Published in final edited form as:

DNA Repair (Amst). 2016 February ; 38: 84–93. doi:10.1016/j.dnarep.2015.11.024.

Roles for mismatch repair family proteins in promoting meiotic crossing over

Carol M. Manhart and Eric Alani*

Department of Molecular Biology and Genetics, Cornell University, 457 Biotechnology Building, Ithaca, NY 14853-2703, USA

Abstract

The mismatch repair (MMR) family complexes Msh4-Msh5 and Mlh1-Mlh3 act with Exo1 and Sgs1-Top3-Rmi1 in a meiotic double strand break repair pathway that results in the asymmetric cleavage of double Holliday junctions (dHJ) to form crossovers. This review discusses how meiotic roles for Msh4-Msh5 and Mlh1-Mlh3 do not fit paradigms established for post-replicative MMR. We also outline models used to explain how these factors promote the formation of meiotic crossovers required for the accurate segregation of chromosome homologs during the Meiosis I division.

Keywords

meiosis; crossing over; Msh4-Msh5; Mlh1-Mlh3; Holliday junction resolution

1. Post-replicative DNA mismatch repair in prokaryotes

DNA mismatch repair (MMR) is a highly conserved mechanism that acts during DNA replication to remove DNA misincorporation errors that result in base-base and loop mismatches. Mutations in MMR genes confer increased mutation rates and have been linked in humans to Lynch syndrome (reviewed in [1,2] and in [3,4], this issue). In *Escherichia coli*, mismatches resulting from DNA polymerase misincorporation events are recognized by MutS. In an ATP-dependent reaction, MutS recruits MutL to activate MutH, a latent methylation-sensitive endonuclease that nicks the newly replicated and transiently unmethylated strand at distances up to several kilobases from the mismatch. The resulting nick promotes excision, polymerization, and ligation, resulting in removal of the mismatch (reviewed in [1] and in [5,6], this issue).

*Corresponding author at: Department of Molecular Biology and Genetics, Cornell University, 459 Biotechnology Building, Ithaca, NY 14853-2703, Tel.: 607-254-4811. eea3@cornell.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Conflict of Interest Statement

The authors declare that there are no conflicts of interest.

2. Eukaryotic MMR

Multiple MutS and MutL homologs (MSH and MLH proteins, respectively) have been identified in eukaryotes, however, no MutH homolog or methylation-dependent nuclease activity has been detected, suggesting that strand discrimination occurs through a different mechanism than seen in *E. coli* (see below). In *Saccharomyces cerevisiae*, base-base and small insertion/deletion mismatches are primarily recognized by the Msh2-Msh6 complex, while large insertion/deletion loops are recognized by Msh2-Msh3 heterodimers [2,7–10] (reviewed in [11] and in [12], this issue). Though not an absolute requirement for MMR, MSH complexes can interact with the replication fork via the replicative processivity clamp (proliferating cell nuclear antigen—PCNA) [13] (and reviewed in [14,15], this issue). In yeast, the Mlh1-Pms1 heterodimer (Mlh1-Pms2 in humans) is the primary MLH component in MMR and is recruited by MSH complexes to mismatched DNA. This interaction initiates downstream excision and re-synthesis steps through either Exo1-dependent or Exo1-independent pathways (reviewed in [16]). There is also a minor MMR pathway that involves mismatch recognition by Msh2-Msh3, followed by interaction with Mlh1-Mlh3, a heterodimer that participates minimally in MMR, but plays a major role in meiotic recombination [17].

MSH proteins form ring-like structures that encircle mismatched and homoduplex DNA [10,18–23] (and reviewed in [6], this issue). These proteins contain highly conserved ATP binding domains. Biochemical, structural, and biophysical studies have shown that MSH conformation and DNA binding are modulated by ATP binding and hydrolysis [24–34]. Differences in nucleotide affinities between MSH subunits have led to a model proposing that in the absence of a mismatch, ADP and ATP are bound to MSH subunits at high and low affinity sites, respectively, with the MSH complex showing a weak affinity for DNA due to the opening and closing of the MSH ring triggered by asymmetric ATP hydrolysis (Figure 1A) [24–34] (and reviewed in [12], this issue). When the MSH complex encounters a mismatch, ADP rapidly dissociates from one subunit. Both subunits are then occupied by ATP, causing the dimer to change conformation and enter a sliding clamp mode (Figure 1B). The ATP-bound sliding clamp complex is then able to recruit downstream repair factors, such as MLH complexes (Figure 1B). Upon interaction with downstream repair signals that presumably initiate excision steps, the MSH-MLH sliding clamp undergoes ATP hydrolysis followed by recycling of MSH complexes.

Structural and biophysical studies on bacterial MutL, yeast Mlh1-Pms1, and human Mlh1-Pms2 have suggested that like the MSH proteins, MLH dimers also encircle DNA [35–41]. Each MutL or MLH monomer consists of a globular N-terminal and C-terminal domain (NTD and CTD, respectively) connected by a flexible linker arm. The NTD of MutL and MLH proteins contains a conserved nucleotide binding site, while the CTD is essential for MLH protein dimerization. In *E. coli*, MutL functions as a matchmaker to facilitate interaction between MutS and the MutH endonuclease. Interestingly, certain bacterial MutL, primarily those that lack MutH strand discrimination mechanisms, and a subset of the eukaryotic MLH proteins display endonuclease activities and contain highly conserved metal-binding motifs (DQHA(X)₂E(E)₄E) in their CTDs that are required for this activity. Two of the four yeast MLH proteins, Pms1 and Mlh3, contain this conserved motif, and

both Mlh1-Mlh3 and Mlh1-Pms1, as well as their human homologs, display endonuclease activity on double stranded DNA [42–46].

Why do a subset of MLH proteins contain an endonuclease activity? Biochemical studies by Modrich and colleagues [43,44] (and reviewed in [47], this issue) have shown that this activity introduces nicks into newly replicated DNA that are used as entry points for exonucleases to promote 5' or 3' directed MMR. This activity may also partially facilitate strand discrimination for downstream repair. The mechanisms that direct the endonucleases to preferentially nick the daughter strand over the template in regions near a mismatch are not fully understood, but interactions within and between MLH proteins and DNA replication components are likely to be important. This is supported by the following: (1) Full length but not the CTD of *B. subtilis* MutL displays *in vitro* endonuclease activity [48]; (2) Structural studies involving *B. subtilis* MutL and yeast Mlh1-Pms1 suggest that MLH proteins undergo large conformational changes that involve nucleotide cofactors, the NTD, the CTD, unstructured linker arm domains, and DNA [40,48,49]; (3) Interactions have been identified between MutL/MLH complexes, the processivity clamp/clamp loader, MSH complexes, and pre-existing discontinuities in the daughter strand [43–45,50,51]; and (4) human Mlh1-Pms2 endonuclease activity is strand-specifically activated by PCNA when properly oriented by the clamp loading complex [43,51].

3. MMR roles for MSH and MLH complexes in meiosis

In addition to the roles outlined in post-replicative MMR, eukaryotic MMR proteins play critical roles in regulating genetic recombination, maintaining genome stability, and expanding trinucleotide repeat sequences (reviewed in [11,52–54]). This review focuses on the role of MMR proteins in facilitating crossing over between homologs to ensure proper chromosome segregation during the first meiotic division. Failures in this process can result in aneuploidy syndromes (e.g. Down, Turner syndromes) resulting from chromosomal nondisjunction events occurring primarily at the first meiotic division (Figure 2) [55].

3.1 Double strand break repair mechanisms

Crossing over between the homolog pairs in meiotic prophase results in the formation of chiasmata that allow for proper chromosome alignment and segregation during the first meiotic division (Figure 2; reviewed in [56]). Failure to form at least one crossover per chromosome pair, referred to as the obligate crossover, can lead to high levels of nondisjunction. As outlined below, extensive studies have been performed to understand how crossing over is controlled and distributed.

In *S. cerevisiae*, the recombination process leading to crossover formation begins in early meiotic prophase with the formation of ~150-200 programmed double strand breaks (DSBs) distributed throughout the genome (Figure 3) [57–65]. DSBs are resected, creating 3' single-stranded tails that are coated with the single-strand binding protein RPA. These tails can invade the double-stranded homologous template in a reaction catalyzed by Rad51, Dmc1, Rad52, and Rad54, creating a displacement-loop (D-loop). Stable heteroduplex DNA intermediates created in this pathway are known as single-end invasion intermediates (SEIs) [66]. Models predict that SEIs, which in baker's yeast are biased to form between homologs,

rather than sister chromosomes [67], can be processed by a number of mechanisms outlined in Figure 3. SEIs can be disassembled by the Sgs1-Top3-Rmi1 helicase/topoisomerase complex (see section 5.2) [68–70], producing non-crossover products by the synthesis-dependent strand-annealing pathway (SDSA), or can be further processed by a second-end capture mechanism facilitated by RPA, Rad52, and Rad54 [71–78], ultimately maturing into a dHJ intermediate. *In vivo* studies suggest that dHJs stabilized by a family of proteins termed the ZMMs (described in section 4.1) are resolved in a biased orientation producing crossover products (Figure 3B) [79–82]. In yeast, a small percentage of crossovers form independently of the ZMM pathway (see section 5 below; Figure 3C). It is important to note that evidence for the recombination intermediates described above has been provided by a combination of genetic, molecular (two-dimensional gel electrophoresis), and biochemical studies [66,67,83].

3.2 MMR in double strand break repair: meiotic gene conversion and heteroduplex rejection

DNA mismatches are formed in heteroduplex DNA during double-strand break repair (DSBR) when the donor and acceptor molecules differ in sequence. The repair of these mismatches by the MMR machinery has been well studied and results in gene conversion when the MMR machinery repairs mismatches on one chromosome to sequences present on the other (reviewed in [79,84,85]). Yeast tetrad analysis and high-throughput DNA sequencing of meiotic progeny have provided valuable insights into how this process occurs [79,85–90]. Numerous studies in yeast have monitored the segregation of genetic markers as a function of their position relative to an initiating DSB site (gene conversion gradient). This relationship is linear for several loci, where there are high levels of aberrant segregation of markers near the DSB site and low levels further away. Strains deficient in MMR (null mutations in *MSH2*, *PMS1*, or *MLH1*) display gene conversion gradients with a more gradual slope; as the distance from the DSB increases, the frequency of aberrant segregations decreases by a smaller amount compared to MMR-proficient strains [85,90–92]. This observation has given rise to a model where discontinuities in DNA direct the orientation of repair analogous to strand-discrimination steps proposed for post-replicative repair. At sites near the initial DSB, the break directs repair to form gene conversions, but mismatches more distant from the DSB are repaired either randomly or are directed by nicks formed through the activity of Holliday junction resolvases to yield a higher frequency of restoration events. It is also likely that the frequency of heteroduplex formation contributes to gradient formation [91]. In addition to their roles in meiotic pathways that result in gene conversion, MMR proteins prevent recombination between divergent DNA sequences (reviewed in [85,93]). This meiotic role is similar to the anti-recombination role seen for MMR proteins during DSBR in non-meiotic cells [94,95] (and reviewed in [96], this issue).

4. A majority of meiotic crossovers depend on a subset of MSH and MLH complexes

As outlined above, eukaryotic MSH and MLH complexes play essential roles in recognizing mismatches in heteroduplex DNA and the subsequent promotion of downstream repair events, including gene conversion and heteroduplex rejection. In addition to these canonical

roles, in some organisms, e.g. *S. cerevisiae* and mammals, a subset of MSH and MLH proteins participate directly in crossover-promoting processes in roles distinct from their MMR functions. These are the Msh4-Msh5 heterodimer, which has no role in the recognition or repair of mismatches, but is vital in meiosis [97–100], and Mlh1-Mlh3, which has a minor role in MMR, but is critical to maintain wild-type levels of crossing over [82,83,101–103].

4.1 Meiotic roles for Msh4-Msh5

Msh4-Msh5 is a member of the ZMM (Zip1-4, Mer3, and Msh4, 5) group of meiotic proteins needed to form crossovers. Early in meiosis, pairing between homologous chromosomes is initiated by strand invasion events facilitated by the synaptonemal complex, composed of proteins that form a zipper-like structure along the lengths of homologous chromosomes (reviewed in [104]). *zmm* mutants are defective in synaptonemal complex formation, suggesting a role for ZMM proteins in their assembly. Additionally, *zmm* mutants have reduced numbers of crossovers, and localization studies suggest that ZMM proteins localize to sites of recombination, and specifically mark sites for crossing over [83,105–109]. Also in baker's yeast, expression of ZMM proteins, including Msh4-Msh5, is specifically induced in meiosis [97,98,110]. These observations suggest roles for ZMM proteins in chromosome pairing and crossover formation.

Msh4 and Msh5 share homology with four of the five domains present in other MSH proteins including the structural domain containing the ATP-binding site. These proteins, however, lack domain I, one of two domains critical for the binding of MSH proteins to mismatch DNA [20,97,98]. Genetic studies have shown that Msh4 and Msh5 are essential for maintaining wild-type levels of crossing over [97–99,102], and Msh4 and Msh5 have early and late meiotic roles. Work in *Sordaria* suggested that Msh4 can act upstream of the other ZMM proteins during the leptotene stage of meiotic prophase to facilitate chromosome pairing [111]. In budding yeast, Msh4-Msh5 has been shown to stabilize strand invasion intermediates in the later zygotene stage [105]. In both mouse spermatocytes and in human oocytes Msh4 foci are seen at all DSB sites during leptotene, but by late pachytene Msh4-Msh5 foci are only found at sites for crossover formation [112].

4.2 Msh4-Msh5 and Mlh1-Mlh3 are involved in a meiotic crossover resolution pathway

Previous studies have indicated that in addition to roles in MMR, Mlh1 is important to form crossovers in meiosis, and Mlh1 and Mlh3 interact in the same meiotic pathway responsible for crossover formation [103,113,114]. Genetic analysis in baker's yeast has defined a pathway involving Msh4-Msh5 and a dHJ resolvase activity suggested to be Mlh1-Mlh3; strains deficient in both Mlh1 and either Msh4 or Msh5 show the same decrease in crossover formation as *mlh1* single mutants [102,115,116]. Furthering the evidence for a Msh4-Msh5/Mlh1-Mlh3 pathway are mouse spermatogenesis data showing that Mlh1-Mlh3 foci form following the appearance of Msh4-Msh5 foci. Msh4-Msh5 foci localize to DSB sites early in meiosis and are reduced in frequency as meiosis progresses. By mid-pachytene Mlh1 foci appear and mostly co-localize with Msh4 foci, and Mlh1 and Mlh3 foci are only present at sites for crossover resolution [117–120].

5. Endonucleases act to resolve Holliday junction intermediates that form prior to crossover resolution in meiosis

As described in section 2, Mlh3 contains a highly conserved metal-binding motif found in MLH proteins that display endonuclease activity. This observation, coupled with localization and genetic analysis of Mlh1-Mlh3, suggest that it acts as a dHJ resolvase. Curiously, Mlh1-Mlh3 does not share conservation with the known structure-selective endonuclease superfamilies: XPF, URI-YIG, and Rad2/XPG.

The conserved metal-binding motif found in *S. cerevisiae* Mlh1-Mlh3 and Mlh1-Pms1 and human Mlh1-Pms2 is required for the endonuclease and MMR functions of these complexes [43,44]. A genetic analysis of this domain in baker's yeast showed that a *mlh3-D523N* mutation, where the highly conserved aspartic acid residue within the DQHA(X)₂E(E)₄E motif was changed to asparagine, conferred an *mlh3-null-like* phenotype in meiosis, illustrating the importance of this putative endonuclease function in the meiotic Mlh1-Mlh3 pathway [101]. Yeast and human Mlh1-Mlh3 display *in vitro* endonuclease activity that is not seen in the purified Mlh1-mlh3-D523N complex. To date cleavage by Mlh1-Mlh3 of synthetic Holliday junctions or related small substrates has not been observed [45,46].

5.1 Characterization of resolvases that act on model Holliday junctions

Cleavage of synthetic Holliday junctions and other branched intermediates constructed from oligonucleotides is considered an important biochemical signature of Holliday junction resolvases. In *S. cerevisiae* (and humans), three eukaryotic nucleases that are capable of nicking such junctions *in vitro* have been identified and characterized using model four-way junctions: Mus81-Mms4 (MUS81-EME1) (XPF family), Slx1-Slx4 (SLX1-SLX4) (URI-YIG family), and Yen1 (GEN1) (Rad2/XPG family) (Figure 4) (reviewed in [121]). *In vitro*, Mus81-Mms4 nicks flapped DNA substrates and four-way Holliday junctions, among other branch structures [122,123]. This complex has been implicated as the primary resolvase in a crossover pathway that does not require ZMM proteins to stabilize crossover intermediates (Figure 3C), and has been suggested to prevent chromosome entanglements in both crossover and non-crossover pathways [102,115,124]. In both *in vitro* and in *in vivo* assays, Mus81-Mms4 is activated by phosphorylation by Cdc5 kinase, suggesting that its meiotic resolvase activity is carefully regulated through post-translational modification [125,126]. Yeast and human Slx1-Slx4 are URI-YIG family endonucleases that are also structure-specific, capable of cleaving forked junctions, 5'-flapped substrates, and four-way Holliday junctions *in vitro* [127–129]. Both Mus81-Mms4 and Slx1-Slx4 are essential for the formation of meiotic crossovers in the absence of Sgs1 [82]. Yen1 is a member of the Rad2/XPG superfamily and has been shown to cleave Holliday junction structures *in vitro* [130,131] and can at least partially compensate for loss of Mus81-Mms4 function *in vivo* [82].

Curiously, the combined activities of the above nucleases account for the formation of only a minority of meiotic crossovers; the majority are dependent on a pathway requiring the MMR complex Mlh1-Mlh3 [82]. In addition to appearing unrelated in homology to the established resolvases, Mlh1-Mlh3 is also distinct in its actions as a putative resolvase.

Resolution of dHJ intermediates by Mus81-Mms4, Slx1-Slx4, and/or Yen1 results in a mixture of crossover and non-crossover products, whereas resolution by Mlh1-Mlh3 results in only crossover products [82].

5.2 Evidence for a Msh4-Msh5, Mlh1-Mlh3, Sgs1-Top3-Rmi1, and Exo1 meiotic crossover pathway

The failure of Mlh1-Mlh3 to conform to paradigms set by previously characterized resolvases suggests that it may be directed and positioned at dHJs in a way that is unique and involves the cooperation of other protein components. Candidates for directing Mlh1-Mlh3 functions are Msh4-Msh5, Sgs1-Top3-Rmi1, and Exo1.

The RecQ family helicase Sgs1 in *S. cerevisiae* (BLM in humans) has been established as a key crossover regulator and is involved in early crossover regulation steps. In meiosis, Sgs1 functions in a complex with type-I topoisomerase (Top3) and Rmi1 [68–70,132–134]. Initially, Sgs1 was assigned as an anti-crossover factor because it facilitates unwinding and decatenation events [73,135–138]. More recently it has been labeled a pro-crossover factor based on the following observations [69,70,82,139]. (1) In *S. cerevisiae*, in the absence of Sgs1, Top3, or Rmi1, SEIs and dHJs accumulate and are primarily resolved by Mus81-Mms4 [69,70,82,139]. (2) In budding yeast Zakharyevich, *et al.* [82] found that in an *mms4 slx4 yen1* triple mutant, crossing over at a meiotic hotspot was reduced to ~70% of wild-type levels. (3) In an *mms4 slx4 yen1 sgs1* quadruple mutant, the number of crossovers at this hotspot was reduced to ~10% of wild-type levels [82]. Similar results were found in strains deficient in Mms4, Slx4, Yen1 and either Top3 or Rmi1 [69,70]. These data suggest that the Sgs1-Top3-Rmi1 complex plays an important role in promoting crossover formation and does so in conjunction with a resolvase that is not Mus81-Mms4, Slx1-Slx4, or Yen1. Consistent with this, when an *mms4 slx4 yen1 mlh3* mutant was made, crossovers were reduced to ~10% of wild-type levels, the same extent seen with the *mms4 slx4 yen1 sgs1* quadruple mutant. These data suggest a pathway where Sgs1-dependent crossovers require Mlh1-Mlh3. Furthermore, non-crossovers were reduced ~four-fold, compared to wild-type, in an *mms4 slx4 yen1 sgs1* quadruple mutant, but in the *mms4 slx4 yen1 mlh3* mutant, non-crossovers form at the same levels as in wild-type strains. These data suggest a role for Sgs1, but not Mlh3, in the formation of non-crossovers. Collectively, these studies indicate that the majority of crossovers form through the actions of either Mlh1-Mlh3 or Mus81-Mms4, with Sgs1-Top3-Rmi1 regulating both [69,70,82,139]. This was consistent with earlier data showing that *mms4 mlh1* double mutants display significant decreases in crossing over compared to *mlh1* or *mms4* single mutants, suggesting that Mlh1-Mlh3 and Mus81-Mms4 are responsible for the majority of meiotic crossovers [102].

The MMR component Exo1 was also shown to be important in the Mlh1-Mlh3 resolution pathway. Exo1, a Rad2/XPG family nuclease, displays 5' to 3' exonuclease activity and an endonuclease activity capable of nicking 5'-single strand flaps. Exo1 has been implicated in a wide variety of cellular processes, including MMR, replication, and recombination. Meiotic crossing over is reduced by as much as two-fold in *S. cerevisiae exo1* strains, suggesting an important role for the protein in at least one meiotic crossover pathway [140–142]. In *S. cerevisiae* Zakharyevich, *et al.* [143] showed that *mlh3* and *exo1* strains

displayed similar levels of SEI and dHJ intermediates; however, crossover formation in both mutants (as well as in an *mlh3 exo1* double mutant) was similarly reduced. These observations support a late resolution step involving both Exo1 and Mlh1-Mlh3. Surprisingly, they observed that Exo1's catalytic activity was not required to maintain wild-type levels of meiotic crossing over, suggesting an exonuclease-independent and perhaps structural role for Exo1 in this pathway. Zakharyevich, *et al.* [82] presents data supporting a structural role for Exo1 in the Mlh1-Mlh3 resolution pathway. These observations are supported by work performed by Borts and colleagues [144] who found Exo1 activity to be important in both early and late stages of meiosis, and that these roles could be genetically separated in the *exo1-D173A* exonuclease mutant which was functional for its late role in meiosis. Together these data suggest that Exo1's exonuclease activity is not required for its late role in promoting meiotic crossovers.

Work in baker's yeast and in mammals have led to the proposal of a pathway where a majority of crossovers occur through the functions of Msh4-Msh5, Sgs1-Top3-Rmi1, Exo1, and the Mlh1-Mlh3 endonuclease. This pathway is subject to crossover interference, a mechanism that ensures that crossovers are spaced non-randomly and that formation of a crossover in a specific region of the chromosome reduces the probability that another crossover will form in a nearby region [83,97,145]. Interestingly, crossovers formed by Mus81-Mms4, Slx1-Slx4, or Yen1 are insensitive to interference mechanisms. The exact processes that confer Mlh1-Mlh3-dependent resolution of dHJs into interference-dependent crossovers are unknown. The formation of crossovers via a dHJ in this Mlh1-Mlh3 pathway requires the actions of a nuclease activity that nicks with a bias between the two junctions; one junction of the dHJ is resolved in one orientation, while the other junction is resolved in an orthogonal orientation (Figure 3B). This finding suggests an asymmetric loading of meiotic protein complexes onto the two Holliday junctions (see below).

5.3 Model for resolution of dHJs into crossover products

Is a Msh4-Msh5/Mlh1-Mlh3 supercomplex critical for dHJ resolution? In MMR, interactions between MSH, MLH proteins, and other repair factors are thought to be important for activating and directing endonuclease activity. Therefore it is reasonable to hypothesize that physical interactions between Msh4-Msh5 and other meiotic protein factors orient Mlh1-Mlh3 to permit asymmetric cleavage of dHJs.

Many studies have been performed to elucidate physical interactions that occur among the proteins implicated in the ZMM-directed dHJ resolution pathway. For example, yeast two-hybrid screens and co-immunoprecipitation assays have suggested that human Sgs1 (BLM) and Mlh1 physically interact [146]. These data were supported by immunoprecipitation studies in meiotic yeast cells where Mlh3 was shown to interact with Sgs1 [147]. Mlh1 has also been shown to physically interact with Exo1, an interaction likely also used in MMR, but perhaps exploited in meiosis as a structural bridge [113,148]. Exo1 is known to interact with MSH proteins during MMR, so conceivably interactions with Msh4 or Msh5 occur during meiosis [149,150]. Physical interactions have also been detected between human Msh4 and human Mlh1 and Mlh3 during meiosis [120,151]. Despite the many interactions that occur, a model for how such interactions convey asymmetry is not immediately clear.

Models suggesting that MSH-MLH interactions are important for biased Mlh1-Mlh3 nicking follow logically from known physical interactions involving MSH and MLH factors and the observation that a majority of crossovers occur via the Msh4-Msh5 pathway. Such models, however, do not account for the fact that other organisms (e.g. *Drosophila* and *Schizosaccharomyces pombe*) do not have Msh4 and Msh5 homologs, yet achieve efficient crossover resolution using endonucleases belonging to the major superfamilies described in section 5.1 [152–155]. Additionally, there are organisms, such as *Caenorhabditis elegans*, that have Msh4 and Msh5 orthologs which are required for nearly all meiotic crossovers, despite the fact that roles for Mlh1-Mlh3 orthologs have not been found [156]. In *C. elegans* crossover resolution occurs with absolute interference (only one crossover per homolog pair) by pathways involving an Slx1-Slx4 ortholog, a Mus81 ortholog, and an additional XPF-1 family endonuclease [157–159]. Redundancy among nucleases and the fact that efficient crossover formation can occur in other organisms in the absence of Msh4-Msh5 or Mlh1-Mlh3 suggests that crossing over can be achieved with different sets and permutations of proteins.

A recent bioinformatic study further suggests that an MSH/MLH complex may not be required for imposing asymmetric nicking of dHJs [124]. Sequencing of recombination products in yeast tetrads derived from *msh4* strains showed that biased nicking of dHJs was retained. However, in a *zip3* mutant (a SUMO E3 ligase and member of the ZMM family), biased cutting was lost. By monitoring crossover-associated gene conversion tracts, Fung and colleagues [124] suggest that Zip3 is involved in promoting second-end capture followed by ligation to form the dHJ, possibly through intermediate stabilization. This is supported by localization data showing late meiotic roles for Zip3 in budding yeast, where late-forming Zip3 foci localize to a subset of crossover sites [106]. These data suggest a model where the asymmetry for dHJ resolution is established by Zip3 and possibly other proteins involved in second-end capture, branch migration, and ligation to form the dHJ (e.g. RPA, Rad52, and Rad54) (Figure 5) [71–78,124]. In the aforementioned bioinformatic study, the retention of biased resolution in the *msh4* mutant suggests that Msh4-Msh5 may act to stabilize strand invasion intermediates and dHJs, and not to asymmetrically direct Mlh1-Mlh3 endonuclease activity [124].

Genetic and molecular studies suggest that Msh4-Msh5 and the other ZMM proteins act to stabilize SEIs and dHJ intermediates [105]. Experiments performed with purified human Msh4-Msh5 suggest that the complex specifically recognizes model four-way junctions constructed from oligonucleotides [160]. Recognition of the junction appears to cause an exchange of ADP for ATP, triggering sliding clamp formation. Msh4-Msh5 can then slide onto the homologous arms of a four-way junction, and appears to encircle two duplex arms of a stacked junction (closed, antiparallel conformation) [160,161]. ATP hydrolysis is triggered upon encountering an open DNA end [161]. Prior to second-end capture, Msh4-Msh5 loaded onto invasion intermediates can encounter open-ended substrate and release. This dissociation can cause iterative loading of Msh4-Msh5 onto the Holliday junction on the invading side of the DSB (Figure 5). Zip3 and proteins that facilitate re-annealing at the opposing side of the DSB may force asymmetry by preventing Msh4-Msh5 from binding to

the other junction. The result of this process is a symmetric DNA structure that is not equivalent in terms of protein occupancy and by extension loading of Mlh1-Mlh3 resolvase.

6. Conclusions and questions for future studies

As described above, not all organisms have co-opted MMR homologs to form meiotic crossovers. This suggests that there are other strategies available to the cell to achieve crossover formation, and that the mechanisms that ensure efficient crossover formation are distinct from MMR.

Biochemical reconstitution of the Mlh1-Mlh3 crossover pathway has been elusive due in part to the difficulty of purifying the many components involved. Purification strategies for yeast and human Sgs1/BLM, Top3, Rmi1, and Exo1 have been available for some time, but only recently have active yeast and human Mlh1-Mlh3 been purified [45,46]. A procedure to purify yeast Msh4-Msh5 is not yet available, despite the fact that the human complex was purified and characterized some time ago. In addition, the full complement of required proteins is not yet established nor is the exact DNA substrate. Requirements for second-end capture proteins (e.g. RPA, Rad52, and Rad54) [71–78] and additional ZMM proteins are not well established. In addition to an incomplete identification of protein components, the biochemical properties of the known crossover promoting components are not well understood. For instance, Mlh1 and Mlh3 each contain nucleotide-binding domains, but roles for ATP hydrolysis by Mlh1-Mlh3 in meiosis are unclear. Genetic analysis suggested that ATP binding, but not hydrolysis by Mlh1, is critical for wild-type levels of crossing over, but another study suggested that hydrolysis is a requirement for both subunits [114,162]. *In vitro* experiments also do not shed light on the role of ATP hydrolysis in Mlh1-Mlh3 function. Purified yeast Mlh1-Mlh3 exhibits a weak ATPase activity; however this activity is not enhanced in the presence of DNA substrate. Furthermore, the complex's *in vitro* endonuclease activity was not activated by the presence of ATP or other nucleotides [45,46]. The possibility that Mlh1-Mlh3's ATPase may need activation by external factors has not been exhaustively studied. In addition, an as yet unidentified co-factor or post-translational modification may clarify the *in vivo* role of Mlh1-Mlh3 ATPase activity.

Currently, Mlh1-Mlh3's *in vitro* endonuclease functions have only been observed on large circular DNA substrates (Figure 4). Mlh1-Mlh3 binding to Holliday junction substrates has been observed *in vitro*, but with preference for an open conformation over stacked junctions, suggesting that other protein components may be required *in vivo* to create DNA substrate in a preferred conformation for Mlh1-Mlh3 binding to occur [46]. The presence of protein aggregates in gel shift experiments with Mlh1-Mlh3 along with the evidence that endonuclease activity has only been observed on large DNA substrates suggest that MLH proteins bind DNA cooperatively [45,46]. This is consistent with filter binding experiments involving various sized DNA substrates and purified yeast Mlh1-Pms1 where cooperative binding was observed [163]. Biochemical analysis of yeast Mlh1-Mlh3, Mlh1-Pms1/2 from yeast and human, and MutH from *E. coli* has shown that sliding clamps, such as MSH proteins and processivity factors, can enhance the nicking activity of these endonucleases, suggesting that interactions or handoff mechanisms may direct nicking [43–45,164].

Specific questions that may elucidate how Mlh1-Mlh3 resolves dHJs into crossover are: (1) What is the role of ATP hydrolysis for Mlh1-Mlh3? (2) Do other factors interact with Mlh1-Mlh3? (3) Are protein-protein interactions between Mlh1-Mlh3 and the other subunits implicated in the pathway essential? (4) Are all of the proteins involved in the pathway identified? (5) Is the role of Exo1 purely structural? (6) There is evidence that post-translational modifications activate resolvases—what if any role does post-translational modification play in activating the pathway? (7) Is Mlh1-Mlh3 capable of making symmetric cuts across a Holliday junction? (8) If Mlh1-Mlh3 does nick a Holliday junction, what activates this activity and where does the protein nick? (9) What roles do chromatin modifications play in this process?

Further bioinformatic approaches and physical assays need to be performed with additional mutant strains to screen for other possible players in the Mlh1-Mlh3 pathway, with particular focus on proteins catalyzing the second-end capture mechanism. Additional biochemical studies further exploring Mlh1-Mlh3's *in vitro* endonuclease activity and searching for factors that may enhance or specify its functions will also be valuable.

Acknowledgements

We thank the members of the Alani laboratory and Jennifer Surtees for helpful comments in preparation of the manuscript. C.M.M. and E.A. are supported by the National Institute of General Medical Sciences of the National Institutes of Health: GM53085 to E.A. and NIH training grant F32 GM112435 to C.M.M. The content of this review is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

References

1. Jiricny J. Postreplicative mismatch repair. *Cold Spring Harb. Perspect. Biol.* 2013; 5:a012633. [PubMed: 23545421]
2. Harfe BD, Jinks-Robertson S. DNA Mismatch Repair and Genetic Instability. *Annu. Rev. Genet.* 2000; 34:359–399. [PubMed: 11092832]
3. Heinen CD. MMR defects and Lynch syndrome: the role of the basic scientist in the battle against cancer. *DNA Repair (Amst)*. 2016 This issue.
4. Sijmons RH, Hofstra RMW. Clinical aspects of hereditary MMR gene mutations. *DNA Repair (Amst)*. 2016 This issue.
5. Putnam CD. Evolution of the methyl directed MMR system in *Escherichia coli*. *DNA Repair (Amst)*. 2016 This issue.
6. Groothuizen FS, Sixma TK. The conserved molecular machinery in MMR structures. *DNA Repair (Amst)*. 2016 This issue.
7. Kolodner RD, Marsischky GT. Eukaryotic DNA mismatch repair. *Curr. Opin. Genet. Dev.* 1999; 9:89–96. [PubMed: 10072354]
8. Marsischky GT, Filosi N, Kane MF, Kolodner RD. Redundancy of *Saccharomyces cerevisiae*. *Genes Dev.* 1996; 10:407–420. [PubMed: 8600025]
9. Sia EA, Kokoska RJ, Dominska M, Greenwell P, Petes TD. Microsatellite instability in yeast: dependence on repeat unit size and DNA mismatch repair genes. *Mol. Cell. Biol.* 1997; 17:2851–2858. [PubMed: 9111357]
10. Srivatsan A, Bowen N, Kolodner RD. Mismatch-specific recruitment of the Mlh1-Pms1 complex identifies repair substrates of the *Saccharomyces cerevisiae* Msh2-Msh3 complex. *J. Biol. Chem.* 2014; 289:9352–64. [PubMed: 24550389]
11. Kunkel TA, Erie DA. DNA mismatch repair. *Annu. Rev. Biochem.* 2005; 74:681–710. [PubMed: 15952900]

12. Hingorani MM. Mismatch binding, ADP-ATP exchange and intramolecular signaling during MMR. *DNA Repair (Amst)*. 2016 This issue.
13. Hombauer H, Campbell CS, Smith CE, Desai A, Kolodner RD. Visualization of eukaryotic DNA mismatch repair reveals distinct recognition and repair intermediates. *Cell*. 2011; 147:1040–53. [PubMed: 22118461]
14. Schmidt TT, Hombauer H. Visualization of MMR complexes using fluorescence microscopy. *DNA Repair (Amst)*. 2016 This issue.
15. Friedhoff P, Li P, Gotthardt J. Protein-protein interactions in DNA MMR. *DNA Repair (Amst)*. 2016 This issue.
16. Goellner EM, Putnam CD, Kolodner RD. Exonuclease 1-dependent and independent mismatch repair. *DNA Repair (Amst)*. 2015; 32:24–32. [PubMed: 25956862]
17. Flores-Rozas H, Kolodner RD. The *Saccharomyces cerevisiae* MLH3 gene functions in MSH3-dependent suppression of frameshift mutations. *Proc. Natl. Acad. Sci. U. S. A.* 1998; 95:12404–9. [PubMed: 9770499]
18. Lamers MH, Perrakis A, Enzlin JH, Winterwerp HH, de Wind N, Sixma TK. The crystal structure of DNA mismatch repair protein MutS binding to a G × T mismatch. *Nature*. 2000; 407:711–7. [PubMed: 11048711]
19. Junop MS, Obmolova G, Rausch K, Hsieh P, Yang W. Composite active site of an ABC ATPase: MutS uses ATP to verify mismatch recognition and authorize DNA repair. *Mol. Cell*. 2001; 7:1–12. [PubMed: 11172706]
20. Obmolova G, Ban C, Hsieh P, Yang W. Crystal structures of mismatch repair protein MutS and its complex with a substrate DNA. *Nature*. 2000; 407:703–10. [PubMed: 11048710]
21. Warren JJ, Pohlhaus TJ, Changela A, Iyer RR, Modrich PL, Beese LS. Structure of the Human MutSα DNA Lesion Recognition Complex. *Mol. Cell*. 2007; 26:579–592. [PubMed: 17531815]
22. Mukherjee S, Feig M. Conformational change in MSH2-MSH6 upon binding DNA coupled to ATPase activity. *Biophys. J.* 2009; 96:L63–L65. [PubMed: 19486659]
23. Qiu R, DeRocco VC, Harris C, Sharma A, Hingorani MM, Erie DA, et al. Large conformational changes in MutS during DNA scanning, mismatch recognition and repair signalling. *EMBO J.* 2012; 31:2528–40. [PubMed: 22505031]
24. Antony E, Hingorani MM. Asymmetric ATP binding and hydrolysis activity of the *Thermus aquaticus* MutS dimer is key to modulation of its interactions with mismatched DNA. *Biochemistry*. 2004; 43:13115–28. [PubMed: 15476405]
25. Antony E, Khubchandani S, Chen S, Hingorani MM. Contribution of Msh2 and Msh6 subunits to the asymmetric ATPase and DNA mismatch binding activities of *Saccharomyces cerevisiae* Msh2-Msh6 mismatch repair protein. *DNA Repair (Amst)*. 2006; 5:153–62. [PubMed: 16214425]
26. Bjornson KP, Modrich P. Differential and simultaneous adenosine di- and triphosphate binding by MutS. *J. Biol. Chem.* 2003; 278:18557–62. [PubMed: 12624105]
27. Monti MC, Cohen SX, Fish A, Winterwerp HHK, Barendregt A, Friedhoff P, et al. Native mass spectrometry provides direct evidence for DNA mismatch-induced regulation of asymmetric nucleotide binding in mismatch repair protein MutS. *Nucleic Acids Res.* 2011; 39:8052–64. [PubMed: 21737427]
28. Kumar C, Eichmiller R, Wang B, Williams GM, Bianco PR, Surtees J. a. ATP binding and hydrolysis by *Saccharomyces cerevisiae* Msh2-Msh3 are differentially modulated by mismatch and double-strand break repair DNA substrates. *DNA Repair (Amst)*. 2014; 18:18–30. [PubMed: 24746922]
29. Sharma A, Doucette C, Biro FN, Hingorani MM. Slow conformational changes in MutS and DNA direct ordered transitions between mismatch search, recognition and signaling of DNA repair. *J. Mol. Biol.* 2013; 425:4192–205. [PubMed: 23973435]
30. V Hargreaves V, Shell SS, Mazur DJ, Hess MT, Kolodner RD. Interaction between the Msh2 and Msh6 nucleotide-binding sites in the *Saccharomyces cerevisiae* Msh2-Msh6 complex. *J. Biol. Chem.* 2010; 285:9301–10. [PubMed: 20089866]
31. Mazur DJ, Mendillo ML, Kolodner RD. Inhibition of Msh6 ATPase activity by mispaired DNA induces a Msh2(ATP)-Msh6(ATP) state capable of hydrolysis-independent movement along DNA. *Mol. Cell*. 2006; 22:39–49. [PubMed: 16600868]

32. Gradia S, Subramanian D, Wilson T, Acharya S, Makhov A, Griffith J, et al. hMSH2-hMSH6 forms a hydrolysis-independent sliding clamp on mismatched DNA. *Mol. Cell.* 1999; 3:255–61. [PubMed: 10078208]
33. Gradia S, Acharya S, Fishel R. The human mismatch recognition complex hMSH2-hMSH6 functions as a novel molecular switch. *Cell.* 1997; 91:995–1005. [PubMed: 9428522]
34. Wilson T, Guerrette S, Fishel R. Dissociation of mismatch recognition and ATPase activity by hMSH2-hMSH3. *J. Biol. Chem.* 1999; 274:21659–64. [PubMed: 10419475]
35. Gorman J, Plys AJ, Visnapuu M-L, Alani E, Greene EC. Visualizing one-dimensional diffusion of eukaryotic DNA repair factors along a chromatin lattice. *Nat. Struct. Mol. Biol.* 2010; 17:932–938. [PubMed: 20657586]
36. Hall MC, Shcherbakova PV, Fortune JM, Borchers CH, Dial JM, Tomer KB, et al. DNA binding by yeast Mlh1 and Pms1: Implications for DNA mismatch repair. *Nucleic Acids Res.* 2003; 31:2025–2034. [PubMed: 12682353]
37. Ban C, Junop M, Yang W. Transformation of MutL by ATP binding and hydrolysis: a switch in DNA mismatch repair. *Cell.* 1999; 97:85–97. [PubMed: 10199405]
38. Guarné A, Ramon-Maiques S, Wolff EM, Ghirlando R, Hu X, Miller JH, et al. Structure of the MutL C-terminal domain: a model of intact MutL and its roles in mismatch repair. *EMBO J.* 2004; 23:4134–4145. [PubMed: 15470502]
39. Kosinski J, Steindorf I, Bujnicki JM, Giron-Monzon L, Friedhoff P. Analysis of the quaternary structure of the MutL C-terminal domain. *J. Mol. Biol.* 2005; 351:895–909. [PubMed: 16024043]
40. Sacho EJ, Kadyrov FA, Modrich P, Kunkel TA, Erie DA. Direct visualization of asymmetric adenine-nucleotide-induced conformational changes in MutL alpha. *Mol. Cell.* 2008; 29:112–21. [PubMed: 18206974]
41. Plys AJ, Rogacheva MV, Greene EC, Alani E. The unstructured linker arms of Mlh1-Pms1 are important for interactions with DNA during mismatch repair. *J. Mol. Biol.* 2012; 422:192–203. [PubMed: 22659005]
42. Guarné A. The functions of MutL in mismatch repair: the power of multitasking. *Prog. Mol. Biol. Transl. Sci.* 2012; 110:41–70. [PubMed: 22749142]
43. Kadyrov FA, Dzantiev L, Constantin N, Modrich P. Endonucleolytic function of MutLalpha in human mismatch repair. *Cell.* 2006; 126:297–308. [PubMed: 16873062]
44. Kadyrov FA, Holmes SF, Arana ME, Lukianova OA, O'Donnell M, Kunkel TA, et al. *Saccharomyces cerevisiae* MutLalpha is a mismatch repair endonuclease. *J. Biol. Chem.* 2007; 282:37181–90. [PubMed: 17951253]
45. V Rogacheva M, Manhart CM, Chen C, Guarne A, Surtees J, Alani E. Mlh1-Mlh3, A Meiotic Crossover and DNA Mismatch Repair Factor, is a Msh2-Msh3-Stimulated Endonuclease. *J. Biol. Chem.* 2014; 289:5664–5673. [PubMed: 24403070]
46. Ranjha L, Anand R, Cejka P. The *Saccharomyces cerevisiae* Mlh1-Mlh3 Heterodimer Is an Endonuclease That Preferentially Binds to Holliday Junctions. *J. Biol. Chem.* 2014; 289:5674–86. [PubMed: 24443562]
47. Kadyrova LY, Kadyrov FA. Endonuclease activities of MutLalpha and its homologs in MMR. *DNA Repair (Amst).* 2016 This issue.
48. Pillon MC, Lorenowicz JJ, Uckelmann M, Klocko AD, Mitchell RR, Chung YS, et al. Structure of the endonuclease domain of MutL: unlicensed to cut. *Mol. Cell.* 2010; 39:145–51. [PubMed: 20603082]
49. Gueneau E, Dherin C, Legrand P, Tellier-Lebegue C, Gilquin B, Bonnesoeur P, et al. Structure of the MutLα C-terminal domain reveals how Mlh1 contributes to Pms1 endonuclease site. *Nat. Struct. Mol. Biol.* 2013; 20:461–8. [PubMed: 23435383]
50. Pillon MC, Miller JH, Guarné A. The endonuclease domain of MutL interacts with the β sliding clamp. *DNA Repair (Amst).* 2011; 10:87–93. [PubMed: 21050827]
51. Pluciennik A, Dzantiev L, Iyer RR, Constantin N, Kadyrov FA, Modrich P. PCNA function in the activation and strand direction of MutLα endonuclease in mismatch repair. *Proc. Natl. Acad. Sci. U. S. A.* 2010; 107:16066–16071. [PubMed: 20713735]
52. McMurray C. Mechanisms of trinucleotide repeat instability during human development. *Nat. Rev. Genet.* 2010; 11:786–799. [PubMed: 20953213]

53. Pinto RM, Dragileva E, Kirby A, Lloret A, Lopez E, St Claire J, et al. Mismatch repair genes Mlh1 and Mlh3 modify CAG instability in Huntington's disease mice: genome-wide and candidate approaches. *PLoS Genet.* 2013; 9:e1003930. [PubMed: 24204323]
54. Bak ST, Sakellariou D, Pena-Diaz J. The dual nature of mismatch repair as antimutator and mutator: for better or for worse. *Front. Genet.* 2014; 5:287. [PubMed: 25191341]
55. Lamb NE, Sherman SL, Hassold TJ. Effect of meiotic recombination on the production of aneuploid gametes in humans. *Cytogenet. Genome Res.* 2005; 111:250–5. [PubMed: 16192701]
56. Marston AL, Amon A. Meiosis: cell-cycle controls shuffle and deal. *Nat. Rev. Mol. Cell Biol.* 2004; 5:983–97. [PubMed: 15573136]
57. Keeney S, Giroux CN, Kleckner N. Meiosis-specific DNA double-strand breaks are catalyzed by Spo11, a member of a widely conserved protein family. *Cell.* 1997; 88:375–84. [PubMed: 9039264]
58. Cao L, Alani E, Kleckner N. A pathway for generation and processing of double-strand breaks during meiotic recombination in *S. cerevisiae*. *Cell.* 1990; 61:1089–101. [PubMed: 2190690]
59. Chen SY, Tsubouchi T, Rockmill B, Sandler JS, Richards DR, Vader G, et al. Global analysis of the meiotic crossover landscape. *Dev. Cell.* 2008; 15:401–15. [PubMed: 18691940]
60. Robine N, Uematsu N, Amiot F, Gidrol X, Barillot E, Nicolas A, et al. Genome-wide redistribution of meiotic double-strand breaks in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* 2007; 27:1868–80. [PubMed: 17189430]
61. Pan J, Sasaki M, Kniewel R, Murakami H, Blitzblau HG, Tischfield SE, et al. A hierarchical combination of factors shapes the genome-wide topography of yeast meiotic recombination initiation. *Cell.* 2011; 144:719–31. [PubMed: 21376234]
62. Buhler C, Shroff R, Lichten M. Genome-wide mapping of meiotic DNA double-strand breaks in *Saccharomyces cerevisiae*. *Methods Mol. Biol.* 2009; 557:143–64. [PubMed: 19799181]
63. Buhler C, Borde V, Lichten M. Mapping meiotic single-strand DNA reveals a new landscape of DNA double-strand breaks in *Saccharomyces cerevisiae*. *PLoS Biol.* 2007; 5:e324. [PubMed: 18076285]
64. Murakami H, Nicolas A. Locally, meiotic double-strand breaks targeted by Gal4BD Spo11 occur at discrete sites with a sequence preference. *Mol. Cell. Biol.* 2009; 29:3500–16. [PubMed: 19380488]
65. Peciña A, Smith KN, Mézard C, Murakami H, Ohta K, Nicolas A. Targeted stimulation of meiotic recombination. *Cell.* 2002; 111:173–84. [PubMed: 12408862]
66. Hunter N, Kleckner N. The single-end invasion: an asymmetric intermediate at the double-strand break to double-holliday junction transition of meiotic recombination. *Cell.* 2001; 106:59–70. [PubMed: 11461702]
67. Schwacha A, Kleckner N. Identification of double Holliday junctions as intermediates in meiotic recombination. *Cell.* 1995; 83:783–91. [PubMed: 8521495]
68. Fasching CL, Cejka P, Kowalczykowski SC, Heyer W-D. Top3-Rmi1 Dissolve Rad51-Mediated D Loops by a Topoisomerase-Based Mechanism. *Mol. Cell.* 2015; 57:595–606. [PubMed: 25699708]
69. Tang S, Wu MKY, Zhang R, Hunter N. Pervasive and Essential Roles of the Top3-Rmi1 Decatenase Orchestrate Recombination and Facilitate Chromosome Segregation in Meiosis. *Mol. Cell.* 2015; 57:607–621. [PubMed: 25699709]
70. Kaur H, De Muyt A, Lichten M. Top3-Rmi1 DNA Single-Strand Decatenase Is Integral to the Formation and Resolution of Meiotic Recombination Intermediates. *Mol. Cell.* 2015; 57:583–594. [PubMed: 25699707]
71. V Nimonkar A, Sica RA, Kowalczykowski SC. Rad52 promotes second-end DNA capture in double-stranded break repair to form complement-stabilized joint molecules. *Proc. Natl. Acad. Sci. U. S. A.* 2009; 106:3077–3082. [PubMed: 19204284]
72. Lao JP, Oh SD, Shinohara M, Shinohara A, Hunter N. Rad52 promotes postinvasion steps of meiotic double-strand-break repair. *Mol. Cell.* 2008; 29:517–24. [PubMed: 18313389]
73. Oh SD, Lao JP, Hwang PY-H, Taylor AF, Smith GR, Hunter N. BLM ortholog, Sgs1, prevents aberrant crossing-over by suppressing formation of multichromatid joint molecules. *Cell.* 2007; 130:259–72. [PubMed: 17662941]

74. Malone RE, Esposito RE. The RAD52 gene is required for homothallic interconversion of mating types and spontaneous mitotic recombination in yeast. *Proc. Natl. Acad. Sci. U. S. A.* 1980; 77:503–7. [PubMed: 6987653]
75. Mortensen UH, Bendixen C, Sunjevaric I, Rothstein R. DNA strand annealing is promoted by the yeast Rad52 protein. *Proc. Natl. Acad. Sci. U. S. A.* 1996; 93:10729–34. [PubMed: 8855248]
76. Shinohara A, Shinohara M, Ohta T, Matsuda S, Ogawa T. Rad52 forms ring structures and co-operates with RPA in single-strand DNA annealing. *Genes Cells.* 1998; 3:145–56. [PubMed: 9619627]
77. Sugiyama T, Kantake N, Wu Y, Kowalczykowski SC. Rad52-mediated DNA annealing after Rad51-mediated DNA strand exchange promotes second ssDNA capture. *EMBO J.* 2006; 25:5539–48. [PubMed: 17093500]
78. Sugiyama T, New JH, Kowalczykowski SC. DNA annealing by RAD52 protein is stimulated by specific interaction with the complex of replication protein A and single-stranded DNA. *Proc. Natl. Acad. Sci. U. S. A.* 1998; 95:6049–54. [PubMed: 9600915]
79. Pâques F, Haber JE. Multiple pathways of recombination induced by double-strand breaks in *Saccharomyces cerevisiae*. *Microbiol. Mol. Biol. Rev.* 1999; 63:349–404. [PubMed: 10357855]
80. Szostak JW, Orr-Weaver TL, Rothstein RJ, Stahl FW. The double-strand-break repair model for recombination. *Cell.* 1983; 33:25–35. [PubMed: 6380756]
81. Allers T, Lichten M. Differential timing and control of noncrossover and crossover recombination during meiosis. *Cell.* 2001; 106:47–57. [PubMed: 11461701]
82. Zakharyevich K, Tang S, Ma Y, Hunter N. Delineation of joint molecule resolution pathways in meiosis identifies a crossover-specific resolvase. *Cell.* 2012; 149:334–47. [PubMed: 22500800]
83. Hunter N. Meiotic Recombination. *Mol. Genet. Recomb. Top. Curr. Genet* (17th ed.). 2006:381–442.
84. Chen J-M, Cooper DN, Chuzhanova N, Férec C, Patrinos GP. Gene conversion: mechanisms, evolution and human disease. *Nat. Rev. Genet.* 2007; 8:762–775. [PubMed: 17846636]
85. Surtees JA, Argueso JL, Alani E. Mismatch repair proteins: key regulators of genetic recombination. *Cytogenet. Genome Res.* 2004; 107:146–59. [PubMed: 15467360]
86. Martini E, Borde V, Legendre M, Audic S, Regnault B, Soubigou G, et al. Genome-wide analysis of heteroduplex DNA in mismatch repair-deficient yeast cells reveals novel properties of meiotic recombination pathways. *PLoS Genet.* 2011; 7:e1002305. [PubMed: 21980306]
87. Mancera E, Bourgon R, Brozzi A, Huber W, Steinmetz LM. High-resolution mapping of meiotic crossovers and non-crossovers in yeast. *Nature.* 2008; 454:479–485. [PubMed: 18615017]
88. Anderson CM, Chen SY, Dimon MT, Oke A, DeRisi JL, Fung JC. ReCombine: a suite of programs for detection and analysis of meiotic recombination in whole-genome datasets. *PLoS One.* 2011; 6:e25509. [PubMed: 22046241]
89. Qi J, Wijeratne AJ, Tomsho LP, Hu Y, Schuster SC, Ma H. Characterization of meiotic crossovers and gene conversion by whole-genome sequencing in *Saccharomyces cerevisiae*. *BMC Genomics.* 2009; 10:475. [PubMed: 19832984]
90. Alani E, Reenan RA, Kolodner RD. Interaction between mismatch repair and genetic recombination in *Saccharomyces cerevisiae*. *Genetics.* 1994; 137:19–39. [PubMed: 8056309]
91. Nicolas A, Petes TD. Polarity of meiotic gene conversion in fungi: contrasting views. *Experientia.* 1994; 50:242–52. [PubMed: 8143798]
92. Detloff P, White MA, Petes TD. Analysis of a gene conversion gradient at the HIS4 locus in *Saccharomyces cerevisiae*. *Genetics.* 1992; 132:113–23. [PubMed: 1398048]
93. George CM, Alani E. Multiple cellular mechanisms prevent chromosomal rearrangements involving repetitive DNA. *Crit. Rev. Biochem. Mol. Biol.* 2012; 47:297–313. [PubMed: 22494239]
94. Chambers SR, Hunter N, Louis EJ, Borts RH. The mismatch repair system reduces meiotic homeologous recombination and stimulates recombination-dependent chromosome loss. *Mol. Cell. Biol.* 1996; 16:6110–20. [PubMed: 8887641]
95. Hunter N, Chambers SR, Louis EJ, Borts RH. The mismatch repair system contributes to meiotic sterility in an interspecific yeast hybrid. *EMBO J.* 1996; 15:1726–33. [PubMed: 8612597]

96. Tlām KC, Lebbink JHG. Functions of MMR in regulating genetic recombination. *DNA Repair (Amst)*. 2016 This issue.
97. Ross-Macdonald P, Roeder GS. Mutation of a meiosis-specific MutS homolog decreases crossing over but not mismatch correction. *Cell*. 1994; 79:1069–80. [PubMed: 8001134]
98. Hollingsworth NM, Ponte L, Halsey C. MSH5, a novel MutS homolog, facilitates meiotic reciprocal recombination between homologs in *Saccharomyces cerevisiae* but not mismatch repair. *Genes Dev*. 1995; 9:1728–39. [PubMed: 7622037]
99. Novak JE, Ross-macdonald PB, Roeder GS. The Budding Yeast Msh4 Protein Functions in Chromosome Synapsis and the Regulation of Crossover Distribution. *Genetics*. 2001; 158:1013–1025. [PubMed: 11454751]
100. Nishant KT, Chen C, Shinohara M, Shinohara A, Alani E. Genetic analysis of baker's yeast Msh4-Msh5 reveals a threshold crossover level for meiotic viability. *PLoS Genet*. 2010; 6:e1001083. [PubMed: 20865162]
101. Nishant KT, Plys AJ, Alani E. A mutation in the putative MLH3 endonuclease domain confers a defect in both mismatch repair and meiosis in *Saccharomyces cerevisiae*. *Genetics*. 2008; 179:747–55. [PubMed: 18505871]
102. Argueso JL, Wanat J, Gemici Z, Alani E. Competing crossover pathways act during meiosis in *Saccharomyces cerevisiae*. *Genetics*. 2004; 168:1805–16. [PubMed: 15611158]
103. Wang TF, Kleckner N, Hunter N. 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. U. S. A.* 1999; 96:13914–9. [PubMed: 10570173]
104. Page SL, Hawley RS. The genetics and molecular biology of the synaptonemal complex. *Annu. Rev. Cell Dev. Biol*. 2004; 20:525–558. [PubMed: 15473851]
105. Börner GV, Kleckner N, Hunter N. Crossover/noncrossover differentiation, synaptonemal complex formation, and regulatory surveillance at the leptotene/zygotene transition of meiosis. *Cell*. 2004; 117:29–45. [PubMed: 15066280]
106. Agarwal S, Roeder GS. Zip3 provides a link between recombination enzymes and synaptonemal complex proteins. *Cell*. 2000; 102:245–255. [PubMed: 10943844]
107. Fung JC, Rockmill B, Odell M, Roeder GS. Imposition of crossover interference through the nonrandom distribution of synapsis initiation complexes. *Cell*. 2004; 116:795–802. [PubMed: 15035982]
108. Lynn A, Soucek R, Börner GV. ZMM proteins during meiosis: Crossover artists at work. *Chromosom. Res*. 2007; 15:591–605.
109. Shinohara M, Oh SD, Hunter N, Shinohara A. Crossover assurance and crossover interference are distinctly regulated by the ZMM proteins during yeast meiosis. *Nat. Genet*. 2008; 40:299–309. [PubMed: 18297071]
110. Pochart P, Woltering D, Hollingsworth NM. Conserved properties between functionally distinct MutS homologs in yeast. *J. Biol. Chem*. 1997; 272:30345–9. [PubMed: 9374523]
111. Storlazzi A, Gargano S, Ruprich-Robert G, Falque M, David M, Kleckner N, et al. Recombination proteins mediate meiotic spatial chromosome organization and pairing. *Cell*. 2010; 141:94–106. [PubMed: 20371348]
112. Lenzi ML, Smith J, Snowden T, Kim M, Fishel R, Poulos BK, et al. Extreme heterogeneity in the molecular events leading to the establishment of chiasmata during meiosis i in human oocytes. *Am. J. Hum. Genet*. 2005; 76:112–127. [PubMed: 15558497]
113. Argueso JL, Smith D, Yi J, Waase M, Sarin S, Alani E. Analysis of conditional mutations in the *Saccharomyces cerevisiae* MLH1 gene in mismatch repair and in meiotic crossing over. *Genetics*. 2002; 160:909–21. [PubMed: 11901110]
114. Hoffmann ER, V Shcherbakova P, Kunkel TA, Borts RH. MLH1 Mutations Differentially Affect Meiotic Functions in *Saccharomyces cerevisiae*. *Genetics*. 2003; 164:81–94. [PubMed: 12618391]
115. de los Santos T, Hunter N, Lee C, Larkin B, Loidl J, Hollingsworth NM. The Mus81/Mms4 endonuclease acts independently of double-Holliday junction resolution to promote a distinct subset of crossovers during meiosis in budding yeast. *Genetics*. 2003; 164:81–94. [PubMed: 12750322]

116. Hunter N, Borts RH. Mlh1 is unique among mismatch repair proteins in its ability to promote crossing-over during meiosis. *Genes Dev.* 1997; 11:1573–82. [PubMed: 9203583]
117. Kolas NK, Svetlanov A, Lenzi ML, Macaluso FP, Lipkin SM, Liskay RM, et al. Localization of MMR proteins on meiotic chromosomes in mice indicates distinct functions during prophase I. *J. Cell Biol.* 2005; 171:447–58. [PubMed: 16260499]
118. Lipkin SM, Moens PB, Wang V, Lenzi M, Shanmugarajah D, Gilgeous A, et al. Meiotic arrest and aneuploidy in MLH3-deficient mice. *Nat. Genet.* 2002; 31:385–90. [PubMed: 12091911]
119. Moens PB, Kolas NK, Tarsounas M, Marcon E, Cohen PE, Spyropoulos B. The time course and chromosomal localization of recombination-related proteins at meiosis in the mouse are compatible with models that can resolve the early DNA-DNA interactions without reciprocal recombination. *J. Cell Sci.* 2002; 115:1611–22. [PubMed: 11950880]
120. Santucci-Darmanin S, Walpita D, Lespinasse F, Desnuelle C, Ashley T, Paquis-Flucklinger V. MSH4 acts in conjunction with MLH1 during mammalian meiosis. *FASEB J.* 2000; 14:1539–47. [PubMed: 10928988]
121. Schwartz EK, Heyer W-D. Processing of joint molecule intermediates by structure-selective endonucleases during homologous recombination in eukaryotes. *Chromosoma.* 2011; 120:109–27. [PubMed: 21369956]
122. Bastin-Shanower SA, Fricke WM, Mullen JR, Brill SJ. The mechanism of Mus81-Mms4 cleavage site selection distinguishes it from the homologous endonuclease Rad1-Rad10. *Mol. Cell. Biol.* 2003; 23:3487–96. [PubMed: 12724407]
123. Mukherjee S, Wright WD, Ehmsen KT, Heyer W-D. The Mus81-Mms4 structure-selective endonuclease requires nicked DNA junctions to undergo conformational changes and bend its DNA substrates for cleavage. *Nucleic Acids Res.* 2014; 1–12.
124. Oke A, Anderson CM, Yam P, Fung JC. Controlling Meiotic Recombinational Repair - Specifying the Roles of ZMMs, Sgs1 and Mus81/Mms4 in Crossover Formation. *PLoS Genet.* 2014; 10:e1004690. [PubMed: 25329811]
125. Schwartz EK, Wright WD, Ehmsen KT, Evans JE, Stahlberg H, Heyer W-D. Mus81-Mms4 functions as a single heterodimer to cleave nicked intermediates in recombinational DNA repair. *Mol. Cell. Biol.* 2012; 32:3065–80. [PubMed: 22645308]
126. Matos J, Blanco MG, Maslen S, Skehel JM, West SC. Regulatory control of the resolution of DNA recombination intermediates during meiosis and mitosis. *Cell.* 2011; 147:158–72. [PubMed: 21962513]
127. Fricke WM, Brill SJ. Slx1-Slx4 is a second structure-specific endonuclease functionally redundant with Sgs1-Top3. *Genes Dev.* 2003; 17:1768–1778. [PubMed: 12832395]
128. Muñoz IM, Hain K, Déclais AC, Gardiner M, Toh GW, Sanchez-Pulido L, et al. Coordination of Structure-Specific Nucleases by Human SLX4/BTBD12 Is Required for DNA Repair. *Mol. Cell.* 2009; 35:116–127. [PubMed: 19595721]
129. Svendsen JM, Smogorzewska A, Sowa ME, Connell BCO, Gygi SP, Elledge SJ, et al. Mammalian BTBD12/SLX4 assembles a Holliday junction resolvase and is required for DNA repair. *Cell.* 2009; 138:63–77. [PubMed: 19596235]
130. Ip SCY, Rass U, Blanco MG, Flynn HR, Skehel JM, West SC. Identification of Holliday junction resolvases from humans and yeast. *Nature.* 2008; 456:357–61. [PubMed: 19020614]
131. Ishikawa G, Kanai Y, Takata KI, Takeuchi R, Shimanouchi K, Ruike T, et al. DmGEN, a novel RAD2 family endo-exonuclease from *Drosophila melanogaster*. *Nucleic Acids Res.* 2004; 32:6251–6259. [PubMed: 15576351]
132. Ira G, Malkova A, Liberi G, Foiani M, Haber JE. Srs2 and Sgs1-Top3 suppress crossovers during double-strand break repair in yeast. *Cell.* 2003; 115:401–11. [PubMed: 14622595]
133. Chang M, Bellaoui M, Zhang C, Desai R, Morozov P, Delgado-Cruzata L, et al. RMI1/NCE4, a suppressor of genome instability, encodes a member of the RecQ helicase/Topo III complex. *EMBO J.* 2005; 24:2024–33. [PubMed: 15889139]
134. Mullen JR, Nallasetth FS, Lan YQ, Slagle CE, Brill SJ. Yeast Rmi1/Nce4 controls genome stability as a subunit of the Sgs1-Top3 complex. *Mol. Cell. Biol.* 2005; 25:4476–87. [PubMed: 15899853]

135. Wu L, Hickson ID. The Bloom's syndrome helicase suppresses crossing over during homologous recombination. 2003; 426:15–19.
136. Jessop L, Rockmill B, Roeder GS, Lichten M. Meiotic chromosome synapsis-promoting proteins antagonize the anti-crossover activity of sgs1. *PLoS Genet.* 2006; 2:e155. [PubMed: 17002499]
137. Cejka P, Plank JL, Dombrowski CC, Kowalczykowski SC. Decatenation of DNA by the *S. cerevisiae* Sgs1-Top3-Rmi1 and RPA complex: a mechanism for disentangling chromosomes. *Mol. Cell.* 2012; 47:886–96. [PubMed: 22885009]
138. Cejka P, Plank JL, Bachrati CZ, Hickson ID, Kowalczykowski SC. Rmi1 stimulates decatenation of double Holliday junctions during dissolution by Sgs1-Top3. *Nat. Struct. Mol. Biol.* 2010; 17:1377–82. [PubMed: 20935631]
139. De Muyt A, Jessop L, Kolar E, Sourirajan A, Chen J, Dayani Y, et al. BLM Helicase Ortholog Sgs1 Is a Central Regulator of Meiotic Recombination Intermediate Metabolism. *Mol. Cell.* 2012; 46:43–53. [PubMed: 22500736]
140. Khazanehdari KA, Borts RH. EXO1 and MSH4 differentially affect crossing-over and segregation. *Chromosoma.* 2000; 109:94–102. [PubMed: 10855499]
141. Tsubouchi H, Ogawa H. Exo1 roles for repair of DNA double-strand breaks and meiotic crossing over in *Saccharomyces cerevisiae*. *Mol. Biol. Cell.* 2000; 11:2221–33. [PubMed: 10888664]
142. Kirkpatrick DT, Ferguson JR, Petes TD, Symington LS. Decreased meiotic intergenic recombination and increased meiosis I nondisjunction in *exo1* mutants of *Saccharomyces cerevisiae*. *Genetics.* 2000; 156:1549–57. [PubMed: 11102356]
143. Zakharyevich K, Ma Y, Tang S, Hwang PY-H, Boiteux S, Hunter N. Temporally and biochemically distinct activities of Exo1 during meiosis: double-strand break resection and resolution of double Holliday junctions. *Mol. Cell.* 2010; 40:1001–15. [PubMed: 21172664]
144. Keelagher RE, Cotton VE, Goldman ASH, Borts RH. Separable roles for Exonuclease I in meiotic DNA double-strand break repair. *DNA Repair (Amst).* 2011; 10:126–37. [PubMed: 21044871]
145. Argueso JL, Kijas AW, Sarin S, Heck J, Waase M, Alani E. Systematic mutagenesis of the *Saccharomyces cerevisiae* MLH1 gene reveals distinct roles for Mlh1p in meiotic crossing over and in vegetative and meiotic mismatch repair. *Mol. Cell. Biol.* 2003; 23:873–86. [PubMed: 12529393]
146. Pedrazzi G, Perrera C, Blaser H, Kuster P, Marra G, Davies SL, et al. Direct association of Bloom's syndrome gene product with the human mismatch repair protein MLH1. 2001; 29:4378–4386.
147. Wang T-F, Kung W-M. Supercomplex formation between Mlh1-Mlh3 and Sgs1-Top3 heterocomplexes in meiotic yeast cells. *Biochem. Biophys. Res. Commun.* 2002; 296:949–53. [PubMed: 12200140]
148. Tran PT, Simon JA, Liskay RM. Interactions of Exo1p with components of MutLalpha in *Saccharomyces cerevisiae*. *Proc. Natl. Acad. Sci. U. S. A.* 2001; 98:9760–5. [PubMed: 11481425]
149. Bertrand P, Tishkoff DX, Filosi N, Dasgupta R, Kolodner RD. Physical interaction between components of DNA mismatch repair and nucleotide excision repair. *Proc. Natl. Acad. Sci. U. S. A.* 1998; 95:14278–83. [PubMed: 9826691]
150. Tishkoff DX, Boerger AL, Bertrand P, Filosi N, Gaida GM, Kane MF, et al. Identification and characterization of *Saccharomyces cerevisiae* EXO1, a gene encoding an exonuclease that interacts with MSH2. *Proc. Natl. Acad. Sci. U. S. A.* 1997; 94:7487–92. [PubMed: 9207118]
151. Santucci-Darmanin S, Neyton S, Lespinasse F, Saunières A, Gaudray P, Paquis-Flucklinger V. The DNA mismatch-repair MLH3 protein interacts with MSH4 in meiotic cells, supporting a role for this MutL homolog in mammalian meiotic recombination. *Hum. Mol. Genet.* 2002; 11:1697–706. [PubMed: 12095912]
152. Yildiz Ö, Majumder S, Kramer B, Sekelsky JJ. *Drosophila* MUS312 interacts with the nucleotide excision repair endonuclease MEI-9 to generate meiotic crossovers. *Mol. Cell.* 2002; 10:1503–1509. [PubMed: 12504024]
153. Baker BS, Carpenter AT. Genetic analysis of sex chromosomal meiotic mutants in *Drosophila melanogaster*. *Genetics.* 1972; 71:255–86. [PubMed: 4625747]

154. Smith GR, Boddy MN, Shanahan P, Russell P. Fission yeast Mus81.Eme1 Holliday junction resolvase is required for meiotic crossing over but not for gene conversion. *Genetics*. 2003; 165:2289–93. [PubMed: 14704204]
155. Hollingsworth NM, Brill SJ. The Mus81 solution to resolution: Generating meiotic crossovers without Holliday junctions. *Genes Dev*. 2004; 18:117–125. [PubMed: 14752007]
156. Zalevsky J, MacQueen AJ, Duffy JB, Kempthues KJ, Villeneuve AM. Crossing over during *Caenorhabditis elegans* meiosis requires a conserved MutS-based pathway that is partially dispensable in budding yeast. *Genetics*. 1999; 153:1271–1283. [PubMed: 10545458]
157. Agostinho A, Meier B, Sonnevile R, Jagut M, Woglar A, Blow J, et al. Combinatorial regulation of meiotic holliday junction resolution in *C. elegans* by HIM-6 (BLM) helicase, SLX-4, and the SLX-1, MUS-81 and XPF-1 nucleases. *PLoS Genet*. 2013; 9:e1003591. [PubMed: 23901331]
158. O'Neil NJ, Martin JS, Youds JL, Ward JD, Petalcorin MIR, Rose AM, et al. Joint molecule resolution requires the redundant activities of MUS-81 and XPF-1 during *Caenorhabditis elegans* meiosis. *PLoS Genet*. 2013; 9:e1003582. [PubMed: 23874209]
159. Saito TT, Lui DY, Kim H-M, Meyer K, Colaiácovo MP. Interplay between structure-specific endonucleases for crossover control during *Caenorhabditis elegans* meiosis. *PLoS Genet*. 2013; 9:e1003586. [PubMed: 23874210]
160. Snowden T, Acharya S, Butz C, Berardini M, Fishel R. hMSH4-hMSH5 Recognizes Holliday Junctions and Forms a Meiosis-Specific Sliding Clamp that Embraces Homologous Chromosomes. *Mol. Cell*. 2004; 15:437–451. [PubMed: 15304223]
161. Snowden T, Shim K-S, Schmutte C, Acharya S, Fishel R. hMSH4-hMSH5 adenosine nucleotide processing and interactions with homologous recombination machinery. *J. Biol. Chem*. 2008; 283:145–54. [PubMed: 17977839]
162. Sonntag Brown M, Lim E, Chen C, Nishant KT, Alani E. Genetic analysis of *mlh3* mutations reveals interactions between crossover promoting factors during meiosis in baker's yeast. *G3 (Bethesda)*. 2013; 3:9–22. [PubMed: 23316435]
163. Hall MC, Wang H, Erie DA, Kunkel TA. High affinity cooperative DNA binding by the yeast Mlh1-Pms1 heterodimer. *J. Mol. Biol*. 2001; 312:637–47. [PubMed: 11575920]
164. Acharya S, Foster PL, Brooks P, Fishel R. The coordinated functions of the *E. coli* MutS and MutL proteins in mismatch repair. *Mol. Cell*. 2003; 12:233–46. [PubMed: 12887908]
165. Miller MP, Amon A, Ünal E. Meiosis I: when chromosomes undergo extreme makeover. *Curr. Opin. Cell Biol*. 2013; 25:687–96. [PubMed: 23916768]
166. Laceyfield S, Murray AW. The spindle checkpoint rescues the meiotic segregation of chromosomes whose crossovers are far from the centromere. *Nat. Genet*. 2007; 39:1273–7. [PubMed: 17828265]

Highlights

- Many organisms have co-opted MMR homologs for meiotic crossing over.
- Mlh1-Mlh3 is an endonuclease that acts in both MMR and meiosis.
- Mlh1-Mlh3 endonuclease activity is required for meiotic crossing over.
- Msh4-Msh5, Mlh1-Mlh3 act in meiotic recombination in a pathway distinct from MMR.
- We outline models for an MLH-mediated Holliday junction resolution pathway in meiosis.

Figure 1. MSH sliding clamps recruit downstream factors required for MMR during DNA replication

The model presented is based on work described in: [24–34]. It should be noted that the associations and disassociations of the factors, as well as stoichiometries are not well established in MMR. (A) MSH dimers (green and blue) have asymmetric nucleotide binding sites (high and low affinity). ATP is indicated by red T and ADP by black D. (1) In the absence of a mismatch, asymmetric ATP binding and hydrolysis results in the opening and closing of the dimeric ring. When ADP is bound to both subunits, the dimer is in the open state and has weak affinity for DNA. Exchange of ADP for ATP (2) is thought to close the ring (3). (B) Recognition of a mismatch causes the MSH complex to exchange ADP for ATP at both sites (1). (2) The MSH dimer changes conformation and enters a sliding clamp mode, moving away from the mismatch (3) to recruit downstream factors, such as MLH proteins (orange and yellow), PCNA (brown), and Exo1 (red), and a search for strand discrimination signals that are hypothesized to involve pre-existing strand discontinuities on either leading or lagging strands. (4) Excision of the mismatch occurs, leaving gaps to be filled in by Pol δ .

Figure 2. Crossing over facilitates proper chromosomal segregation in Meiosis I

Figure is based on models presented in [56,165,166]. During meiosis, homologs (blue and red) are duplicated, forming sister chromatids which are held together by cohesin (1). Recombination between the homologs forms chiasma (2, top, and magnified inset). Normal segregation proceeds when the crossover and cohesion between sister chromosomes allows for proper alignment and attachment to the spindle poles (3-4, top). Crossovers that do not occur (2, bottom) or do not occur in the correct location can cause segregation to the same spindle pole, resulting in nondisjunction (3-4, bottom).

Figure 3. Multiple pathways for strand invasion intermediates

In early prophase I, Spo11 catalyzes the formation of ~150-200 DSBs that undergo 5' to 3' end resection. The 3' single-stranded tails can then invade the homolog to form a D-loop in a reaction catalyzed in part by RPA, Rad52, Rad51, Dmc1, and Rad54. The resulting D-loop intermediate can be: (A) unwound by Sgs1-Top3-Rmi1, followed by synthesis and re-annealing to form non-crossovers (SDSA pathway); (B) stabilized by ZMM proteins (Zip3 and Msh4-Msh5) to enable second-end capture (catalyzed in part by RPA, Rad52, and Rad54), DNA synthesis, branch migration, and ligation, forming a dHJ that is resolved asymmetrically by Mlh1-Mlh3 to form only crossover products; (C) form dHJ independent of ZMM stabilization resolved by Mus81-Mms4 (or Slx1-Slx4 or Yen1) to give a mixture of crossover and non-crossover products.

Mus81-Mms4 (XPF)

Slx1-Slx4 (URI-YIG)

Yen1 (Rad2/XPG)

Mlh1-Mlh3

Figure 4. Substrate specificities of *S. cerevisiae* dHJ resolvases

Figure is adapted and based on data reviewed in [121] and determined for Mlh1-Mlh3 in [45,46]. As described in section 5.1, Mus81-Mms4, Slx1-Slx4, and Yen1 are structure-specific endonucleases that cleave branched structures at discrete locations (green triangle). Mus81-Mms4 displays weak endonuclease activity on continuous four-way junctions (grey) relative to pre-nicked junctions and other indicated branched structures. Slx1-Slx4's *in vitro* nicking positions are currently not determined (ND) on model replication forks. Nicking of model four-way junctions and other branched structures by Mlh1-Mlh3 has not been observed, and activity on supercoiled or open circle DNA substrate has been observed to be non-specific (NS).

Figure 5. Model for creating an asymmetric intermediate to facilitate biased cleavage
 Model is discussed in section 5.3 and is based in part on work presented by Fishel and colleagues, Fung and colleagues, and Kowalczykowski and colleagues [71,124,160], Msh4-Msh5 (purple) binds to invasion intermediates (1). Encountering open-ended duplex allows ATP to be hydrolyzed and the clamp to dissociate (2), allowing iterative loading of Msh4-Msh5 prior to second end capture (3-4). The second-end capture action (5) and its facilitating proteins (e.g. RPA, Rad52, and Rad54) (grey), likely including Zip3 (green) create an asymmetric substrate for the dHJ resolving proteins (6-7).