Mutation of the ATPase Domain of MutS Homolog-5 (MSH5) Reveals a Requirement for a Functional MutS\(\gamma\) Complex for All Crossovers in Mammalian Meiosis

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ABSTRACT During meiosis, induction of DNA double strand breaks (DSB) leads to recombination between homologous chromosomes, resulting in crossovers (CO) and non-crossovers (NCO). In the mouse, only 10% of DSBs resolve as COs, mostly through a class I pathway dependent on MutS\(\gamma\) (MSH4/MSH5) and MutL\(\gamma\) (MLH1/MLH3), the latter representing the ultimate marker of these CO events. A second Class II CO pathway accounts for only a few COs, but is not thought to involve MutS\(\gamma\)/MutL\(\gamma\), and is instead dependent on MUS81-EME1. For class I events, loading of MutL\(\gamma\) is thought to be dependent on MutS\(\gamma\), however MutS\(\gamma\) loads very early in prophase I at a frequency that far exceeds the final number of class I COs. Moreover, loss of MutS\(\gamma\) in mouse results in apoptosis before CO formation, preventing the analysis of its CO function. We generated a mutation in the ATP binding domain of Msh5 (Msh5GA). While this mutation was not expected to affect MutS\(\gamma\) complex formation, MutS\(\gamma\) foci do not accumulate during prophase I. However, most spermatocytes from Msh5GA/GA mice progress to late pachynema and beyond, considerably further than meiosis in Msh5\(-/-\) animals. At pachynema, Msh5GA/GA spermatocytes show persistent DSBs, incomplete homolog pairing, and fail to accumulate MutL\(\gamma\). Unexpectedly, Msh5GA/GA diakinesis-staged spermatocytes have no chiasmata at all from any CO pathway, indicating that a functional MutS\(\gamma\) complex is critical for all CO events regardless of their mechanism of generation.

KEYWORDS MutS homolog meiosis mouse crossing over homologous recombination crossover designation prophase I

INTRODUCTION

MSH5 (MutS homolog 5) belongs to the DNA mismatch repair (MMR) family of proteins that perform multiple DNA repair activities, most prominently the correction of mispaired bases that result from erroneous DNA replication (Modrich and Lahue 1996). Like other family members, MSH5 acts with a MutS homolog partner, specifically with MSH4, to form the MutS\(\gamma\) heterodimer (Bocker et al. 1999). Unlike other MutS heterodimers, MutS\(\gamma\) does not participate in mismatch correction in somatic cells, but instead acts exclusively during meiotic prophase I in budding yeast (Pochart et al. 1997), mice (Edelmann et al. 1999; de Vries et al. 1999; Kneitz et al. 2000; Santucci-Darmanin and Paquis-Fluckinger 2003), humans (Bocker et al. 1999), plants (Higgins et al. 2008), and worms (Zalevsky et al. 1999). Indeed, the heterodimer was named MutS\(\gamma\), with the “\(\gamma\)” referring to “germ cell” (Kolas and Cohen 2004). Importantly, mutation of either MutS\(\gamma\) subunit results in infertility in humans and mice (Edelmann et al. 1999; de Vries et al. 1999; Kneitz et al. 2000; Carlosama et al. 2017).

Prophase I is the defining stage of meiosis, encompassing the unique events that give rise to pairing and equal segregation of homologous chromosomes at the first meiotic division. In early prophase I, homologous chromosomes undergo a physical tethering process.
known as \textit{synapsis}. Synapsis is mediated by the proteinaceous structure called the Synaptonemal Complex (SC) whose status defines the sub-stages of prophase I: leptonema, zygonema, pachynema, diplonema, and diakinesis. Synapsis is dependent on, and facilitated by, homologous recombination, which is triggered by the formation of DNA double strand breaks (DSBs) by the topoisomerase-like SPO11 protein and its co-factors (Keeney et al. 1997; Baudat et al. 2000; Romanienko and Camerini-Otero 2000; Keeney 2008; Kim et al. 2016; Robert et al. 2016a; Robert et al. 2016b). DSBs ends undergo resection to reveal 3’ single-strand tails that become coated with the replication protein A (RPA) which protects the potentially fragile ssDNA molecule and impairs secondary structure formation. RPA is gradually replaced by the RecA family members, RAD51 and DMC1, which promote strand invasion to search for homology in opposing chromosomes (Hunter 2015; Gray and Cohen 2016). Strand invasion results in a nascent intermediate known as a displacement loop (D-loop) (Hunter 2015), which may be resolved via multiple distinct, yet overlapping, pathways that result in either a crossover (CO) or a non-crossover (NCO) (Gray and Cohen 2016). In mouse, the majority (approximately 90%) of the 250+ DSBs that form are processed to become NCOs (Cole et al. 2014), the remaining 10% being resolved as COs. In yeast, NCOs arise at temporarily earlier time points than do the CO repair products (Allers and Lichten 2001; Baudat and de Massy 2007; Jessop and Lichten 2008; Kaur et al. 2015).

COs can arise from several pathways downstream of DSB formation, and result in reciprocal exchange of DNA between maternal and paternal homologs, giving rise to the chiasmata that ensure equal segregation of chromosomes at the first meiotic division. Following D-loop formation, a metastable structure known as a single end invasion (SEI) arises, followed by second end capture of the other side of the DSB, to produce a double Holliday Junction (dHJ). These events are promoted through stabilization of the SEI structure by the ZMM group of proteins, of which the MutSy constituents are members, along with Zip1-4, Mer3, and Spo16 (Lynn et al. 2007). Once formed, the dHJ must then be resolved via the action of resolvases which cleave the dHJs to release the recombined homologous chromosomes. In mouse, this is the major Class I crossover pathway, accounting for 90% of all COs, and involves resolution of the dHJ by the Mut\textsubscript{L\gamma} heterodimer, consisting of the MMR proteins MLH1 and MLH3 (Edelman et al. 1996; Hunter and Borts 1997; Wang et al. 1999; Lipkin et al. 2002; Svetlanov et al. 2008; Nishant et al. 2008). In mouse, at least one other CO pathway has been described, known as the class II pathway. Class II events account for fewer than 10% of COs in the mouse and these are dependent on the MUS81-EME1 endonuclease (Oh et al. 2008; Holloway et al. 2008). This pathway does not involve canonical dHJ formation but instead may resolve a diverse set of repair intermediates that would not ordinarily be strong substrates for the class I machinery.

Mut\textsubscript{L\gamma} and MutSy are present on the SC during late pachynema, at a frequency and distribution that resemble class I CO numbers (Santucci-Darmanin and Paquiss-Fluckinger 2003). This suggests that, similar to other MMR complexes, MutSy functions to recruit Mut\textsubscript{L\gamma} to the SC during pachynema. However, MutSy foci first appear on meiotic chromosome cores in zygonema, prior to Mut\textsubscript{L\gamma} localization, and at frequencies that far exceed the final CO tally (approximately 150 foci, or 10-fold higher than the final Mut\textsubscript{L\gamma} count). These cytogenetic differences in MutSy/Mut\textsubscript{L\gamma} appearance suggest additional early functions for MutSy that are distinct from its interactions with Mut\textsubscript{L\gamma}. Indeed, the meiotic phenotype of mice lacking components of either complex underscores the temporally distinct roles for each heterodimer. Prophase I spermatocytes from \textit{Mlh\textsubscript{1}\textsuperscript{-/-}} and \textit{Mlh\textsubscript{3}\textsuperscript{-/-}} male mice show normal early progression of meiosis, with cells progressing all the way through prophase I. However, by diplonema, mostly univalent chromosomes are observed in these mutants, with a 90% reduction in chiasmata frequency and loss of spermatocytes prior to the first meiotic division (Edelman et al. 1996; Lipkin et al. 2002). By contrast, loss of \textit{Msh\textsubscript{4}} or \textit{Msh\textsubscript{5}} results in an earlier loss of prophase I progression, with almost complete failure of homologous synapsis, and cell death prior to pachynema (Edelman et al. 1999; de Vries et al. 1999; Kneitz et al. 2000). Thus, MutSy plays an essential role in early events of DSB repair prior to, and distinct from, its proposed role in recruiting Mut\textsubscript{L\gamma}.

\textit{In vitro} studies have demonstrated that the human and yeast MutSy heterodimer can bind to D loops, HJs, single-stranded overhangs and other DNA substrates (Snowden et al. 2004; Lahiri et al. 2018). Binding to junctions enhances stability of these structures, while binding to single-stranded DNA promotes displacement of the overhang that could potentially allow for nucleoprotein filament formation involving, for example, RPA (Lahiri et al. 2018). Like all MutS heterodimers, MSH4 and MSH5 each possess an ATPase domain that, upon substrate binding, promotes ADP to ATP exchange and subsequent formation of a sliding clamp that can encircle DNA and translocate away from the binding site, potentially allowing further rounds of MutSy binding and translocation (Snowden et al. 2004, 2008). To explore MSH5 ATPase function \textit{in vivo}, we mutated a highly conserved residue within the P-loop domain of mouse \textit{Msh5} (G to A mutation at residue 596, termed \textit{Msh5\textsuperscript{GA}}), which has been shown previously to affect ATP binding by MutS homologs. A similar mutation in \textit{S. cerevisiae} has no effect on the dimerization with its wild-type (WT) MSH4 partner, but reduces crossing-over and spore viability (Nishant et al. 2010). Based on this study, we anticipated that the G-to-A mutation within the MSH5 ATP binding domain would not affect MutSy complex formation. Interestingly, although spermatocytes in \textit{Msh5\textsuperscript{GA}} mice fail to progress beyond zygonema, a subset of \textit{Msh5\textsuperscript{CA/CA}} spermatocytes escape this fate, progressing through prophase I and entering metaphase I. Thus, this mutant allele allowed for the first time an investigation of the role of MSH5 in crossing over during the prophase I. Interestingly, diakinesis-staged chromosomes from spermatocytes of \textit{Msh5\textsuperscript{CA/CA}} mice show exclusively univalent chromosomes and a complete absence of chiasmata, including those residual chiasmata that would presumably arise from the class II CO (MUS81-EME1) pathway. Such residual chiasmata are always observed in mice lacking key class I CO mediators, such as \textit{Mlh\textsubscript{1}\textsuperscript{-/-}} and \textit{Mlh\textsubscript{3}\textsuperscript{-/-}} animals (Edelman et al. 1996; Lipkin et al. 2002; Kolas et al. 2005). These observations indicate that the ATPase domain of MSH5 is essential for MutSy activity early in DSB repair, and that mutation of this domain results in disrupted homolog interactions and aberrant DNA repair, leading to a failure to form any COs at the end of prophase I. Thus, loss of a functional MutSy complex impacts CO formation regardless of the chosen pathway for CO generation.

\textbf{MATERIALS AND METHODS}

\textbf{Generation of Msh5\textsuperscript{GA} mice}

The mouse \textit{Msh5} genomic locus was cloned from a P1 mouse ES cell genomic library (Genome Systems) (Edelman et al., 1999). A 3.6 kb genomic \textit{HindIII} fragment of mouse \textit{Msh5} spanning exons 17-25 was inserted into pBluescript SK vector. Positive clones were identified by PCR. The G596A mutation and an analytic \textit{BglII} restriction site, were generated by site-directed mutagenesis in exon 19. A loxp\textsuperscript{f} flanked PGK hygromycin/neomycin cassette was inserted into the \textit{MscI} site in intron 19. The targeting vector was linearized at the single \textit{NotI} site and electroporated into WW6 ES cells. After selection in hygromycin, resistant colonies were isolated and screened by PCR. Positive clones were identified and injected into C57BL/6j blastocysts to produce chimeric
animals. The PGK hygromycin/neomycin cassette was deleted by Cre-loxP-mediated recombination after mating of chimeric mice to Zp3Cre recombinase transgenic females (C57BL/6J). F1 offspring were genotyped and heterozygote animals were intercrossed to generate F2 homozygous mutant Msh5GAGA mice and appropriate controls. Previously generated Msh4+/+ and Msh5+/+ mice were used for cross breeding studies to provide Msh5+/- null mice for comparison (Edelmann et al. 1999; Kneitz et al. 2000). All Msh4+/-, Msh4-/-, Msh5+/-, Msh5-/- and Msh5GA/GA mice used in these studies were backcrossed more than 10 times onto a C57BL/6J genetic background. Due to loss of the allele, Msh5+/- null mice were not available in the latter half of these studies.

**Genotyping of Msh5GA mice**

Reverse transcription-PCR was performed on total RNA isolated from mouse tails with forward primer 5’ – AGACCTGCACTGTGAGATCCG – 3’ (5’-18d-3’) located in exon 16 and reverse primer 5’. TT-GGTGCTACAAAGACGTG-3’ located in exon 22 using the One Tube reverse transcription-PCR reaction kit (Roche) according to the manufacturer’s instructions. The following cycling conditions were used: 30 min at 50°C (1 cycle); 2 min at 94°C (1 cycle); 45 s at 60°C (1 cycle). The resulting 480 bp PCR product was subsequently restricted with BlpI.

**Care and use of experimental animals**

Mice were maintained under strictly controlled conditions of light and temperature, with ad libitum access to food and water. All experiments were conducted with prior approval from the Albert Einstein College of Medicine and Cornell Institutional Animal Care and Use Committees. At least six mice per genotype were used for all studies.

**Histological analysis and TUNEL staining of mouse testis**

Testes from 12-week old mice were fixed in Bouin’s fixative for 6 hr at room temperature or 10% formalin overnight at 4°C, and then washed in 70% ethanol. Fixed and paraffin-embedded tissues were sectioned at 5 μm. Hematoxylin and eosin (H&E) staining and TUNEL staining and were performed as described previously (Holloway et al. 2008, 2010), the latter using Apoptag-peroxidase kit (Millipore).

**Chromosome preparation and spreads**

The testes were decapsulated and incubated in hypotonic extraction buffer (HEB; 30 mM Tris, pH 8.2, 50 mM sucrose, 17 mM trisodium citrate dihydrate, 5 mM EDTA, 0.5 mM DTT, and 0.5 mM PMSF) for 1 hr on ice. About three to five millimeters length of seminiferous tubule was transferred into a drop of 20 μl hypotonic sucrose (100 mM, pH 8.2). After adding another drop of 20 μl of sucrose the tubule was macerated and the cell suspension was pipetted up and down for about 3-4 times. Remaining tubule fragments were removed from the cell suspension. Slides were coated with 1% paraformaldehyde containing 0.15% Triton X. 20 μl of the cell suspension were dispersed across the surface of one slide containing a layer of fixative. Slides were transferred to a humid chamber for 1-2 hr at room temperature and then allowed to air dry. Slides were washed three times for 3 min (0.4% Kodak Photo-Flo 200 in water) and air-dried and stored at -80°C until use, not longer than 2 weeks.

**Immunofluorescence**

The slides were washed in 0.4% Kodak Photo-Flo 200 in PBS and 0.1% Triton X-100 in PBS for 5 min each, blocked for 10 min in 10% antibody dilution buffer (ADB) in PBS (ADB: 3% bovine serum albumin, 0.05% Triton in 1 x PBS) followed by an overnight incubation in primary antibodies (at varying concentrations in ADB; Supplementary Table 1) at room temperature in a humid chamber. Slides were washed as described earlier and incubated for 1 h at 37°C in secondary fluorochrome conjugated antibodies in the dark. Primary and secondary antibodies used are listed in Supplementary Table 1. All secondary antibodies were raised specifically against Fc fraction, Fab-fraction purified and conjugated to Alexafluor 594, 488, or 647.

**FIJI Image J Macro for SYCP1 & SYCP3 track measurements**

An Image J macro was created using the available tools in ImageJ. Images were first converted to TIFF files, with DAPI in blue, SYCP3 in red, and SyCP1 in green. The script used was as follows:

```java
selectWindow(title);
setTool("freehand");
r直播("Clear Side");
r直播("Split Channels");
selectWindow(title + " (red)");
setAutoThreshold("RenyiEntropy dark");
r直播("Convert to Mask");
selectWindow(title + " (green)");
setAutoThreshold("RenyiEntropy dark");
r直播("Convert to Mask");
selectWindow(title + " (red)");
r直播("Skeletonize");
r直播("Analyze Skeleton (2D/3D)", "prune=[shortest branch] calculate show display");
selectWindow(title + " (green)");
r直播("Skeletonize");
r直播("Analyze Skeleton (2D/3D)", "prune=[shortest branch] calculate show display");
```

**Spermatocyte diakinesis spread preparations to observe chiasmata**

Diakinesis chromosome spreads were prepared as previously described (Holloway et al. 2008, 2014). Slides were stained with 20% Giemsa for 2.5 min, washed, air-dried and mounted with Permount.

**Data Availability**

All mice, plasmids, and reagents created as part of this study are available on request. Supplemental material available at Figshare: https://doi.org/10.25387/g3.7934312.

**RESULTS**

**Generation of Msh5G596A mutant mice**

We generated a mouse line bearing a mutation that disrupts the conserved Walker “type A” motif GXXXXGKS/T (G refers to the modified G596 amino acid residue) in the ATPase domain of MSH5, which is important for ATP binding (Figure S1A). The targeting vector introduces a glycine to alanine change at amino acid residue 596 into Msh5GA/GA mice and appropriate controls (Holloway et al. 2008). The subsequent generation of Msh5G596A mutant mice was generated in the Msh5 coding regions that overlaps with the mutation (Figure S1B). Transmission of the mutant Msh5GA allele was confirmed by PCR genotyping of genomic tail DNA and subsequent

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restriction of the associated Blp1 site (Figure S1C,D). Likewise, RT-PCR and subsequent Blp1 restriction digestion confirmed the expression of the mutant transcript in Msh4\(^{2/2}\) and Msh5\(^{2/2}\) mice (Figure S1D). In addition, while homologous null mice lack all detectable MSH5 protein, the mutant MSH5\(^{GA}\) protein signal was found in testis extracts from both Msh4\(^{2/2}\) and Msh5\(^{2/2}\) mice at levels similar to wildtype (WT) mice (Figure S1E). In all subsequent studies, Msh5\(^{2/2}\) mutant animals were compared to Msh5\(^{+/+}\) (WT) littermates, as well as Msh4\(^{2/2}\) and/or Msh5\(^{2/2}\) mice (Edelmann et al. 1999; Kneitz et al. 2000). All alleles of Msh4 and Msh5 were maintained on a C57Bl/6J background.

**Msh5\(^{GA/GA}\) mice exhibit severely impaired meiotic progression, reduced testis size, and no spermatooza**

Similar to Msh5\(^{-/-}\) and Msh4\(^{-/-}\) mice, Msh5\(^{GA/GA}\) mice are infertile as a result of defects in meiotic prophase I. By contrast, Msh5\(^{GA/+}\) females and males are fertile (not shown), with no change in testis weights in Msh5\(^{GA/+}\) males compared to WT littermates (Figure 1A). Homozygous mutant Msh5\(^{GA/GA}\) males display a 40% reduced testis size compared to their WT littermates (Figure 1A) associated with complete loss of epididymal spermatooza (Figure 1B). Sperm production in Msh5\(^{GA/+}\) males was similar to that of WT animals (Figure 1B).

Hematoxylin and Eosin (H&E) staining of testis sections from WT adult male mice showed normal cell populations within the seminiferous epithelium, while spermatogenesis was severely disrupted in Msh5\(^{GA/GA}\) testis sections (Figure 1C). Testis sections from Msh5\(^{GA/GA}\) male mice contained Leydig cells, Sertoli cells, and spermatagonia, and spermatocytes, along with a high proportion of TUNEL-positive apoptotic germ cells within the seminiferous tubules (Figure 1C). Most notably, testis sections from Msh5\(^{GA/GA}\) males contained pachytene and post-pachytene spermatocytes (Figure 1C, bottom panels, arrow), including cells that were clearly at metaphase I (Figure 1C, bottom arrows, asterisks). This is in contrast to our previous observations in Msh5\(^{+/+}\) and Msh4\(^{-/-}\) males, in which the majority of the spermatocyte pool is lost at or prior to entry into pachynema (Figure S2A) (Edelmann et al. 1999; Kneitz et al. 2000). Seminiferous tubules from WT and Msh5\(^{GA/+}\) males have an average of one or less than one TUNEL-positive cell per tubule section, while in Msh5\(^{GA/GA}\) males, TUNEL-positive cell frequencies were higher, at 2.7 TUNEL-positive cells per tubule (Figure S2B). The important difference between the histological appearance of Msh5\(^{GA/GA}\) tubules and that of Msh5\(^{-/-}\) and Msh4\(^{-/-}\) males is the increased progression into pachynema and the appearance of metaphase cells in the tubules of Msh5\(^{GA/GA}\) males.

A functional ATP binding domain of MSH5 is important for early homolog interactions and complete homolog synapsis

To assess progression through prophase I, immunofluorescence (IF) staining was performed on chromosome spreads of spermatocytes from Msh5\(^{+/+}\) and Msh5\(^{GA/GA}\) adult male mice using antibodies against components of the SC, SYCP3 and SYCP1 (Figure 2A). In leptotene, the SC begins to form, with SYCP3 localization appearing in a punctate pattern along synapsed chromosomes. Such a staining pattern was evident on leptotene chromosome preparations from mice of all genotypes (Figure 2A). Upon entry into zygonema, the transverse filaments and central element of the SC begin to assemble, as shown by the localization of SYCP1 between the chromosome axes on chromosome spreads from both Msh4\(^{+/+}\) and Msh5\(^{GA/GA}\) adult male mice (Figure 2A). By pachynema, when autosomes in Msh5\(^{+/+}\) adult males are now fully synapsed along their entire lengths, the first signs of synapsis failure become evident in Msh5\(^{GA/GA}\) animals.

While WT pachytene cells contain 20 discrete synapsed homologs, spermatocytes from Msh5\(^{GA/GA}\) animals show variable degrees of synapsis, coupled with frequent occurrences of inappropriate synapsis between more than two chromosome partners (Figure 2A, arrowheads), indicating non-homologous synapsis events between multiple chromosomes, but also some occurrences of apparently normal homolog synapsis. Thus, in order to stage these spermatocytes from Msh5\(^{GA/GA}\) animals, we defined certain criteria for each prophase I substage. Zygotene and diplotene spermatocytes, which often look similar, were distinguished based on the length of the SC (longer in zygonema), differences in telomeric ends of the chromosomes (more bulbous in diplonema), and by H1t localization (see below). A “pachytene-like” stage was defined as having ≥4 discrete synapsed chromosome pairs, either wholly or partially, along with a more condensed SC appearance across all chromosomes. Using these criteria, we observed many cells in a pachytene-like stage, and beyond, in Msh5\(^{GA/GA}\) animals. The aberrant synapsis phenotype observed in Msh5\(^{GA/GA}\) spermatocytes range in severity, with some pachytene-like cells showing synapsis defects across the majority of homolog pairs, while other pachytene-like cells showed defects among a few homolog pairs.

Utilizing Image J software, we obtained quantitative measurements of synapsis across our mouse model. For each cell, we measured the total track length of SYCP3 signal and compared it to the total track length of SYCP1 to obtain the percent synapsis (SYCP1/SYCP3 X 100). For this analysis, we used Msh4\(^{-/-}\) mice as a comparison with Msh5\(^{GA/GA}\) males because the original reports suggested slightly higher levels of synapsis than observed in Msh5\(^{-/-}\) mice, and because Msh5\(^{-/-}\) mice are no longer available. Since MSH4 and MSH5 always act as a hetero-dimer, Msh4\(^{-/-}\) mice reflect overall MutS\(\gamma\) function. Previous descriptions of Msh4\(^{-/-}\) males indicated no pachytene entry, an observation that was based on the 20 independently synapsed homologs as defined by WT pachytene. In the current study however, we defined pachytene-like as ≥4 synapsed or partially synapsed chromosomes. Under these criteria, we observe pachytene-like cells in both Msh4\(^{-/-}\) males and in Msh5\(^{GA/GA}\) males.

The average synapsis in WT spermatocytes during pachynema, remembering the XY chromosome pair in males is only synapsed at the autosomal region, is 86.5 ± 7.2% (Figure 2B) with Msh5\(^{GA/+}\) spermatocytes showing similar synapsis rates at 86.3 ± 4.2% (Figure 2B). Overall there is a remarkable degree of synapsis in Msh5\(^{GA/GA}\) animals, with spermatocytes exhibiting an average of 43.2 ± 12.4%, and some cells achieving up to 76% of chromosome axes. By contrast, synapsis in Msh5\(^{-/-}\) animals is less than 5% in two previous reports (Edelmann et al. 1999; de Vries et al. 1999). The level of synapsis in Msh5\(^{GA/GA}\) males is comparable to that of Msh5\(^{GA/GA}\) males, at 47.4 ± 12.7% synapsis. Synapsis in Msh4\(^{-/-}\) males was slightly lower than Msh5\(^{GA/GA}\) males, at 44.9 ± 11.3% synapsis (Figure 2B). Importantly, synapsis in Msh5\(^{GA/GA}\) spermatocytes is similar to that seen in Msh5\(^{GA/GA}\) homozygous mutant animals, while synapsis in Msh5\(^{GA/+}\) spermatocytes is similar to WT, indicating that the Msh5\(^{GA}\) allele is recessive and not causing a dominant negative effect.

To further assess the degree of synapsis in different mice, the number of independently synapsed homologs were counted in each pachytene-like cell from Msh5\(^{GA/GA}\), Msh5\(^{GA/+}\), and Msh4\(^{-/-}\) males (Figure S3). In none of these cases are cells from mutant testes able to achieve a wild-type pachytene configuration of 20 independently synapsed homologs. While some Msh4\(^{-/-}\) pachytene cells were only able to achieve as many as 12 independently synapsed homologs, only 4.8% of the pachytene-like population had more than 10 independently synapsed homologs. The degree of synapsis is significantly greater in Msh5\(^{GA/GA}\) and Msh5\(^{GA}\) spermatocytes, with instances of cells achieving up to
15 independently synapsed homologs occurring in each genotype, and Msh5GA/GA having 5.6% homologs having more than 10 independently synapsed homologs and Msh5GA/+ having 11.6% homologs having more than 10 independently synapsed homologs (Figure S3). Thus, we observe a greater degree of synapsis in spermatocytes from Msh5GA/GA or Msh5GA/+ males compared to that of Msh4−/− cells suggesting that the presence of the mutant MSH5GA protein allows for more proficient early homolog pairing and progression through later stages of prophase I. Thus, homolog pairing and/or synapsis initiation/progression does not rely on a fully functional MutSg heterodimer.

The histone marker, H1t, allows for differentiation of pachytene cells into “early” and “late”, since H1t only associates with the latter population (Wiltshire et al. 1995). Moreover, H1t positive staining allows for differentiation of pachytene cells into “early” and “late”, since H1t only associates with the latter population (Wiltshire et al. 1995). Moreover, H1t positive staining allows...
for differentiating between zygote and diplote-like cells in Msh5GA/GA males. Despite the incomplete synopsis and inappropriate synopsis between multiple chromosomes in spermatocytes from Msh5GA/GA animals, these cells are competent to achieve a mid-pachytene-like stage of meiosis, at least as assessed by acquisition of H1t signal (Figure 2C). Synapsis mutants (Msh5GA/GA, Msh5GA/−, Msh4−/−) do not achieve the normal 20 independently synapsed homologs as observed in WT. However, the localization of H1t to these mutants suggests that they achieve a pachytene-like stage. To compare prophase I populations across genotypes, we looked at the total number of H1t-positive cells in prophase I (Figure 2D). Across all prophase I cells in WT males, we observe that 52.3 ± 14.1% of cells are H1t-positive. Surprisingly, our mutant animals gave values similar to WT: in Msh5GA/GA animals we observe a 64.4 ± 10.1% H1t positive prophase I population, in Msh4−/− 63.2 ± 12.9%; Msh5GA 63.3 ± 5.8%. The Msh51/+ spermatocytes are the only population for which we observed a lower, albeit not statistically different H1t-positive prophase I pool of 40.0 ± 5.3%. Overall, we observed a comparable prophase I progression in Msh5GA/GA mutant spermatocytes and in Msh4−/− spermatocytes, although the degree of synopsis observed in these mutants is markedly different.

**MutSγ association with the synaptonemal complex is drastically reduced in spermatocytes From Msh5GA/GA males**

In WT mice, MSH4 and MSH5 localize on chromosome cores of the SC from zygonema through pachynema, with approximately 200 foci in zygonema, reducing progressively through until late pachynema.
We investigated whether MutSy localization on SCs was affected by loss of a functional ATP binding domain within MSH5. To this end, chromosome spreads from Msh5<sup>+/−</sup>, Msh5<sup>+/GA</sup>, and Msh5<sup>GA/GA</sup> male mice were subjected to IF staining using antibodies against MSH4 and SYCP3. MSH4 localization in early prophase I cells looks comparable between Msh5<sup>+/−</sup> and Msh5<sup>+/GA</sup> adult males, with abundant foci associated with early SC structures in zygonema (Figure 1D). Interestingly, in spermatocytes from Msh5<sup>GA/GA</sup> males, there appears to be a dramatically decreased association of MSH4 to the SC and an observable increase in MSH4 foci not associated with the SC in zygotene and pachytene nuclei (Figure 1D). Overall the intensity of MSH4 staining in zygotene and pachytene spermatocytes from Msh5<sup>GA/GA</sup> males is lower than that of WT littermates, although some foci are clearly associated with the SC at both zygonema and pachynema (Figure 1D, arrows). Further examples of MSH4 staining at this stage are provided in Figure S4 which provide additional evidence of a broader but fainter distribution of MSH4 signal in spermatocytes from Msh5<sup>GA/GA</sup> males.

The ATP binding domain of MSH5 is essential for timely progression of DSB repair events

To assess progression of DSB repair through prophase I, IF was performed on chromosome spreads from Msh5<sup>+/−</sup>, Msh5<sup>+/GA</sup>, and Msh5<sup>GA/GA</sup> adult littermates using antibodies against γH2AX, a phosphorylated histone variant that marks sites of DSB (Figure 3A). Spermatocytes from Msh5<sup>+/−</sup> animals show a strong γH2AX signal during leptotena and zygonema of prophase I indicating normal induction of DSBs, with loss of the γH2AX signal at pachynema signaling progression of DSB repair (Figure 3A). As expected, the γH2AX signal is intensified on the sex chromosomes at pachynema, a phenomenon that is not related to DSB formation (Turner et al. 2004); (Figure 3A, top row). Spermatocytes from Msh5<sup>GA/GA</sup> animals show a similarly strong γH2AX signal during leptotena and zygonema, indicating DSBs are induced at the expected time. Unlike in Msh5<sup>+/−</sup> cells, however, γH2AX signal is retained on autosomes throughout prophase I in Msh5<sup>GA/GA</sup> cells, indicating persistent DNA damage (Figure 3A, bottom row).

During DSB repair, one of the earliest common intermediate steps involves strand invasion and homology search, which is mediated by the RecA homologs, RAD51 and DMC1. MutSy has been suggested to participate in stabilization of these strand invasion events in vitro (Snowden et al. 2004). During leptotena in WT spermatocytes, RAD51 foci are observed on axial elements of the SC in high numbers (Figure 3B,C), and similar numbers of RAD51 foci are observed on leptotene spreads from Msh5<sup>GA/GA</sup> spermatocytes. As WT cells progress from zygonema to pachynema, RAD51 foci numbers drop dramatically, reflecting the repair of DSBs.

The RAD51 focus numbers in Msh5<sup>GA/GA</sup> and Msh5<sup>GA/−</sup> spermatocytes remain significantly elevated above that of WT spermatocytes throughout prophase I (P < 0.0001, Figure 3C). Interestingly, the RAD51 focus counts at zygonema and pachynema are significantly higher in Msh5<sup>GA/−</sup> spermatocytes than in homozygous mutant Msh5<sup>GA/GA</sup> spermatocytes (P < 0.0001, Figure 3C), indicating more DSB repair activity during this stage in the presence of only one copy of ATPase defective Msh5. At diplonema, WT spermatocytes have lost all RAD51 foci, but these foci remain significantly higher in Msh5<sup>GA/GA</sup> and Msh5<sup>GA/−</sup> spermatocytes, albeit at lower frequency to that seen in pachynema (P < 0.0001). At this stage, RAD51 counts in Msh5<sup>GA/GA</sup> and Msh5<sup>GA/−</sup> spermatocytes are not statistically different from each other. Importantly, spermatocytes from Msh5<sup>GA/−</sup> males are similar to WT with few abnormalities and normal dynamics of RAD51 loss (Figure 3C, S3B).

Taken together, these data demonstrate the presence of the ATP binding-defective MSH5<sup>GA</sup> protein is critical for normal progression of DSB repair. Alternatively, it is possible that the high rate of RAD51 foci observed at pachynema in Msh5<sup>GA/GA</sup> results from additional induction of DSBs through prophase I, but the current tools preclude our ability to differentiate between these two options. Importantly, the presence of only one GA allele on a WT background (Msh5<sup>GA/−</sup>) results in normal temporal dynamics of RAD51 loss, while the presence of one GA allele on a null background (Msh5<sup>GA/−</sup>) results in a significantly more delayed processing of DSBs, as characterized by RAD51 accumulation and loss. These observations argue strongly against a dominant negative effect of the GA point mutation.

An intact MSH5 ATP binding domain is essential for formation of all classes of crossover

MutSy recruits the Mut-Lγ complex during pachynema as part of a canonical class I CO machinery. IF staining using antibodies against MLH3 was compared across genotypes (Figure 4A). In WT and Msh5<sup>GA/−</sup> mice during pachynema, MLH3 appears on chromosome cores at a frequency that correlates with final class I CO numbers (Figure 4A, top row), but is absent in spermatocytes from Msh5<sup>GA/−</sup> males (Figure S5B). In pachytene-like spermatocytes from Msh5<sup>GA/GA</sup> males, MLH3 foci do not form on chromosome cores (Figure 4A). Occasional very faint signal was observed throughout the chromatin, as well on the SC cores, when the microscope intensity gain is increased, but it was not possible to obtain reliable images depicting this weak signal. Nonetheless, such staining was never observed in chromosome spread preparations from Msh5<sup>GA/−</sup> males, suggesting that this weak staining might be specific for MLH3 protein (Figure S5B). Given the diffuse and faint nature of this staining, it cannot be determined if this MLH3 signal is associated with sites of DSB repair. Thus, a fully functional MSH5 protein is required for appropriate association of the Mut-Lγ with the synaptonemal complex and establishment of nascent class I CO sites.

To assess crossing over across the genome, diakinesis spreads were prepared to assess chiasma formation (Holloway et al. 2010). In WT males, each bivalent chromosome pair had at least one chiasma (Figure 4B). Since a small number of spermatocytes from Msh5<sup>GA/GA</sup> males are capable of progressing into diakinesis, we were able to count chiasmata in these homozygous mutant mice (Figure 4B,C). Unexpectedly, diakinesis-staged cells from Msh5<sup>GA/GA</sup> males displayed exclusively univalent chromosomes and did not form any chiasma (Figure 4B,C). Thus, normal MSH5 ATP processing is essential for all crossover formation in mammals. Such analysis has not been possible in Msh5<sup>GA/−</sup> males because spermatocytes from these mice fail to reach diakinesis, and die predominantly in zygonema.

Discussion

The data presented herein demonstrate that intact MutSy function is required for normal prophase I progression in male meiosis. Importantly, this work is the first to show a definitive requirement for an intact MutSy heterodimer in crossing over in the mouse and, unexpectedly, that MutSy is critical for all crossovers regardless of their route of generation from DSB precursors. These observations were made possible by the fact that the mutation in the MSH5 ATP binding domain can allow for limited progression through to the end of prophase I, whereas most spermatocytes from Msh5<sup>GA/−</sup> mice die prior to pachynema (Edelmann et al. 1999; de Vries et al. 1999). Mutation of the ATP binding domain within Msh5 results in normal DSB induction but prolonged RAD51 installation on chromosome cores, either due to delayed DSB repair or due to extended DSB initiation through...
prophase I. As a result, we demonstrate a greater degree of synapsis observed in spermatocytes from Msh5GA/GA or Msh5GA/− males compared to that of Msh4−/− cells, suggesting that the presence of the MSH5GA protein allows for more proficient early homolog pairing, or that the SC is established more robustly in the presence of defective MutSg heterodimer than in the complete absence of any heterodimer.

Data presented herein also demonstrate altered distribution of MutSy throughout the nucleus of Msh5GA/GA prophase I spermatocytes, with significant localization off the SC, and a reduction in overall MSH4 signal on chromosome cores. These results indicate that the MSH5 ATP binding domain is essential for the recruitment and retention of MutSy on SC cores from zygonema through until pachynema. Loss of ATP binding in Msh5GA/GA mutants is predicted to result in a clamp protein that is unable to slide along DNA, and thus is unable to allow successive rounds of MutSy loading. In our Msh5GA/GA mutants we see a dramatic reduction in MSH4 signal along chromosome cores, suggesting either minimal loading of MutSy complex onto the DNA and/or enhanced (but not complete) degradation of the complex. Thus, the low amount of MSH5GA-MSH4 heterodimer that can associate with the SC may still provide some stabilization between homologs, allowing for small amounts of synapsis in Msh5GA/GA animals. However, without the MSH5 ATP domain, the normal function of MutSy in SC establishment and/or DSB repair processing is abolished. Taken together, we conclude that early DSB repair events and synapsis are perturbed in our Msh5GA/GA mutants, but that some progression remains possible. Importantly, these observations suggest that the ATP binding domains of both MutSy subunits must be intact in order to facilitate a complete repertoire of MutSy functions, which is not

**Figure 3** DNA damage persists in Msh5GA/GA spermatocytes throughout prophase I. (A) Immunofluorescent staining of γH2AX (red) on chromosome spreads of Msh5+/+ and Msh5GA/GA littermates. (B) DNA repair marker RAD51 (red) on Msh5+/+ and Msh5GA/GA chromosome spreads persists throughout prophase I. (C) Quantitation of RAD51 foci associated with the SC of chromosome spreads during leptonema (n = 13 and 14, respectively, for Msh5+/+ and Msh5GA/GA males; P = 0.88 by Mann-Whitney), zygonema (n = 30 and 29, respectively; P = 0.14 by Mann-Whitney), pachynema (n = 26 and 35, respectively; P < 0.0001 by Mann-Whitney), and diplonema (n = 11 each; P < 0.0001 by Mann-Whitney). RAD51 counts were also assessed for Msh5GA− males at each stage and were significantly different to that of Msh5GA/GA males at zygonema and pachynema (P < 0.0001 by Mann-Whitney), and statistically different to Msh5+/+ males at all stages (P < 0.0001 by Mann-Whitney) except leptonema (P = 0.86 by Mann-Whitney).
Figure 4  No crossovers form in Msh5GA/GA spermatocytes. (A) Immunofluorescence staining of MLH3 (green) on SYCP3-stained SC cores (red) in adult pachytenic Msh5+/+ and Msh5GA/GA spermatocytes show localization of MLH3 to SC as expected in wild type and no MLH3 localization to the SC in Msh5GA/GA males. (B) Giemsa staining of diakinesis preparations from Msh5+/+ and Msh5GA/GA littermates showing normal chiasmata in wild type cells, with 20 bivalent chromosomes, and all univalent chromosomes in spermatocytes from Msh5GA/GA males. (C) Chiasmata counts for Msh5+/+ (n = 22), Msh5vGA/ (n = 23), and Msh5GA/ (n = 15) littermates (P < 0.0001, unpaired t-test). Each circle symbol represents a different cell, while the red overlay lines depict the average ± SD.

surprising given the fact that MSH5 has been shown to bind ATP with a higher affinity than MSH4 (Snowden et al. 2008).

In the mouse, MutSα accumulation on SCs in zygonema is in excess of the final number of MutLβ foci, but the two heterodimeric complexes are shown to localize at similar frequencies by late pachytena, albeit with number of MutSα foci remaining slightly higher than MutLβ (Novak et al. 2001; Santucci-Darmanin and Paquis-Fluckinger 2003). The earlier and more abundant localization of MutSα in zygonema implies that MutLβ is recruited to only a subset of MutSα sites upon entry into pachytena, with the remaining sites that fail to accumulate MutLβ presumably being processed to become NCO events via other repair pathways. Thus, the higher numbers of MutSα foci in zygotene and early pachytenic mouse spermatocytes, together with the earlier loss of spermatocytes in Msh5−/− animals compared to Mlh3−/− or Mlh1−/− mice, implies a role for MSH4 and MSH5 in DSB processing at an early intermediate stage for multiple repair pathways. Such a possibility is supported by our data showing that diakinesis preps from Msh5GA/GA spermatocytes display no chiasmata (Figure 4), which indicates that a functional MutSα complex is essential for all CO, acting at a stage that is upstream of both class I and class II CO designation, and thus may be a common intermediate for all CO pathways early in prophase I. Indeed, both class I and class II crossovers arise from a common DNA repair intermediate structure downstream of RAD51/DMC1 activity.

While the class II CO pathway, which in mice involves MUS81-EME1 (Holloway et al. 2008; Schwartz and Heyer 2011), is not traditionally viewed to be dependent on the ZMM class of proteins, and persists in mice lacking either Mlh1 or Mlh3, our data indicate that a functional MSH5 protein is required to promote both classes of CO. Conversely, while we briefly considered the possibility that the mutant MutSα complex may bind irreversibly to DSB repair intermediates that might otherwise have been processed via the Class II pathway, thus blocking the recruitment of appropriate class II repair factors, this does not appear to be the case since severely reduced MSH4 signal is observed on the SC, while no meiotic phenotype is observed in Msh5GA/ males, arguing against a dominant negative effect. Thus, loss of appropriate loading of MutSα on the SC is sufficient to prevent any CO processing, regardless of the pathway of repair. This argues against current dogma that states that ZMM proteins, of which MSH4 and MSH5 are family members, do not operate outside of the class I machinery. While our current data do not currently provide a mechanism by which MutSα can orchestrate both CO pathways in mammals, studies from other organisms provide interesting insight into potential mechanisms. In *Tetrahymena thermophila*, for example, which has no SC, COs are exclusively of the class II variety, requiring Mus81-Mms4, but not the canonical ZMM family. Despite the absence of class I CO events, MSH4 and MSH5 are essential for appropriate CO levels in this species, leading to the conclusion that these proteins function outside (or upstream) of the canonical class I CO pathway (Shodhan et al. 2014) (Figure 5).

In SC-bearing organisms, where class I and class II CO events occur in tandem to differing degrees, ZMM proteins appear to function exclusively in the metabolism of the former class of COs. In *S. cerevisiae*, CO assignment occurs prior to SC assembly, and the number of MSH5 foci observed in this species corresponds well with the final tally of class I COs (Agarwal and Roeder 2000) (Figure 5). However, this does not appear to be the case for organisms such as *C. elegans*, in which only class I COs occur. Yokoo et al. have
proposed that the installation of MSH-5 in worms represents a “CO licensing” stage during which the protein initially accumulates at a supernumerary frequency along the chromosome cores (Yokoo et al. 2012). These foci then diminish in number as the cell progresses through pachynema in C. elegans, accumulating the pro-crossover factor COSA-1 only once the final number of class I events is achieved. Thus, the final appearance of COSA-1 and MSH-5 bound foci at six sites across the worm genome represents the final “designation” of presumptive class I CO sites (Yokoo et al. 2012) (Figure 5).

In the mouse, the same excessive number of MutSγ foci appear somewhat earlier in prophase I, at or soon after the completion of the axial elements in early zygonema, and these too get pared down through zygonema and pachynema coincident with the progression of CO designation. Loss of the entire MSH5 protein results in a failure to accumulate MutSγ or to complete synopsis in zygonema, resulting in cell death prior to pachynema or, at the very most, aberrant progression through pachynema (Edelmann et al. 1999; Knietz et al. 2000). Thus in the mouse, CO licensing is tightly linked to appropriate synopsis and may reflect the requirement for distinct rearrangements in SC architecture by the MutSγ complex, similar to that proposed for C. elegans (Pattabiraman et al. 2017). However, in the current study, we find that loss of a functional ATPase domain in one component of MutSγ, MSH5, allows for partial synopsis implying that any structural changes to the SC can be orchestrated in the absence of full ATPase activity of the MutSγ complex. Under such circumstances, all COs are lost, regardless of their final pathway of biogenesis. Thus, CO processing through both the class I and class II pathways is dependent on a fully functional MutSγ heterodimer, but may not be dependent on any SC changes induced by MutSγ in zygonema.

Our data suggest either that functional activity of class II machinery depends on the presence and processing of class I COs (an indirect requirement perhaps involving more discrete localized changes in the SC state at the DSB site), or that loading of class II pathway mediators requires the presence of MutSγ at these sites (a direct requirement for loading of MutSγ prior to recruitment of class II repair factors). In either case, this would infer that MutSγ is required for CO licensing for both pathways and/or lies upstream of the licensing decision. This is not surprising given that, in the mouse, no fewer than 60% of the DSB sites become loaded with MutSγ (or 150 out of 250), and only a minor fraction of these licensed sites (approximately 20%) will become COs of the class I or class II variety (Knietz et al. 2000; Cole et al. 2012). Thus, there is an over-abundance of available sites for crossing over and, suggesting that MutSγ loads as efficiently onto NCO-destined DSB repair intermediates as it does onto CO-destined DSB repair intermediates. Though the implication of this promiscuous MutSγ binding is not yet understood, it suggests that, while CO licensing in worms is achieved by MSH-5 association, this may not be the case in the mouse since MutSγ association with DSB repair intermediates appears to be more promiscuous than in worm and yeast.

Taken together, our analysis of a point mutant mouse for Msh5 has allowed us for the first time to explore late prophase I roles for MSH5 in DSB repair and homologous recombination. Our observations demonstrate that the large number of MutSγ sites found in early prophase I may serve as intermediates for both class I and class II CO events, and indeed for NCO events. Moreover, unlike the situation in yeast, the early loading of MutSγ in mouse spermatocytes suggests progressive NCO formation through prophase I. Given that MutLγ is restricted to class I CO events, these data suggest a functional distinction between the roles of MutSγ and MutLγ in DSB repair during mammalian meiosis, and open the door for additional roles for MutSγ in orchestrating/overseeing DSB repair in the mammalian germline. In light of the role of other heterodimeric MutS complexes in recruiting a diverse array of repair pathways, we envisage that MutSγ serves a similar purpose in the context of DSB repair during mammalian meiosis, serving as a point of dialog between multiple repair pathways to achieve genome stability.

ACKNOWLEDGMENTS

The authors acknowledge, with extreme gratitude, the technical support of Mr. Peter L. Borst. We thank Dr. Uwe Werling for help in generating the mutant mouse line, and we thank the members of the Cohen lab for their technical advice and for critical feedback on.
Pattabiraman, D., B. Roelens, A. Woglar, and A. M. Villeneuve, 2017 Meiotic recombination modulates the structure and dynamics of the synaptonemal complex during C. elegans meiosis. PLoS Genet. 13: e1006670. https://doi.org/10.1371/journal.pgen.1006670

Pochart, P., D. Woltering, and N. M. Hollingsworth, 1997 Conserved properties between functionally distinct MutS homologs in yeast. J. Biol. Chem. 272: 30345–30349. https://doi.org/10.1074/jbc.272.48.30345

Robert, T., A. Nore, C. Brun, C. Maffre, B. Crimi et al., 2016a The TopoVIB-Like protein family is required for meiotic DNA double-strand break formation. Science 351: 943–949. https://doi.org/10.1126/science.aad5309

Robert, T., N. Vrielynck, C. Mézard, B. de Massy, and M. Grelon, 2016b A new light on the meiotic DSB catalytic complex. Semin. Cell Dev. Biol. 54: 165–176. https://doi.org/10.1016/j.semcdb.2016.02.025

Romanienko, P. J., and R. D. Camerini-Otero, 2000 The mouse Spo11 gene is required for meiotic chromosome synapsis. Mol. Cell 6: 975–987. https://doi.org/10.1016/S1097-2765(00)00097-6

Santucci-Darmanin, S., and V. Paquis-Flucklinger, 2003 Les homologues de MutS et de MutL au cours de la méiose chez les mammifères. Med. Sci. (Paris) 19: 85–91. https://doi.org/10.1051/medsci:200319185

Schwartz, E. K., and W.-D. Heyer, 2011 Processing of joint molecule intermediates by structure-selective endonucleases during homologous recombination in eukaryotes. Chromosoma 120: 109–127. https://doi.org/10.1007/s00412-010-0304-7

Shodhan, A., A. Lukaszewicz, M. Novatchkova, and J. Loidl, 2014 Msh4 and Msh5 function in SC-independent chiasma formation during the streamlined meiosis of Tetrahymena. Genetics 198: 983–993. https://doi.org/10.1534/genetics.114.169698

Snowden, T., S. Acharya, C. Butz, M. Berardini, and R. Fishel, 2004 hMSH4-hMSH5 recognizes Holliday Junctions and forms a meiosis-specific sliding clamp that embraces homologous chromosomes. Mol. Cell 15: 437–451. https://doi.org/10.1016/j.molcel.2004.06.040

Snowden, T., K.-S. Shim, C. Schmutte, S. Acharya, and R. Fishel, 2008 hMSH4-hMSH5 adenosine nucleotide processing and interactions with homologous recombination machinery. J. Biol. Chem. 283: 145–154. https://doi.org/10.1074/jbc.M704060200

Svetlanov, A., F. Baudat, P. E. Cohen, and B. de Massy, 2008 Distinct functions of MLH3 at recombination hot spots in the mouse. Genetics 178: 1937–1945. https://doi.org/10.1534/genetics.107.084798

Turner, J. M. A., O. Aprilekova, X. Xu, R. Wang, S. Kim et al., 2004 BRCA1, histone H2AX phosphorylation, and male meiotic sex chromosome inactivation. Curr. Biol. 14: 2135–2142. https://doi.org/10.1016/j.cub.2004.11.032

Wang, T. F., N. Kleckner, and N. Hunter, 1999 Functional specificity of MutL homologs in yeast: evidence for three Mlh1-based heterocomplexes with distinct roles during meiosis in recombination and mismatch correction. Proc. Natl. Acad. Sci. USA 96: 13914–13919. https://doi.org/10.1073/pnas.96.24.13914

Wiltshire, T., C. Park, K. A. Caldwell, and M. A. Handel, 1995 Induced premature G2/M-phase transition in pachytene spermatocytes includes events unique to meiosis. Dev. Biol. 169: 557–567. https://doi.org/10.1006/dbio.1995.1169

Yokoo, R., K. A. Zawadzki, K. Nabeshima, M. Drake, S. Arur et al., 2012 COSA-1 reveals robust homeostasis and separable licensing and reinforcement steps governing meiotic crossovers. Cell 149: 75–87. https://doi.org/10.1016/j.cell.2012.01.052

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

Communicating editor: K. McKim