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Review

Mismatch repair proteins, meiosis, and mice: understanding the complexities of mammalian meiosis

Anton Svetlanov and Paula E. Cohen*

Department of Molecular Genetics, Albert Einstein College of Medicine, Bronx, NY 10461, USA

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Abstract

Mammalian meiosis differs from that seen in lower eukaryotes in several respects, not least of which is the added complexity of dealing with chromosomal interactions across a much larger genome (12 MB over 16 chromosome pairs in *Saccharomyces cerevisiae* compared to 2500 MB over 19 autosome pairs in *Mus musculus*). Thus, the recombination machinery, while being highly conserved through eukaryotes, has evolved to accommodate such issues to preserve genome integrity and to ensure propagation of the species. One group of highly conserved meiotic regulators is the DNA mismatch repair protein family that, as their name implies, were first identified as proteins that act to repair DNA mismatches that arise primarily during DNA replication. Their function in ensuring chromosomal integrity has also translated into a critical role for this family in meiotic recombination in most sexually reproducing organisms. In mice, targeted deletion of certain family members results in severe consequences for meiotic progression and infertility. This review will focus on the studies involving these mutant mouse models, with occasional comparison to the function of these proteins in other organisms.

Keywords: Meiosis; Recombination; Mismatch repair; MLH1; MLH3; MSH4; MSH5; Synaptonemal complex; Knockout; Mouse

Introduction

Meiosis is the specialized cell cycle that gives rise to haploid gametes (or spores in plants) for sexual reproduction. It is common to all sexually reproducing organisms and is characterized by a single round of DNA replication followed by two rounds of division. In meiosis I, homologous chromosomes, each derived from one parental genome are paired together, first by the formation of a proteinaceous structure called the synaptonemal complex and subsequently by the physical interaction of DNA molecules through reciprocal recombination at sites of crossing over (or chiasmata). At metaphase I, the homolog pairs line up along the midplate of the cell, attaching to the spindle microtubules at their centromeres, each pair of sister chromatids remaining together throughout. At anaphase, the chiasmata are released, allowing the sister chromatids to move in tandem to the same pole, while the sister chromatids of the homolog move to the opposing pole. Thus, this reductional division results in a halving of the 4n chromosome number to 2n in preparation for a second equational division that results up to 4x1n gametes. Meiosis II more closely resembles mitotic cell division (without an intervening S phase) in that sister chromatids are separated at metaphase.

Prophase I is the defining stage of meiosis in that it is unique from mitotic prophase and in that it encompasses many of the I unique features of meiosis, including formation of the synaptonemal complex (SC), pairing of homologous (maternal and paternal) chromosomes, and formation of chiasmata between homologs. These features are common to almost all meiotic species, and are in place to ensure that homologous chromosomes find each other, pair, and remain together until the first meiotic division. There are some exceptions: for example, *Schizosaccharomyces pombe*, *Aspergillus nidulans*, and *Drosophila melanogaster* male spermatocytes, all of which undergo meiosis, do not assemble SCs and do not exhibit reciprocal recombination. Similarly, while the molecular and cytological events are common to all meiotic species, some distinct differences are apparent. For example, homolog pairing and SC formation occur before the initiation of recombination in *Drosophila* females, but in the reverse for species such as *Saccharomyces cerevisiae* and mice [1,2].

* Corresponding author. Department of Molecular Genetics, Albert Einstein College of Medicine, 1300 Morris Park Avenue, Bronx, NY 10461. Fax: +1-718-430-3248.
E-mail address: pcohen@aecom.yu.edu (P.E. Cohen).

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Our knowledge of the processes of meiotic prophase I has traditionally emerged from studies of simple unicellular organisms such as yeast, as well as from plants, flies, and worms. More recently, with technological advances in cytology and genetics, it has been possible to explore the intricacies of mammalian meiosis. Studying such processes is not easy in view of the endocrine and physiological complexities associated with larger organisms. In addition, studies in mammals preclude the ability to identify and isolate all four products of a single meiotic event. Despite these difficulties, the past 10 years or so have seen an overwhelming increase in our knowledge of meiotic events in mammals, more specifically in mice. This review will focus on these recent studies, with occasional reference to analogous events in yeast, flies, and other organisms. More particularly, we have chosen to focus on one family of proteins comprising the mismatch repair pathway because this system provides a good example of the power of using mouse models for meiotic studies. Moreover, the MMR family is highly conserved throughout eukaryotes, and its members are involved in meiotic recombination in yeast, worms, mice, and humans. However, it appears that in larger eukaryotes, their function has altered to accommodate the complexities of dealing with recombinant events over a larger genome.

The synaptonemal complex and sub-stages of prophase I

The synaptonemal complex (SC) is a specialized structure that arises during prophase I and functions to tether homologous chromosomes together until crossover structures are stabilized and can ensure maintenance of pairing until metaphase. In mice, as in yeast, the SC begins to form in leptonema, the first stage of prophase I. At this time, chromosomes are long, uncompacted structures consisting of sister chromatid pairs held together by a ring-like cohesin complex [3]. Meiotic sister chromatid cohesion is partially dissolved in the chromosomal arms at later stages of meiosis I to allow crossovers, but unlike in mitosis, is maintained at centromeres until meiosis II, and these particularities are reflected in existence of meiotic cell-specific forms cohesins, such as mammalian SMC1β and Rec8, and associated protein complexes [4,5].

Axial elements, consisting primarily of the proteins synaptonemal complex proteins 2 and 3 (SCP2 and SCP3, respectively, also known as SYCP2 and SYCP3), begin to form along each chromatid pair, firstly at sites of initial interaction, and then forming a continuous filament along the entire pair. The axial elements form a chromosome core from which loops of chromatin extend out in a perpendicular array. The importance of SCP3 in synapsis and meiotic progression has been underscored by the observation that *Scp3*$^{-/-}$ male mice are sterile due to apoptotic loss of germ cells during prophase I. Spermatocytes from these mice enter prophase I, but fail to accumulate axial elements of the SC, resulting in a failure of homolog synapsis [6]. In addition, the failure to construct a functional SC in the absence of SCP3 results in altered distribution of proteins involved in recombination, highlighting the intricate relationship between structural and functional aspects of chromatin organization during meiosis. In *Scp3*$^{-/-}$ females, however, mature oocytes can be obtained, but are severely aneuploid. Thus, oocytes survive beyond spermatocytes in *Scp3*$^{-/-}$ mice, although the requirement for SCP3 in chiasma formation and maintenance of chromosomal integrity eventually results in failure of proper chromosome segregation [7].

By the time the next stage of prophase I, zygonema, is attained, the two axial elements are complete and the third component of the SC, the central element, begins to accumulate between the axial elements. The central element, composed of the protein SCP1, functions to zipper the two homologs together in a process termed synapsis. By the end of zygonema, the central element is fully formed and stretches the entire length of the axial (now termed lateral) elements. The cells then enter the longest phase of prophase I, pachynema, which in mice last several days. During pachynema, chromosomes remain completely synapsed (Fig. 1) and continue to condense/compact to their shortest length, before entering diplonema. At this time, the central element begins to break down and the chromosomes repel one another, their lateral elements remaining intact through this time. At diplonema, as chromosomes move apart, their crossover structures become apparent, and these maintain the chromosomes in their appropriate pairs. Eventually, as diplonema progresses, the lateral elements begin to dissipate such that, by the end of diplonema, no SC components are apparent (except for residual protein at the centromeres).

In addition to SCP1-3 and the cohesins, other proteins have also been identified along chromosome cores during prophase I. More recently, the FK506 binding protein, FKBP6, has been identified as a novel component of the SC [8]. FKBP6 belongs to the immunophilin family, but is the first in this group to be associated with a meiotic function. In mouse germ cells, FKBP6 localizes to meiotic chromosome cores and regions of homologous chromosome synapsis and interacts with SCP1 [8]. Targeted inactivation of *Fkbp6* in mice results in male sterility, associated with a complete absence of normal pachytene spermatocytes. At the chromosome level, loss of FKBP6 results in abnormal pairing and misalignments between homologous chromosomes, nonhomologous partner switches, and autosynapsis of X chromosome cores in meiotic spermatocytes. Similar to *Scp3*$^{-/-}$ females, fertility and meiosis are normal in *Fkbp6* mutant females. Thus, FKBP6 is a component of the synaptonemal complex essential for sex-specific fertility and for the fidelity of homologous chromosome pairing in meiosis.

The SC also functions as a docking site for proteins that accumulate at nascent recombination sites. These sites, termed meiotic nodules (MN), appear at leptonema, at the time when the initiating events of recombination, the forma-

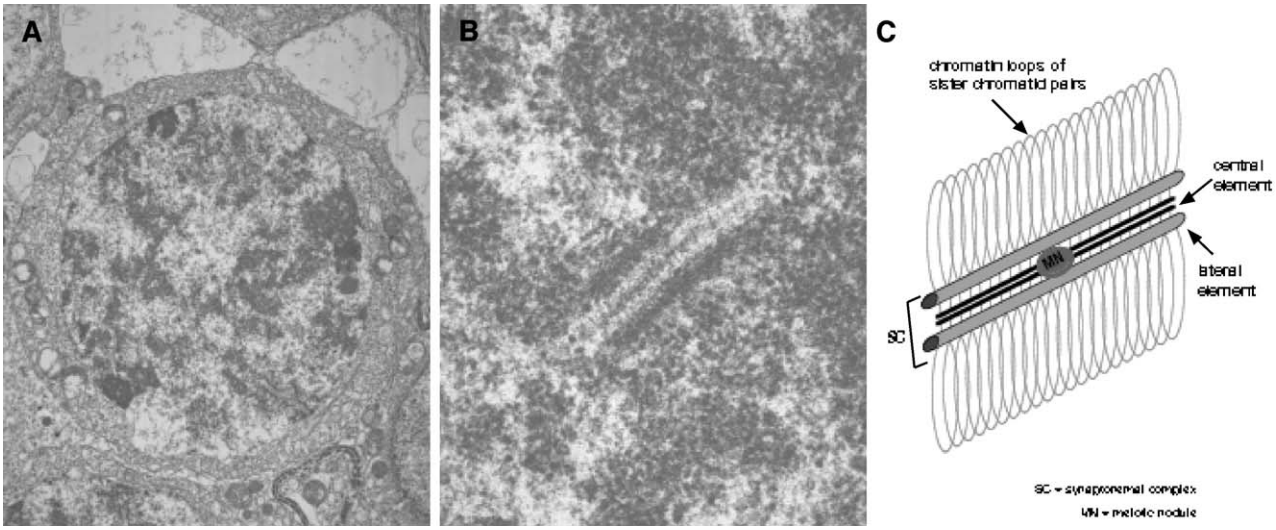

Fig. 1. Synaptonemal complex (SC) structure in mouse spermatocytes. (A) Electron micrography of a mouse spermatocyte during pachynema, showing a partial length of synaptonemal complex attached to the nuclear membrane (×16,200); (B) higher magnification view of SC structure (×135,000); (C) cartoon of SC structure.

tion double-strand breaks (DSBs), occur. As discussed below, the composition and frequency of meiotic nodules is dynamic, changing as cells progress from leptonema through until pachynema where they ultimately become the sites of crossing over, and they represent the functional complexes that mediate homology recognition and recombination events throughout prophase I. That their frequency is intimately linked with SC length and, in turn, that SC formation is dependent on their processing, suggests a functional interaction between SC dynamics and recombination events, as discussed in a recent review by Kleckner et al. [9].

Initiation and processing of recombination in meiosis

Much of our current knowledge of recombination processes during meiosis has emerged from studies in organisms such as *S. cerevisiae*, *Sordaria macrospora*, *D. melanogaster*, *Coprinus cinereus*, and *Caenorhabditis elegans*. In such studies, the relatively simple genome organization and size has facilitated the examination and identification of recombination hotspots, along with the tracking of and correlation with recombination intermediates. In all cases, it appears the recombination is initiated by the formation of DSBs within one DNA molecule, and is mediated by the conserved topoisomerase, Spo11 [10–13]. In mammals, failure to introduce the double-strand breaks into homologs leads to failure of recombination and synapsis, and ultimately meiosis failure, as exhibited in *Spo11*$^{-/-}$ mice [14–17], while reintroduction of DSB breaks by irradiation or cisplatin treatment rescues the meiotic defect [17].

The next step in recombination process, 5′–3′ resection of DNA, generating 3′ overhangs, requires action of an exonuclease whose exact identity remains unknown, but may be associated with the Mre11/Rad50/Nbs1 complex (at least in yeast), also known to process the DNA ends in other types of DNA repair. Once the 3′ overhangs are generated, they become available for homology search and strand invasion into homologous chromosome. Homologs of bacterial RecA protein are indispensable at this stage, and in both yeast and mammals are represented by RAD51, DMC1, and associated proteins. These proteins associate with DNA forming rigid protein–DNA structure (nucleoprotein filament), making the resected DNA ends amenable for homology search and heteroduplex formation. The appearance of RAD51 and DMC1 nuclear foci coincides with the DSB formation and they disappear as synapsis progresses. Proof that meiotic DSBs in mice are processed in a similar fashion to that in yeast has emerged from studies of spermatocytes from wild-type and *Spo11*$^{-/-}$ mice, which show that DSBs are resected to produce 3′ overhangs that ultimately become the site of attachment of the RecA homologs, DMC1, and RAD51 [18]. Additionally, loss of DMC1, in *Dmc1* null mice, results in infertility with gross chromosome pairing defects and apoptosis at or before pachynema [19,20].

Once the 3′ overhangs invade an opposing double-strand DNA molecule (single end invasion), a D-loop structure is formed, and is then extended by DNA synthesis. This strand is then capable of capturing the 5′ end of the strand on the opposite side of the DSB, resulting in the formation of a double Holliday junction (dHJ) structure. Nicking of each HJ, by as yet unidentified "resolvases" (Mus81 being one candidate in yeast; [21]) in one or other orientation results in resolution of the structure as a crossover or a non-crossover (reviewed in Refs. [22,23]). Alternatively, non-crossovers can arise as a result of a failure to progress through the complete D-loop stage, such that the invading strand is displaced after DNA synthesis and re-anneals to the distal end of the DSB (synthesis-dependent strand annealing,

SDSA; [23]). Such processes remain poorly understood in mammals as a result of our reduced ability to observe these intermediate structures.

One fundamental difference in recombination progression between yeast and mice lies in the number of initiating DSB events. In yeast, most, if not all, of the DSBs that occur at early leptonema give rise to crossover structures, through a mechanism that is thought to favor dHJ formation. In mice, however, the number of DSBs formed is in the order of 300 per nucleus in both male and female germ cells, greatly exceeding the final number of recombination sites (1–3 per chromosome, or 24–28 per nucleus). These excessive DSB numbers are thought to promote homology recognition and to ensure appropriate pairing before synapsis. However, this modification of the role of DSBs in early prophase I suggests the need for mechanisms to select future crossover sites as a subset of the DSB events. Those DSBs that are not selected to become crossovers (or which are actively not selected) are presumably repaired through non-recombination pathways. Indeed, proteins with anti-recombination activity (such as Bloom syndrome mutated, BLM, helicase) also localize with SC at prophase in mammals [24,25]. Finally, both the total number and the distribution of crossover sites along the chromosomes are strictly regulated in a process known as interference. In yeast, interference is thought to be established at the strand invasion stage, but in mice, the over-abundance of DSB sites must be taken into account, and would suggest that interference is intimately involved in the selection/deselection of DSBs for the crossover pathway.

Table 1
Summary of eukaryotic MMR genes and the phenotypes of mouse mutants (if known)

Bacterial gene	Yeast gene	Mouse gene	Role in mammalian MMR	Role in mammalian Meiosis
MutS	*MSH1*[a]	–	n/a	n/a
	MSH2	*Msh2*	Base: base mispairs and insertion/deletion loops	Not known[b]
	MSH3	*Msh3*	Insertion/deletion loops	Not known[b]
	MSH4	*Msh4*	None	Processing recombination intermediates
	MSH5	*Msh5*	None	Processing recombination intermediates
	MSH6	*Msh6*	Base: base mispairs and insertion/deletion loops	Not known[b]
MutL	*MLH1*	*Mlh1*	Base: base mispairs and insertion/deletion loops	Establishing and/or maintaining crossovers
	MLH2	*Pms1*	Not known/none	Not known/none
	MLH3	*Mlh3*	(Insertion/deletion loops)	Establishing and/or maintaining crossovers
	PMS1	*Pms2*	Base: base mispairs and insertion/deletion loops	Involved in male meiosis, but role unclear[c]
MutH	–	–	Methyl directed nicking	n/a

[a] Mitochondrial protein not present in mammals.
[b] No obvious meiotic phenotype in null mice.
[c] Females fertile; n/a = not applicable.

The multiple functions of the mismatch repair family

Eukaryotic mismatch repair

The MutHLS mismatch repair (MMR) family was first characterized in bacteria in its capacity to repair mismatches that arise primarily as a result of errors during replication and to inhibit recombination between divergent sequences (antirecombination). The system was first described in bacteria, where it consists of homodimeric MutS complexes that scan the DNA for mismatches, bind to DNA at the mismatch, and form an active repair intermediate complex in a multi-step reaction involving bending of DNA an binding of ATP. Following mismatch recognition in bacteria, a MutL homodimer presumably links the MutS-DNA intermediate to exonuclease MutH, thereby activating the latter to degrade the daughter strand containing the mismatch, which is followed by resynthesis of excised DNA fragment and religation of DNA, utilizing specialized DNA polymerase and DNA ligase activities.

The MutS and MutL components of MMR system are highly conserved across species (Table 1) and in yeast are represented by six MutS homologs, Msh1-6, and four MutL homologs, Mlh1, Mlh2, Mlh3, and Pms1 (for post-meiotic segregation). In mammals, orthologs of MSH2 through MSH6 exist, along with all four MutL homologs. Rather confusingly, the mammalian ortholog of yeast Mlh2 is known as PMS1, and the ortholog of yeast Pms1 is known as PMS2 in mammals. Similar to prokaryotic repair, these seem to work in dimeric form, but in this case as heterodimers, and with particular heterodimers specialized in particular types of repair or antirecombination (for review, see Refs. [26,27]). Furthermore, the eukaryotic MMR system has evolved to perform functions not directly related to DNA repair, particularly in meiosis, as discussed further in this review.

As in prokaryotes, heterodimers of MutS homologs function as mismatch recognition and DNA binding complexes, while the MutL homolog heterodimers function as adaptor complexes, either for initiating downstream events and/or signaling to the cell cycle and checkpoint machinery [26,27]. During mammalian MMR, when a mismatch arises, heterodimers of MSH2 with either MSH6 (together called MutSα) or MSH3 (called MutSβ) recognize different mismatches (Table 1), together encompassing a wide range of possible mismatch structures. In a subsequent step, a heterodimer of MutL homologs binds to the MSH complex, consisting of MLH1 together with either PMS2 (MutLα) or MLH3 (MutLβ). Again, biochemical evidence points to-

wards different substrate specificities for each MutL heterodimer, with the MutLα complex being the principal MutL heterodimer participating in MMR events.

One of the major functions of the MutL heterodimer is to signal to downstream repair events, including the machinery that will ultimately remove and repair the aberrant DNA sequence. To this end, several exonucleases (such as exonuclease I, EXO1), DNA polymerases (δ and ε), replication factors (RPA, PCNA, and RFC) are potential downstream effectors. In addition, the MutL heterodimers also function as molecular switches and can recruit and activate the apoptotic machinery in cases where cell death is a preferable event to chromosomal instability.

Localization of MMR proteins in meiotic cells and the phenotypes of MMR knockout mice

The MutS homologs

MSH4 and MSH5 were both identified in budding yeast as meiosis-specific members of the MutS homolog family [28–31]. Similar to their repair counterparts, MSH4 and MSH5 form heterodimeric complexes with each other, but they lack mismatch detection and repair activity as a result of the absence of the appropriate amino acid residues. Furthermore, the structure defined by the MSH4–MSH5 heterodimer is such that it allows access of a large heteroduplex-like structure to pass through it [32,33]. Indeed, evidence in yeast points to their role in the recognition of specialized structures such as Holliday junctions [30], suggesting a role for this heterodimer in the resolution of these recombination intermediates.

Localization of MSH4 on meiotic chromosomes from male mice indicates that MSH4 loads onto chromosome cores in zygonema, in numbers that far outweigh the number of eventual reciprocal recombinant events [34,35]. MSH4 appears on meiotic nodules that are positive for RAD51 (P.E. Cohen, unpublished observations), and persists even as RAD51 is lost from the nodules at the onset of pachynema. At this time, MSH4 begins to colocalize with the single-stranded binding protein, replication protein A (RPA) [36], although the precise nature of the MSH4-RPA interaction, if any, is unclear at the current time. However, RPA has been implicated in somatic cell MMR events [37] and is thought to participate in meiotic events downstream of RAD51 in mice and yeast [38–40]. By mid-pachynema, MSH4 foci numbers have declined yet further to a level approximately double that of the number of reciprocal recombination events, and at least a subset of these interact with the MutL homologs, MLH1 and MLH3 [35], as described below.

Mouse mutants lacking *Msh4* or *Msh5* result in infertility in both male and female mice as a result of meiotic arrest at zygonema [34,41,42]. DSB formation occurs normally in spermatocytes from $Msh4^{-/-}$ or $Msh5^{-/-}$ males, as demonstrated by the normal accumulation of phosphorylated histone H2AX at leptonema (P.E. Cohen, unpublished observations), and RAD51 hyperlocalizes to chromosome cores at this time [34]. By late zygonema, chromosomes from *Msh4*- and *Msh5*-deficient mice fail to synapse appropriately at zygonema and into early pachynema. Some nuclei show partial pairing (70% of all spermatocyte nuclei in $Msh4^{-/-}$ males and 10% of all nuclei in $Msh5^{-/-}$ males), but the majority of these pairing events are between nonhomologous chromosomes [34]. These germ cells fail to enter pachynema and die by apoptosis, resulting in testes that are entirely devoid of post-leptotene spermatocytes.

Female mice lacking MSH4 or MSH5 exhibit similar meiotic disruption to that seen in the males. However, the physiological consequence of this pre-pachytene meiotic failure is much more severe in the females. Oocytes die by apoptosis around the time of birth, such that the ovary is completely devoid of germ cells by day 4 postpartum [34,42]. In the absence of oocytes, the ovary degenerates gradually over the first few months of postnatal life, and by 4 months of age, the residual ovarian structure consists of large cyst-like structures containing few stromal cells [34]. Such degeneration is common to meiotic mutants that arrest early in prophase I, including $Spo11^{-/-}$ and *mei1* mutant females [16,43], although the extent of the ovarian degeneration is somewhat variable. In both $Msh4^{-/-}$ and $Msh5^{-/-}$ females, however, the loss of the entire oocyte pool results in complete destruction of the ovarian structures within the first 8 to 10 weeks of postnatal life.

The MutL homologs

The major MutL homologs active in meiosis both in yeast and mammals are MLH1, MLH3, and PMS2 (or Pms1 in yeast). Of these, DNA repair studies suggest the existence of MLH1–MLH3 and MLH1–PMS2 dimer pairs with different activities. Yeast data implicate the Mlh1–Mlh3 heterodimer in crossover pathway, as yeast *mlh1* and *mlh3* mutants are defective in crossover formation [44,45]. In mice and humans, MLH1 and MLH3 form distinct foci along the arms of synapsed homologs during prophase I, appearing at mid and late pachynema (Fig. 2 and Refs. [46–50]). In mice, the number of MLH1 foci is 1–2 foci per chromosome, and these persist through until diplonema. The number and distribution of these foci exactly matches that of the final number of chiasmata, and are localized to electron dense meiotic nodules [36,47]. Okadaic acid treatment of early prophase I spermatocytes results in the precocious induction of chiasmata formation and diplotene progression [51], and these sites also accumulate MLH1 and MLH3 [52]. Thus, both of these proteins are absolute markers of reciprocal recombination in mammals.

Biochemical and immunofluorescent studies both point to a functional interaction between MLH1 and MLH3 in mammalian germ cells. In addition, it appears that a common feature of MutL homolog heterodimers is that their recruitment to MutS complexes is dependent on their heterodimerization. Given these observations, it was previously assumed that MLH1 resided at crossover sites in

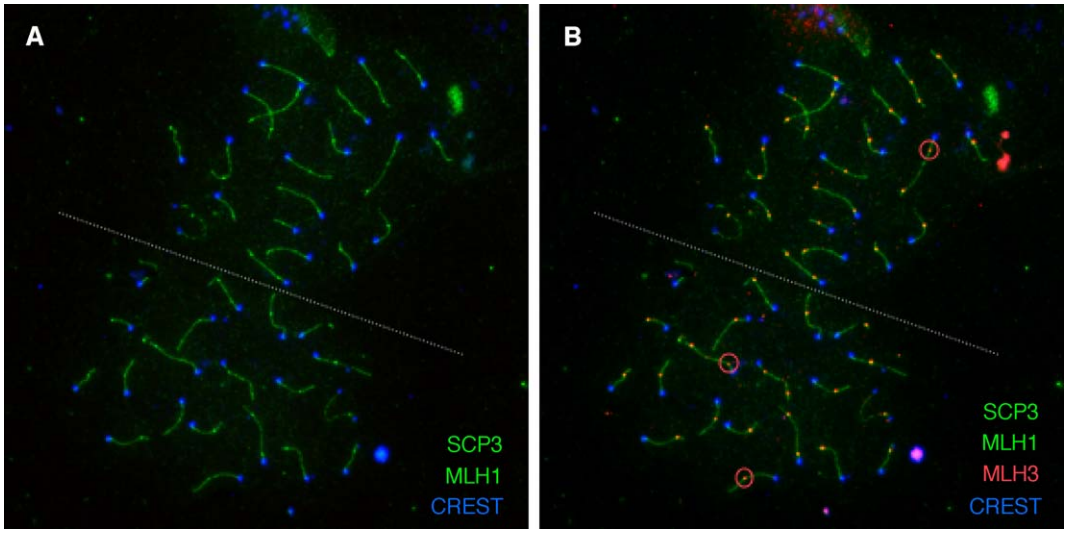

Fig. 2. Localization of MLH1 and MLH3 on meiotic chromosomes during pachynema of prophase I in the mouse. (A) FITC and CY5 channels only, showing SCP3 and MLH1 labeling in green and centromere labeling in blue; (B) Same image as in (A), but with TRITC channel overlay, showing MLH3 foci in red. Note the occasional MLH3-only sites (red circles). Image is taken from spermatocytes spreads from adult wild-type testes.

murine germ cells only in the context of its association with MLH3 (or perhaps PMS2), and vice versa. Interestingly, however, this does not appear to true for all foci, since immunolocalization of MLH1 and MLH3 on spermatocytes chromosomes from wild-type mice indicates that MLH3 is found occasionally at foci in the absence of MLH1 (Fig. 2 and Ref. [49]). This suggests one of two possibilities that are not mutually exclusive: either MLH3 appears at crossovers before (and recruits) MLH1, or MLH3-only foci represent a novel class of meiotic nodule that is involved in non-reciprocal recombinant events.

Mlh1 inactivation in mice results in male and female infertility [48,53]. *Mlh1* null spermatocytes progress through to pachytene apparently normally, as assessed by the accumulation of SC components, acquisition and loss of RAD51 and RPA, and the appearance of electron dense meiotic nodules. Following entry into diplonema, however, as the central element of the SC begins to break down, the chromosomes are no longer held together at their chiasmata, such that, by metaphase, almost all chromosomes are seen as univalents. As a result, the cells are actively eliminated by apoptosis, resulting in complete absence of mature sperm.

In $Mlh1^{-/-}$ females, ovarian structures appear normal, and oocytes are observed at all stages of folliculogenesis [53]. Oocytes from $Mlh1^{-/-}$ females reach metaphase but are very frequently aneuploid, as a result of congression failure and inappropriate spindle loading at the first meiotic division [46,53]. These oocytes fail both in vitro and in vivo fertilization. This phenotype is clearly different to that seen in *Msh4* and *Msh5* null animals, in which the earlier failure of chromosome pairing results in apoptotic cell death at or before pachynema. With the failure of MLH1-related events at pachynema in *Mlh1* null animals, however, these oocytes avoid this checkpoint and are able to progress through to diplotene and dictyate arrest. Thus, in adult $Mlh1^{-/-}$ females, the ovaries look normal because oocytes are still in dictyate arrest, and it is not until meiosis resumes after ovulation that problems become evident.

A similar phenotype is observed in MLH3-deficient mice in that chromosome synapsis occurs normally, and DSB processing progresses through until pachynema [49,54]. However, an important distinction between the two phenotypes is revealed however by electron microscopic studies of meiotic chromosomes and metaphase analysis of spermatocytes: MLH3-deficient chromosomes completely lack meiotic nodules and MLH1, while MLH1-deficient chromosomes seem to retain at least some of the meiotic nodules and associated MLH3 protein (N.Kolas, A. Svetlanov and P. Cohen, unpublished observations and Ref. [54]). Taken together with the fact that the number of MLH3 fluorescent foci seem to be in some excess of that of MLH1 and arise somewhat earlier in pachynema, these observations point to a possibility of an MLH1-independent role for MLH3, or perhaps an independent mechanism of its localization to meiotic chromosomes. Whatever that role or mechanism may be, both proteins are critical for success of recombination process at the dual-labeling foci, and it remains to be discovered what the ultimate outcome is at sites of MLH3-only foci.

Another member of the mammalian MutL homolog family, PMS2, plays significant but less well-understood role in mammalian meiosis. Interestingly, that role seems to be sexually dimorphic, since *Pms2*-null male mice are infertile, while $Pms2^{-/-}$ females retain their fertility [55]. In yeast, *PMS1* inactivation leads to defects in the repair of DNA mismatches arising during heteroduplex formation and reduced spore viability. The meiotic role of mammalian ortholog PMS2 has proven to be harder to pinpoint, espe-

cially since data on PMS2 meiotic localization are lacking. Our analysis of *Pms2* null mice shows, however, that this role is not related to recombination per se (P. Cohen, A. Svetlanov and N. Kolas, unpublished observations), but nevertheless affects prophase I progression in male, but not female, germ cells.

Other MMR proteins

Exonuclease 1 (Exo1) is a 5′–3′ exonuclease that interacts with MutS and MutL homologs and has been implicated in the excision step downstream of the MMR recognition step. Thus, Exo1$^{-/-}$ cells are defective for mismatch repair activity, resulting in elevated microsatellite instability and increased mutation rates. In addition, Exo1$^{-/-}$ mice are sterile as a result of post-pachytene defects during prophase I. Chromosome synapsis and SC formation are completely normal in both male and female *Exo1*$^{-/-}$ germ cells and MLH1–MLH3 foci accumulate normally during pachynema in these mice. However, the chromosomes undergo a dynamic loss of chiasmata during metaphase I, resulting in meiotic failure and apoptosis. Thus, even though MLH1 and MLH3 accumulate normally at crossover structures in *Exo1*$^{-/-}$ germ cells, their maintenance is still not assured, suggesting that factors downstream of MLH1–MLH3 are still required to ensure appropriate segregation at metaphase I.

Perspectives: functions of the MMR pathway in mammalian meiosis

MLH1 and MLH3 are now established to be the ultimate markers of reciprocal recombination events in mice and humans. One of the major issues that remains to be resolved is how such sights are selected from amongst the 350 or so initiating events in mouse spermatocytes. The answer is not as simple as recruitment by MSH4–MSH5 complexes since these associate with all (or almost all) early nodules soon after DSB formation. The decline in MSH4–MSH5 numbers, therefore, most likely represents a gradual selection of reciprocal recombination events (or deselection of DSB sites to become resolved by alternative pathways). However, the fact that there are approximately double the number of MSH4 sites as crossovers at mid-pachynema indicates that not all MSH4–MSH5 sites are destined to become crossovers and, conversely, that MSH4–MSH5 localization alone is not sufficient to ensure that a DSB site will become a reciprocal recombination site. Thus, of the total number of MSH4 sites at mid-pachynema, only approximately half will become reciprocal events, acquiring both MLH1 and MLH3, while a small number of them will recruit only MLH3, and the remainder do not associate with either MLH1 or MLH3 (Fig. 3). Thus, of these three species of nodule, the first will resolve as crossovers, the second as non-crossovers, and the third, presumably, as non-crossover repair (gene conversion) events. Whether there is a qualitative difference between the second and third group is uncertain. Alternatively, the MLH3-only group could represent a destabilized version of the first (MLH1^{+} MLH3^{+}) group.

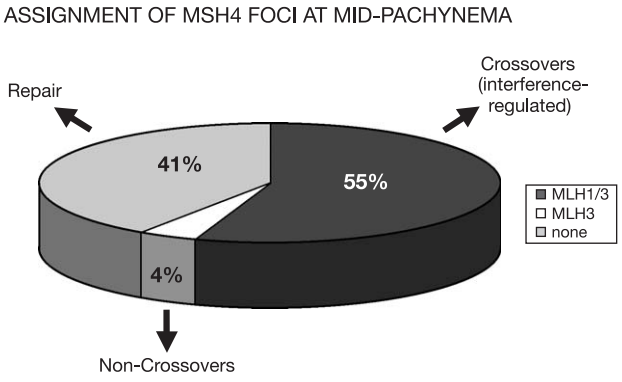

Fig. 3. Analysis of MSH4 foci along mouse spermatocytes SCs during mid-pachynema. Total number of MSH4 foci is based on data from Kneitz et al. [34]. Percentages of total MSH foci that are associated with MLH1 and MLH3 (black), MLH3-only (white) or neither MutL homolog (grey) are shown. These numbers are based on extensive quantitation of MLH1 and MLH3 foci in our laboratory (data not shown).

In most meiotic species, it is now established that crossovers are not randomly distributed, but are instead subject to certain rules; firstly, each chromosome (or chromosome arm) must have one crossover at the very least; and secondly, the probability that one crossover will be placed close to another is lower than the expected placement due to random distribution. This latter phenomenon is known as crossover interference and is thought to be mediated, at least in part, by the SC itself, since species lacking SCs, also lack crossover interference [56]. In yeast, it has been demonstrated that crossovers that are regulated by interference are Msh4-dependent, while those that do not exhibit interference are instead dependent on Mus81 (in conjunction with Mms4) [57]. The absence of any information relating to a mammalian ortholog for Mus81 precludes a similar model for mouse recombination, but it raises several interesting points. In yeast, only a subset of reciprocal events are associated with Msh4, and it is these that are subject to interference. However, in mice, it appears that all the reciprocal recombinant events are dependent on MSH4, and that some non-reciprocal sites are also loaded with MSH4. Thus, if interference rules only apply as they do in yeast, then all these MSH4-positive sites will be subject to interference. This is quite unlikely because of the distribution of MLH1–MLH3 sites that we see at pachynema in the mouse. Thus it appears that in mice, the loading of MSH4 onto meiotic nodules is not sufficient to ensure interference regulation, and this might only come later, once MLH3 loads. It can be assumed that the selection of MSH4 sites by MLH3–MLH1 would be subject to interference rules, since we observe only the expected 1–2 MLH1–MLH3 foci per chromosome. Further analysis of how DSB sites are selected and then deselected post-RAD51, along with MSH4–MSH5 loading and dynamics will be important for establishing how these sites are selected/deselected through early

to mid prophase I, and where interference regulation might be established in the mammalian system.

The mechanism of MMR proteins participation in meiotic recombination is unknown and is a new and exciting area of study. It seems probable that interactions similar to that of MutS–MutL and their homologs take place here as well. Indeed, such interactions are supported by experimental data [35,36,58]. As crossover resolution must involve DNA unwinding in some form, the recently found interaction of yeast Mlh1–Mlh3 complex with that of Sgs1–Top3 (helicase-topoisomerase) is intriguing [59]. In addition, MLH1 interacts with the Sgs1 ortholog, BLM, in mouse somatic cells, as part of a larger chromosome surveillance complex [60], and MSH4 might also interact biochemically with BLM in mammalian germ cells, suggesting a role for MSH4/MLH1/BLM signaling in the recruitment of meiotic nodules for crossovers. Also of note is the possible involvement of and interactions with exonucleases, such as Exo1 in crossover formation and/or resolution [61–63], and the essential requirement for such interactions in maintaining crossover structures through until metaphase. Future studies will help to further elucidate the mechanisms of mammalian recombination and will continue to provide fascinating insights into regulatory differences across eukaryotic species.

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