* in a screen for meiotically-induced genes (Szankasi and Smith, 1995). Further studies revealed that it plays a role in mutation avoidance in both *S. pombe* and *S. cerevisiae* (Johnson et al., 1998; Qiu et al., 1998; Rudolph et al., 1998), and that it interacts biochemically with Msh2, Mlh1 and Pms1 (Tishkoff et al., 1997; Rudolph et al., 1998; Tran et al., 2001). Interestingly, the enhanced mutation rate in *exo1* yeast mutants carrying weak mutator mutations in *mlh1*, *pms1* and *msh2* suggest an additional role for EXO1 in stabilizing the MMR complex at mismatch sites (Amin et al., 2001).

The role of yeast Exo1 in meiosis was demonstrated by the observation that *exo1* mutant *S. pombe* and *S. cerevisiae* strains exhibit increased meiosis I non-disjunction and reduced recombination (Borts et al., 2000; Khazanehdari and Borts, 2000; Kirkpatrick et al., 2000; Tsubouchi and Ogawa, 2000). However, its role has been the subject of considerable debate (reviewed by Hoffmann and Borts, this issue). In *S. cerevisiae*, Exo1 acts in the same pathway as Msh4 for intergenic recombination and crossing over, but appears to affect spore viability independently of both Msh4 and Msh5 (Khazanehdari and Borts, 2000; Kirkpatrick et al., 2000). On the basis of these, and other data, it was suggested that Exo1 functions to process a subset of recombination intermediates by generating single-stranded tails, while MSH4/5 functions to resolve recombination intermediates and to establish crossover interference (Khazanehdari and Borts, 2000).

Like its yeast counterpart, human EXO1 exhibits 5'-3' exonuclease activity and interacts biochemically with MSH2 and MLH1 (Tishkoff et al., 1998; Lee and Wilson, 1999; Lee Bi et al., 2002). Mouse EXO1 is highly expressed in lymphoid tissue and meiotic cells (Lee et al., 1999), possesses 5'-3' exonuclease activity, and is required for the repair of base:base and single-base insertion/deletion mismatches (Wei et al., 2003). Interestingly, extracts from *Exo1* mutant mouse ES cells are deficient in both 5' and 3' directed repair, indicating that EXO1 is required for both 5'-3' and 3'-5' directed mismatch repair. A similar observation was made for human EXO1, but in this case, the 3' activity was MLH1-dependent, while the 5' activity was not (Genschel et al., 2002; Genschel and Modrich, 2003).

Both male and female *Exo1* mutant mice are sterile as a result of meiotic failure (Fig. 7). However, both spermatocytes and oocytes progress through early prophase I with wild-type levels of recombination, as demonstrated by the appropriate accumulation of MLH1 at meiotic nodules during pachynema (Wei et al., 2003). Spermatocytes from *Exo1*^{-/-} males progress until metaphase I but, while a spindle is evident at this stage, the chromosomes appear to be misaligned at the metaphase plate and are more likely to be found as univalents, rather than bivalent pairs (Wei et al., 2003). This failure of spindle alignment results in checkpoint activation and a switch to apoptosis.

The phenotype of *Exo1* null mice suggests that this exonuclease functions in post-pachytene events and not in early processing of DSBs, as appears to be the case for its yeast ortholog. However, these findings do not rule out an earlier, less essential, or partially redundant, role for EXO1 in mammalian meiosis. Alternatively, other exonucleases might substitute for EXO1 during early DSB processing in mammalian germ cells, including MRE11. However, the exonuclease activity of MRE11 occurs in the 3'-5' direction, suggesting perhaps that the endonuclease activity of MRE11 might instead substitute for EXO1 at this early stage, in combination with its binding partners, RAD50 and NBS (Moreau et al., 2001).

Postulating the functions of the MMR proteins in mammalian meiosis

The DSB repair (DSBR) model (Szostak et al., 1983) has undergone considerable evolution since it was first proposed, and has gained more acceptance as intermediates of reciprocal recombination, double-strand breaks, 5' resected DNA, single-end invasion molecules and double Holliday junctions have all been physically identified and temporally defined in yeast (Schwacha and Kleckner, 1995; Paques and Haber, 1999). Recent evidence has demonstrated that the DSB initiating events can be resolved, following single-end invasion, through non-crossover (gene conversion) and crossover events that are processed through temporally and mechanistically-distinct pathways (Paques and Haber, 1999; Allers and Lichten, 2001b; De Los Santos et al., 2003). Thus, DSB formation, resection and strand invasion result in one of two pathways. In the first, second-end capture, DNA synthesis and ligation result in mature dHJs that may be resolved as crossovers or non-crossovers, depending on the polarity of cutting at each HJ (see Hoffmann and Borts, this issue, for further details). This first route encompasses the more traditional DSBR model. In the second scheme, known as synthesis-dependent strand annealing (SDSA), the invading strand is displaced and, following DNA synthesis, can anneal with the other, non-resected, DSB end (Allers and Lichten, 2001a). Following further DNA synthesis and ligation, only non-crossover products are obtained. In both models, all possible outcomes involve heteroduplex DNA, but it is only in the DSBR model that such heteroduplex DNA is actually incorporated into the recombination intermediate structures. This fact is important when considering the role of MMR proteins in processing of such intermediates and in the final resolution of recombination via these two mechanisms.

The preceding adjustment to our understanding of yeast recombination prompts us to reevaluate the role of the MMR proteins in gene conversion and reciprocal recombination events. Since the majority of DSBs in yeast are resolved as dHJ-related reciprocal events following stabilization of these structures by Msh4-Msh5, it has been suggested that Msh4-Msh5 binding might bias dHJ resolution towards reciprocal events, perhaps by ensuring second-end capture and/or by promoting dHJ formation, or by restricting access to the SDSA pathway. In addition, once the DSBR pathway is selected, presumably a similar mechanism must ensure that dHJs are resolved to pro-

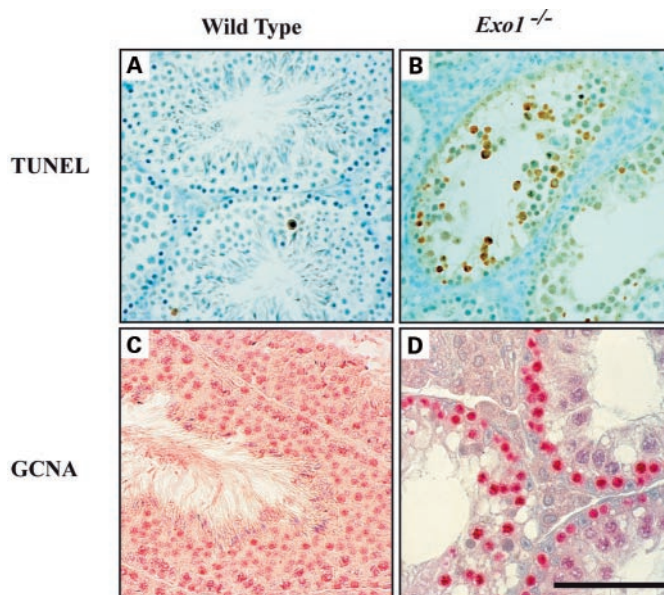


Fig. 7. Immunohistochemistry of post-pubertal testes from mice with a targeted disruption of mismatch repair specific Exonuclease I. **(A)** TUNEL apoptosis labeling of a testis section from a wild-type C57BL/6J mouse. Few TUNEL-positive cells (brown) are evident. **(B)** TUNEL apoptosis labeling of a testis from mice with a point mutation in *Exo1*. Metaphase I is apparently disrupted in these mice, as they have abnormal spindle structures and high levels of apoptosis. The testes are devoid of spermatids and spermatozoa, and both male and female mice are sterile. **(D)** GCNA1 staining of a wild-type C57BL/6J mouse testis section. **(E)** GCNA of *Exo1*^{-/-} testis sections indicates that the testes have apparently normal numbers of Sertoli cells, spermatogonia and primary spermatocytes, however they are devoid of post-prophase I cells. Bar = 100 μ m.

duce crossovers rather than non-crossovers. The mechanisms for such biasing are unclear at present.

In mammals the number of DSBs, as recognized by RAD51/DMC1 foci at zygonema, outnumber the eventual crossovers by 10-fold (Ashley et al., 1995; Cohen and Pollard, 2001; Moens et al., 2002). The number of crossovers amounts to approximately 24 in each spermatocyte (Baker et al., 1996; Anderson et al., 1999) and approximately 27 in oocytes (M. Lenzi, A. Svetlanov, N.K. Kolas, and P.E. Cohen, manuscript in preparation). The mechanisms by which the 250–350 or so DSBs become pared down to 24–27 crossover events is unclear, but it seems highly plausible that gene conversion events might be a way to reduce/resolve a proportion of the DSB numbers. In addition, gene conversion could account for the redistribution of polymorphisms within the mammalian genome (Fogel and Mortimer, 1969). That these events might, at least initially, involve MSH4 is illustrated by the observation of >150 MSH4 foci on SC cores at zygonema, a number that declines as the number of meiotic nodules gets pared down through to mid-pachynema, at which time the number of MSH4 foci is approximately 47 (Kneitz et al., 2000). By contrast, in yeast, approximately 55 Msh4-Msh5-positive foci are observed at pachynema, compared to an average of 90 eventual crossovers that occur in this species (Novak et al., 2001). Indeed, yeast studies have indicated that Msh4-positive

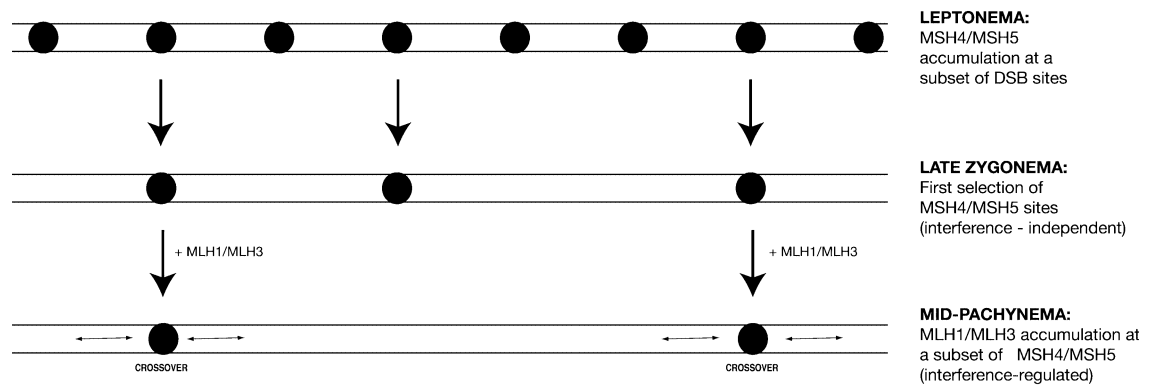


Fig. 8. Model for MMR protein-directed selection of crossover sites during prophase I of mammalian meiosis. During leptone-ma, MSH4 appears to bind to some of the sites of double-strand breaks, which exceed the eventual number of crossovers by 10-fold. Of the approximately 150 MSH4 foci on mammalian SCs at zygonema we propose that about 100 are “deselected” from the pool of possible reciprocal recombinants. The 47 foci observed during mid-pachynema would then mark the sites of double Holliday junctions, each of which can be resolved to either a non-crossover or crossover (marked by 25–27 MLH1/MLH3 localization) product, the distribution of which is regulated by interference.

foci might only represent a subset of reciprocal recombinant events. The conserved endonuclease complex Mus81/Mms4 may direct one relatively minor class of crossovers primarily on smaller chromosomes, which are not subject to interference. Accordingly, *S. pombe* have no Msh4 or Msh5 homologs, and no SC, and their crossover sites are not subject to interference, instead occurring through the Mus81 and Eme1 (the *S. pombe* homolog of Mms4) pathway (Boddy et al., 2001; De Los Santos et al., 2003).

Therefore the differences between yeast and mice are as follows: in yeast, few non-crossover events are seen, and the number of reciprocal recombination events observed exceeds the final number of Msh4 foci, suggesting two species of crossover event (one that is Msh4-dependent and one that is not). In mice the dynamics of recombination appear to be quite different. Firstly, the number of DSBs at leptone-ma is approximately 10-fold higher than the eventual number of reciprocal recombination events that arise at pachynema. Of these 250 or so DSB sites, only about 150 acquire MSH4 (and presumably MSH5) at zygonema (Fig. 8). We would propose that these 150 sites represent the total number of substrates for strand invasion and further processing of recombination, with the other 200+ sites being repaired by other means. Of the sites that undergo further processing, approximately 70% become resolved prior to entry into pachynema as assessed by a decline to some 47 MSH4 sites observed at pachynema. Following the yeast two-pathway model (DSBR and SDSA), one could envisage that the 100 or so sites resolved prior to pachynema are substrates for the SDSA recombination mechanism. The remaining 30% (47/150) would then enter the DSBR pathway, forming joint molecules and dHJ structures. Accordingly, if the resolution of dHJs toward non-crossovers or crossovers occurs with equal probability, one would predict that half of these 47 DSBR sites become crossovers and the other half become non-crossovers. Consistent with this, approximately 53% of the total pachy-tene-MSH4 sites (25/47) become reciprocal recombination events, as assessed by the accumulation of both MLH1 and

MLH3, while the other 47% presumably become resolved as non-crossovers (Fig. 8).

In such a scenario, mouse MSH4 would not bias resolution towards crossovers, as is the case for yeast Msh4, nor would it specifically associate with double Holliday junctions, but instead perhaps an earlier occurring DNA joint molecule structure. Similarly, unlike yeast, such a model would imply that MSH4 alone is not sufficient to establish interference. Instead, this model suggests that it is MLH3 localization that biases the system towards crossover formation, and that the mechanisms responsible for this loading are what define interference in mouse germ cells. The molecular basis for this mechanism remains unclear, but it is possible that helicases such as MER3 or BLM (together with TOPBII α) may mediate a switch towards the SDSA pathway at zygonema, possibly by preventing second-end capture and/or by encouraging annealing to the original DSB strand. Indeed in yeast the action of Msh4 on interference might be mediated in part by its interaction with the RecQ helicase, Sgs1. In mice too, it appears that MSH4 interacts with the RecQ helicase, BLM, in spermatocyte extracts (P.E. Cohen, unpublished observations).

The working model is that mammals and other large genome organisms have high rates of DSB induction at the onset of prophase I which must be resolved by the completion of prophase I, with only a tenth resulting in crossovers. That there is yet no satisfactory way of tracking mammalian meiosis in culture, nor for isolating their recombination intermediates, forces us to rely on extrapolation from yeast and biochemical data, both of which are limited in that they do not address the level of genome complexity. In addition, such comparative biology fails to address the functions of mammalian-specific meiotic genes such as Mei1. That MSH4/MSH5 has been proposed to bias dHJ toward crossing over does not fit with the observation in mammals that MSH4 localizes to two to three times more sites than there are crossovers. Thus we propose that a subset of MSH4/5 sites, bound to DNA joint molecule intermediates, are marked, perhaps by BLM, and “deselected”

from the total number of sites that can become dHJ. Thus, the 47 MSH4 sites at pachytene are the subset bound to mature dHJs. By an as yet undetermined mechanism MLH3 is targeted to a subset of these dHJs in a manner consistent with positive interference, and these sites go on to acquire MLH1 and be resolved as reciprocal recombinant/crossover events. As such

the DNA recognition function of the mismatch repair family of proteins have been employed to influence the outcome of DSB repair. Future studies addressing these hypotheses should significantly advance our understanding of meiosis in complex organisms.

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