Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Genetics & Development

Crossover or non-crossover outcomes: tailored processing of homologous recombination intermediates

Aurore Sanchez[1,3], Giordano Reginato[1,2,3] and Petr Cejka[1,2]

DNA breaks may arise accidentally in vegetative cells or in a programmed manner in meiosis. The usage of a DNA template makes homologous recombination potentially error-free, however, recombination is not always accurate. Cells possess a remarkable capacity to tailor processing of recombination intermediates to fulfill a particular need. Vegetatively growing cells aim to maintain genome stability and therefore repair accidental breaks largely accurately, using sister chromatids as templates, into mostly non-crossovers products. Recombination in meiotic cells is instead more likely to employ homologous chromosomes as templates and result in crossovers to allow proper chromosome segregation and promote genetic diversity. Here we review models explaining the processing of recombination intermediates in vegetative and meiotic cells and its regulation, with a focus on MLH1–MLH3-dependent crossing-over during meiotic recombination.

Addresses
[1] Institute for Research in Biomedicine, Università della Svizzera italiana (USI), Faculty of Biomedical Sciences, Bellinzona, Switzerland
[2] Department of Biology, Institute of Biochemistry, Eidgenössische Technische Hochschule (ETH), Zürich, Switzerland

Corresponding author: Cejka, Petr (petr.cejka@irb.usi.ch)
[3] These authors contributed equally.

Current Opinion in Genetics and Development 2021, **71**:39–47

This review comes from a themed issue on **Homologous recombination in meiosis and repair**

Edited by **Eric C Greene** and **Rodney J Rothstein**

https://doi.org/10.1016/j.gde.2021.06.012

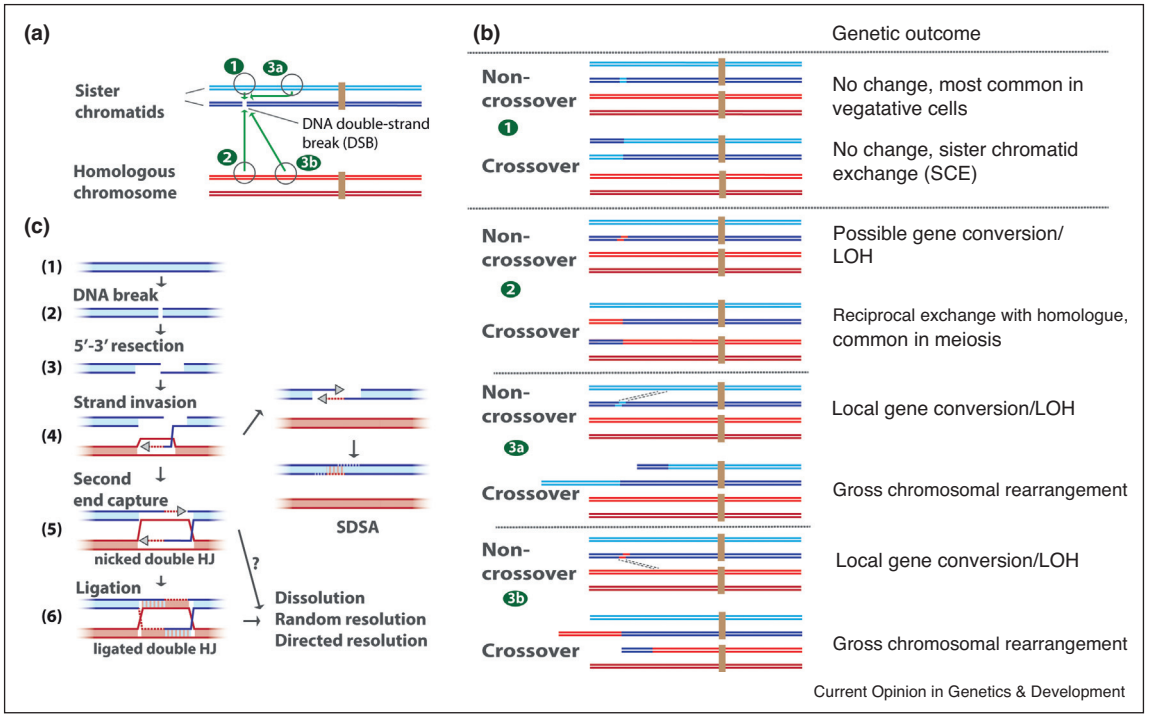
Introduction

DNA double-strand breaks (DSBs) can be repaired by non-homologous end-joining (NHEJ) or homologous recombination (HR). NHEJ is template-independent, and thus frequently leads to mutations at the break site. In cases when multiple DSBs are present, NHEJ can join the wrong pieces of DNA together, leading to gross genome rearrangements. HR is instead template-directed, and therefore largely accurate [1]. Mutations during HR can arise during DNA synthesis, which has a much higher error rate compared to DNA replication [2], or when DNA sequence other than from the same locus on the sister chromatid is used as a template. Allelic recombination represents an event whereby the broken DNA is repaired according to a sequence from the same locus on the sister chromatid (Figure 1a, point 1), or from the same locus, but on the homologous chromosome (Figure 1a, point 2). Allelic recombination with the sister chromatid is the most common recombination process in vegetative cells that generally leads to faithful outcomes, since sister chromatids have the identical sequence. Usage of the homolog as a template, which is rare in vegetative cells, can lead to a loss of heterozygosity (LOH). Such LOH events have been linked to cellular transformation [1]. In meiotic cells, there is a bias towards the use of a homolog as a template at the expense of the sister chromatid, which is achieved by preferential disruption of intermediates arising between the sister chromatids to favor interhomolog recombination [3,4]. A repair event that utilizes a template from a different locus, such as in repetitive DNA regions or between sequences that underwent duplications, is termed ectopic recombination, or non-allelic homologous recombination (NAHR), and is often mutagenic (Figure 1a, points 3a and 3b).

Each of the above recombination events, irrespectively of the template used, may result in crossover (CO) or non-crossover (NCO) products (Figure 1b). CO is a recombination outcome in which the arms of the recombining DNA molecules exchange. COs between sister chromatids are defined as sister chromatid exchanges (SCEs). Although not mutagenic *per se*, elevated SCEs are harbingers of genome instability. COs taking place between homologs are detrimental since they may lead to LOH after mitotic division, which could ultimately reveal the expression of recessive alleles. COs occurring during NAHR automatically yield gross chromosomal rearrangements, which include chromosome translocations, deletions, duplications and inversions (Figure 1b). During NCO events, on the other hand, DNA sequences flanking the recombination site are not exchanged. Nevertheless, local transfer of genetic information between the recombining molecules can occur, leading to gene conversion tracts. NCOs between allelic sites on sister chromatids are accurate, whereas NCOs between homologs or ectopic sites can reveal the expression of recessive and deleterious alleles upon gene conversion.

Mechanistically, HR is initiated by resection of the DSB ends, leading to 3′-ssDNA overhangs. These overhangs are bound by RecA family strand exchange proteins that

Figure 1



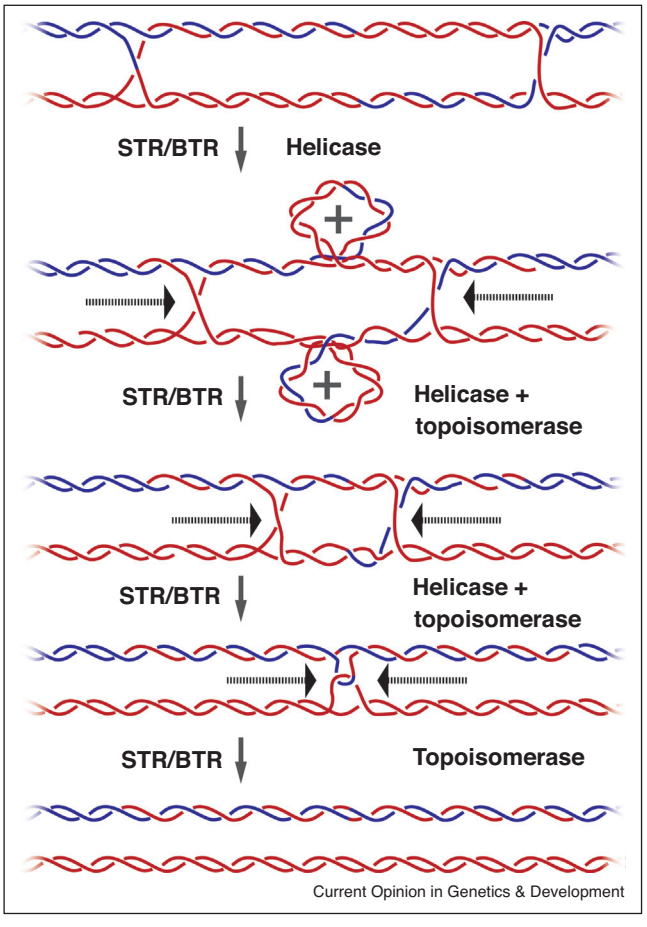
Overview of recombination-based DSB repair pathways and their genetic outcomes.
(a) DSBs can be repaired based on templates from various parts of the genome. HR-mediated repair of a DSB can be allelic if the respective template position is on a sister chromatid (1, most common) or on a homologous chromosome (2, uncommon in vegetative cells, more common in meiotic cells). In ectopic recombination, the template is located at a different locus in the genome (3a and 3b, rare event).
(b) Genetic outcomes of HR-mediated repair events depending on the template sequence position depicted in (a). Each event can result in crossover or non-crossover products. For simplicity, genetic transfer from the template into the broken DNA was depicted, although, depending on the pathway used, the transfer can occur in both directions (not shown). Crossover with sister chromatid is defined as a sister chromatid exchange (1, bottom). Crossovers with the homologous chromosome constitute typical meiotic crossovers (2, bottom). Crossovers with ectopic sites have the most serious consequences in terms of genome instability (3a and 3b, bottom parts).
(c) Main steps in DSB repair by HR. First, the DSB is resected to expose a 3′-terminated overhang (3), which subsequently invades the template DNA forming the D-loop (4). In the synthesis-dependent strand-annealing (SDSA) subpathway, the D-loop is disrupted and the newly synthetized strand anneals with the exposed ssDNA from the other side of the break. Gaps are filled-in by DNA synthesis and the integrity of the DNA molecule is restored by ligation. The template molecule is unaffected in this process (right side). In the canonical DSB repair pathway (DSBR) the displaced strand of the template molecule is annealed to the second end of the broken DNA molecule. Subsequent DNA synthesis results in the formation of a double Holliday junction intermediate, nicked (5) or ligated (6), which can be processed by the dissolution, random resolution or directed resolution pathways.

include RAD51 and DMC1, which catalyze the search, pairing and invasion of the template DNA, leading to the formation of a D-loop intermediate (Figure 1c). In vegetatively growing cells, the main recombinase is RAD51, which is less proficient upon imperfect pairing with the template, thereby reducing the likelihood of inter-homologue recombination. In meiosis, the key recombinase is instead DMC1, which can better tolerate mismatches in the heteroduplex, licensing also inter-homologue recombination, while RAD51 has only an accessory role [5]. The invading 3′-end at D-loops then primes DNA synthesis. In synthesis-dependent strand annealing (SDSA) subpathway, the joint molecule intermediate is subsequently dismantled, and annealed back to the resected second end of the broken DNA (Figure 1c). SDSA leads to exclusively NCOs. Only the originally broken DNA contains heteroduplex DNA, while template DNA in SDSA remains unchanged. Because of its limited mutagenic potential, SDSA constitutes a major recombination pathway in vegetative cells. SDSA is also frequently used in meiotic cells, where it accounts for the majority of NCOs [3]. Here, we focus on mechanisms of processing of joint molecule intermediates arising in the canonical DSB repair pathway (DSBR) [1,6]. In DSBR, the D-loop is stabilized and the second end of the broken DNA is then annealed to the displaced strand (termed as second-end capture). After DNA synthesis and DNA ligation, the double Holliday junction (dHJ) is formed (Figure 1c). This recombination intermediate can be processed by at least three diverse mechanisms, including dissolution, unbiased resolution or crossover-directed resolution as discussed in the next sections.

Dissolution: non-crossover products only

Dissolution likely represents the default mode of dHJ processing in vegetatively growing cells. Mechanistically, dissolution involves convergent migration of the two HJs until the junctions collapse on each other and the DNA molecules separate into exclusively NCO products (Figure 2) [7–10]. Similarly as in SDSA, only the originally broken DNA contains heteroduplex DNA, but in a different configuration, while the template remains unchanged. HJ migration in dissolution is powered by the motor activity of a RecQ family helicase, such as Bloom (BLM) in human cells or Sgs1 in *Saccharomyces cerevisiae*. As dHJs in a chromosomal setting are topologically constrained, initial helicase-driven convergent branch migration gives rise to positive supercoiling between the junctions, which would stall further movement (Figure 2). To this point, BLM associates with the type IA topoisomerase TopoIIIα and OB-fold proteins RMI1 and RMI2 in humans, while Sgs1 binds Top3-Rmi1 in yeast (BTR/STR complex, also known as the dissolvasome) to relieve the torsional stress. Only the concerted action of BLM/Sgs1 and TopoIIIα/Top3 allows the convergent branch migration to proceed [7,10,11]. The most critical phase of dissolution occurs when the two junctions are in close proximity, and the two DNA molecules are held together by a few remaining linkages. Rmi1 was found to stabilize the open gate conformation of Top3, which likely increases the time available for strand passage [10,12]. The stabilization of the reaction intermediate by Rmi1 becomes particularly important during the last steps of dissolution. Top3-Rmi1 also promote the motor activity of Sgs1, highlighting the functional integration of the complex [13,14]. A similar interplay was also observed with the human proteins [15].

Figure 2



Overview of the dissolution process leading to non-crossovers. In dissolution, the two HJs are migrated towards each other by the motor activity of the helicase component of the dissolvasome (Sgs1 in yeast and BLM in human cells). The convergent branch migration creates positive supercoiling between the junctions (2), which is relaxed by the type IA topoisomerase activity of the complex (Top3 in yeast or TopoIIIα in human cells), allowing thus further migration (3). Yeast Rmi1 or human RMI1–RMI2, respectively, stimulate the last steps of the reaction when the junctions are close to each other (4). Ultimately, the two branch points collapse on each other and the molecules are decatenated by TopoIIIα/Top3 (5). The template is unchanged in this process.

Mutations in BLM are associated with the Bloom syndrome, an autosomal recessive disorder characterized by cancer predisposition. At the cellular level, BLM-deficient cells display highly elevated levels of COs. These rearrangements are thought to result from unbiased resolution of undissolved dHJs by structure-specific endonucleases, which are employed as a backup in BLM-deficient cells (see below), leading to LOH, thus explaining the cancer risk. Mutations in TopoIIIα cause a Bloom syndrome-like disorder, underpinning the functional interaction with the BLM helicase [16].

Sgs1 activity was found to be controlled in a cell cycle dependent manner. Sgs1 is stimulated by cyclin-dependent kinase (CDK)-catalyzed phosphorylation, which enhances its activity in S-phase, indicating that dissolution can likely take place early in the cell cycle [17]. Whether BLM is regulated in a similar manner remains undefined. BLM physically and functionally interacts with TopBP1, which was described to stabilize its protein levels in S-phase [18], although this result was later disputed [19]. Nevertheless, the interaction of BLM and TopBP1 is required to prevent SCEs [18,19], but the underlying mechanism of potential TopBP1 requirement for dissolution is not clear. Activation of Sgs1 and hence dissolution early in the cell cycle, as observed in yeast [17], may explain why it is likely a dominant mechanism of dHJ processing in vegetative cells. In mitotic and meiotic cells, the STR complex also facilitates non-crossovers by disrupting or dissolving D-loops to promote SDSA [20,21]. In genetic experiments, however, it is often impossible to unambiguously distinguish whether NCOs arise from SDSA or dHJ dissolution [20,22••]. Top3-Rmi1, with or without Sgs1, were also shown to disentangle pathologically intertwined meiotic chromosomes, which

likely involves their decatenation activity [7,21,23]. It is not established whether, or to which extent, dHJ dissolution occurs in meiotic cells [22••].

Unbiased resolution: a random mixture of crossover and non-crossover products

The mechanism of unbiased resolution of HJs by structure-specific endonucleases (SSNs) involves simple endonucleolytic cleavage at or near the branch points of the DNA junctions in both vegetative and meiotic cells [24,25]. Canonical dHJs are thought to be cleaved in an unbiased manner; therefore, depending on the orientation of the cuts, both COs and NCOs are produced (Figure 3a). Both the originally broken and the template DNA molecules contain heteroduplex DNA, in contrast to dissolution.

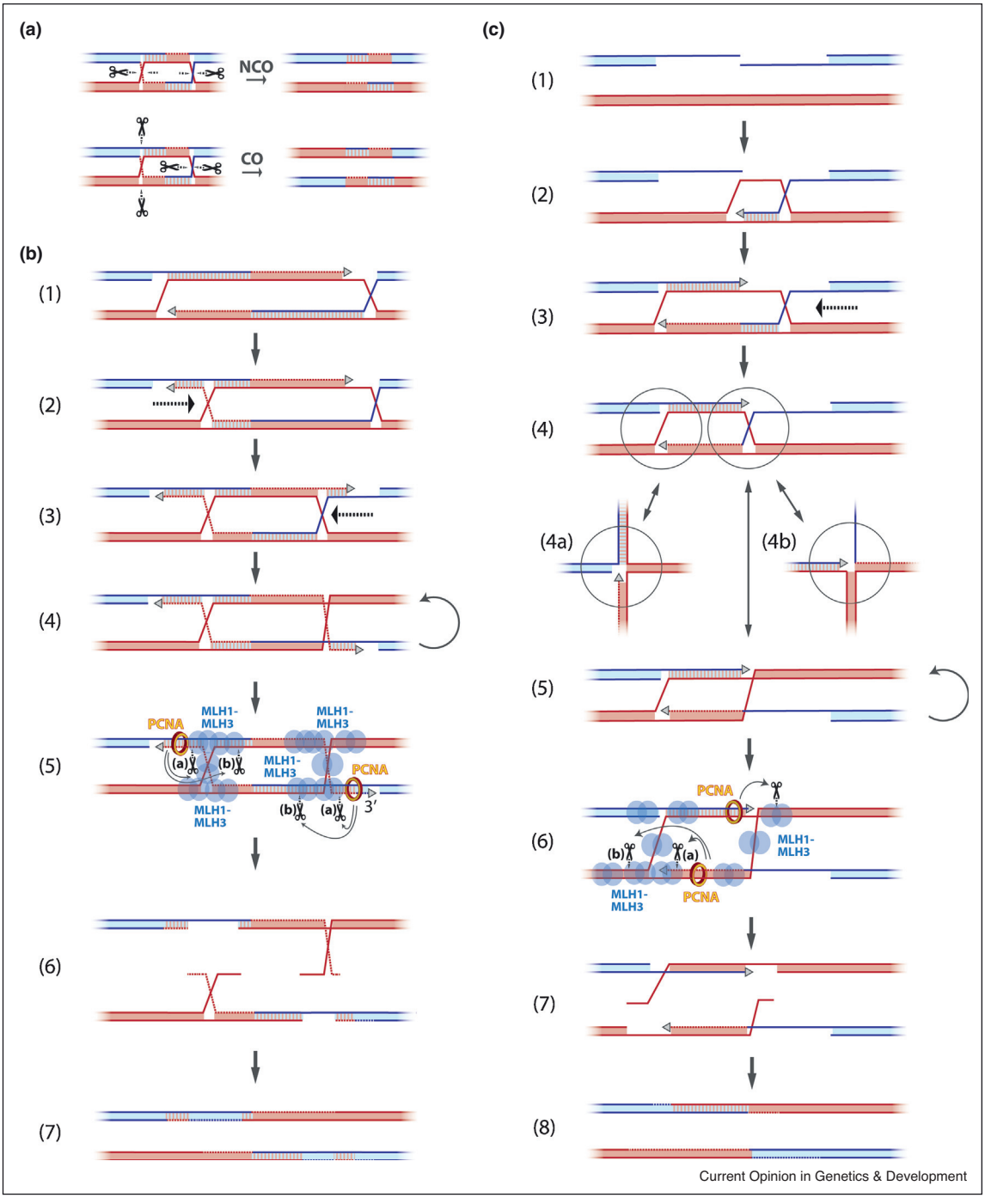
Enzymes closest to a prototypical resolvase in eukaryotes are yeast Yen1 and human GEN1 [26]. GEN1/Yen1 is activated very late in the cell cycle. In S-phase, Yen1 is phosphorylated by CDK, which limits its nuclear import and inhibits its catalytic activity. Only in anaphase of M-phase, Cdc14 dephosphorylates Yen1 to activate it, which indicates that Yen1 is used as a last resort to process structures generated by homologous recombination such as HJs or anaphase bridges [27–29]. Human GEN1 is instead primarily regulated by nuclear exclusion independently of phosphorylation, and gains access to DNA upon the breakdown of the nuclear envelope in M-phase [30]. Untimely and premature activation of Yen1 leads to genome instability [31•]. In meiotic cells, Yen1 becomes fully activated late in meiosis II, showing that it provides a backup for the disentanglement of unresolved recombination intermediates also in meiosis [31•]. MUS81–EME1 or MUS81–EME2 (or Mus81–Mms4 in yeast cells) and SLX1–SLX4/Slx1–Slx4 are additional enzymes capable to process HJs. In yeast, the catalytic activity of Mus81 is activated upon sequential phosphorylation of Mus81–Mms4 by kinases that operate in the G2-M phase, including M-CDK, Cdc5 and Dbf4-dependent kinase (DDK) [24,32–34]. As with Yen1, premature activation of Mus81 leads to genome instability [35]. To prevent any aberrant Yen1 or Mus81 activity in the next cell cycle, their function must be subsequently extinguished, which is achieved by SUMO-targeted modification of Yen1 by Slx5–Slx8, or phosphorylation of Mms4 by the STUbL-Esc2-Cullin8 E3 ubiquitin ligase complexes, respectively, leading to Yen1 and Mus81 degradation [36,37]. Slx1–Slx4 is activated upon formation of the heterocomplex. The Mus81–Mms4 and Slx1–Slx4 dimers act mostly independently of each other in yeast, while human MUS81–EME1 synergizes with SLX1–SLX4 [38]. To cleave HJs, SLX1 makes the first cut, and MUS81 subsequently cleaves the opposite strand. The activity of the MUS81–EME1–SLX1–SLX4 complex is further stimulated by physical interactions with the nucleotide excision repair factors XPF–ERCC1 and the mismatch repair (MMR) heterodimer MSH2–MSH3 (MutSβ) [39,40]. As the resolution enzymes are thought to be activated later than BTR/STR, they mostly act on structures that escaped the attention or were unsuitable for the dissolvasome, making them a second choice for joint molecule processing to avoid as much as possible CO formation in mitotic cells.

Biased resolution in meiosis: crossovers only

In many organisms, the majority of meiotic COs are dependent on the enzymatic activity of the mammalian/yeast MLH1–MLH3/Mlh1–Mlh3 (MutLγ) nuclease, which processes recombination joint molecules exclusively into COs [41,42]. COs resulting from directed resolution are more evenly spaced along the chromosomes than expected from a random distribution, a phenomenon known as CO interference [3]. CO interference facilitates proper chromosome segregation to prevent aneuploidy, and helps repress deleterious COs in centromere/telomere proximal regions [43,44]. Two closely spaced crossovers largely cancel each other out in terms of a genetic outcome, so interference may also help maximize diversification of the recombined molecules. Implementation of interfering CO formation is dependent on the activity of the ZMM proteins, originally identified in yeast, but now found in many other eukaryotes [45]. The ZMM proteins comprise, among others, the synaptonemal complex and SUMO ligase proteins such as Zip1, Zip2, Zip3 and Zip4, Spo16, the Mer3 helicase and the Msh4–Msh5 (MutSγ) complex. The Msh4–Msh5 heterodimer is related to mismatch recognition factors Msh2–Msh6 (MutSα) and Msh2–Msh3 (MutSβ), although it has no MMR function *per se* [45]. Recombinant MutSγ was found to bind HJs and their precursors, on which it forms a sliding clamp. MutSγ clamp is thought to embrace adjacent homologous DNA arms to stabilize recombination intermediates [46]. The levels of MutSγ must be tightly controlled to guarantee its proper function [47].

MutLγ is not described as a ZMM protein, although it is similarly required for CO formation, together with EXO1/Exo1 [41,48]. MutLγ is also related to MMR proteins. In fact, the MLH1/Mlh1 subunit is shared with the key MMR factors MLH1–PMS2 (MutLα) in humans or Mlh1–Pms1 (MutLα) in yeast. MutLγ has a minor function in MMR, and recently was shown to act pathologically in triplet repeat expansion together with MutSβ [49,50,51•,52,53]. The endonuclease activity of MLH3 was also implicated during the late step of homologous recombination in mammalian somatic cells [54]. However, the key physiological function of MutLγ lies in the directed resolution pathway in meiosis, and its deficiency leads to infertility in humans [55,56].

Figure 3



Overview of random and crossover-biased resolution pathways.
(a) In unbiased resolution of double Holliday junctions, the two branch points can be cleaved by SSNs symmetrically with respect to one another (top), resulting in a non-crossover (NCO), or asymmetrically (bottom), resulting in a crossover event (CO). Because of the random nature of this process, SSN-mediated resolution of dHJs in mitotic cells leads to the formation of both NCO and CO products. For simplicity, only one of the two possibilities for CO and NCO formation is shown. The model explains generation of COs in mitotic cells.
(b) A model for crossover-directed processing of double Holliday junctions in meiosis. Following the formation of an unligated dHJ (1), the junctions partially migrate towards each other (2, 3). Structures depicted in (3) and (4) are isoforms that reflect possible chromosome arm rotation around the junction. PCNA is loaded at strand discontinuities on the outer side of the junctions and directs DNA cleavage by MLH1–MLH3 (5). If junctions are ligated, both (a) and (b) cuts are required (in total at least 4 cuts per dHJ). If junctions remained unligated, only (b) cuts are needed (5). The recombining DNA molecules ultimately separate upon branch migration or unwinding of the DNA stretch between the branch points and incision sites, or after nucleolytic degradation of the same DNA region (6). Separated DNA molecules are filled-in by DNA synthesis and sealed by

The mechanism employed by the directed resolution pathway is likely related to MMR. In MMR, MutSα or MutSβ bound to a mismatch help activate the MutLα nuclease. The key strand discrimination signal is a discontinuity in the newly replicated strand, which, by definition, contains the mismatch [57]. The replication clamp proliferating cell nuclear antigen (PCNA) plays a crucial role to activate the MutLα nuclease to nick the newly synthesized strand. Mechanistically, the strand to be incised is determined by the specific loading orientation of PCNA directed by the nick [58]. Therefore, in MMR, the MutLα nuclease cleaves DNA in a biased manner, followed by EXO1-mediated exonucleolytic excision of the DNA stretch from the incision site past the mismatch [57]. Similar asymmetric DNA cleavage by MutLγ has been likely repurposed in meiotic recombination to bias joint molecule processing towards COs [59••,60••].

MutLγ is an endonuclease that nicks one strand of a DNA duplex, but it does not have a structure-specific nuclease activity, despite it is preferentially binding HJs [53,61–63]. *In vitro*, the endonuclease activity of MutLγ is stimulated by MutSγ, EXO1 and the clamp loader replication factor C (RFC) together with PCNA [59••,60••]. The activation by EXO1 is in agreement with genetic observations in yeast and mice, which indicated a structural function of EXO1 to promote MLH3-dependent COs [48,64]. Exo1 in *S. cerevisiae* also helps recruit the Polo kinase Cdc5, which may provide an additional way to facilitate the activation of MutLγ [65,66]. Exo1 thus appears to be a central regulator of MutLγ-dependent COs, able to coordinate CO formation stimulation, chromatin remodeling and cell cycle control through its interaction with MutLγ, the chromatin remodeler Chd1 and the Cdc5 kinase, respectively [65,66].

The physical and functional interaction of MutLγ with PCNA likely determines the directionality of DNA cleavage. In experiments with negatively supercoiled dsDNA *in vitro*, RFC loads PCNA indiscriminately, promoting random DNA nicking by MutSγ–EXO1–MutLγ [59••,60••]. During recombination, PCNA assists Polδ in DNA synthesis, which only extends the broken DNA strand. PCNA may be retained asymmetrically at the joint molecule intermediates until the resolution stage to provide the critical discrimination signal. Indeed, RFC and PCNA were found to localize to prospective crossover sites along meiotic chromosomes [59••,60••,67]. Additionally, whereas dHJs are symmetric structures, they arise from D-loops that are asymmetric. Using structured illumination microscopy in *Caenorhabditis elegans*, one distinct population of MutSγ was observed on early recombination intermediates. Later, as dHJs formed, MutSγ relocated into two distinct foci, possibly to sites internal to the two HJs [68•]. Notably, MLH1–MLH3 is absent in *C. elegans*, and CO-directed resolution is instead catalyzed by SSNs. However, such regulated distribution of MutSγ will likely be common to other organisms. In case MutSγ is located asymmetrically with respect to the junctions, it might provide additional signal for directional DNA cleavage. The presence of MutSγ between the junction points of HJs may also prevent convergent branch migration by dissolution, preventing thus NCOs [68•].

Although the exact structures of the meiotic recombination intermediates and their occupancy by MutLγ and cofactors are not known, several models can be proposed to explain CO-directed processing (Figure 3b and c) [60••,69•]. The model depicted in Figure 3b, originally proposed by Hunter and colleagues [60••], employs partial convergent branch migration. PCNA is then loaded on either side of the junctions to direct DNA cleavage by MutLγ, reminiscent of MMR. Figure 3c shows a related model, in which PCNA, retained after DNA synthesis, directs cleavage from sites between the junction points. It should be also considered that not all dHJs may be fully ligated, limiting the number of MutLγ-dependent nicks required to resolve the junctions, and removing also topological constraints during the processing [69•]. Furthermore, the unligated nicks may serve as discrimination sites themselves. Residual nicks depicted in Figure 3c could also explain CO-directed processing in organisms that rely on SSNs instead of MutLγ, such as in *C. elegans*. Prematurely activated yeast Yen1 could partially compensate for CO defects in *mlh3* cells and promoted CO-biased resolution when expressed during meiotic pachytene, at which point CO-designated HR intermediates have formed [31•]. This shows that SSNs can drive biased processing when ZMM proteins are intact, even in organisms that possess MutLγ. Indeed, cutting the DNA strand at junction points opposite to nicks, a known activity of SSNs, would also result in CO-biased processing (Figure 3c). Both models envision the

(Figure 3 Legend Continued) ligation (7). In this model, PCNA directs MLH1–MLH3 to nick DNA 'upstream', that is, analogously to canonical mismatch repair. However, a new molecule of PCNA would be likely needed to be loaded after branch migration at step 5.
(c) Alternative model for crossover-biased processing of double Holliday junctions. To accommodate for one-sided events suggested from sequencing studies (see text), the D-loop is initially migrated (2, 3) before second end-capture and DNA synthesis takes place (4). Structures depicted in 4, 4a/b and 5 are equivalent. PCNA remaining after DNA synthesis between the junctions directs the MLH1–MLH3 nuclease to nick DNA. The model depicts unligated junctions, which lack the topological constrains of ligated junctions, and only require 2 cleavage events (6). SSNs could also resolve the intermediate into COs, upon cutting the strand opposite to strand discontinuities (4a and 4b). Following the cuts, DNA gets separated and repair process completes as in (b). In this model, the MLH1–MLH3 catalyzed nicking takes place downstream of PCNA, in contrast to canonical mismatch repair. However, the model employs the same molecule of PCNA as used for DNA synthesis. Models in (b) and (c) are theoretical concepts that require experimental validation.

possibility of multiple cleavage events adjacent to the junction by MutLγ, in accord with sequencing of meiotic recombination products resulting from complex events in yeast [22••]. In the canonical DSB repair model (Figures 1c and 3a), dHJ resolution yields heteroduplex DNA tracts on both sides of the initiating DSB. However, extensive branch migration depicted in the model (Figure 3c) would give rise to heteroduplex DNA tracts only on one side, as seen in yeast and mice [22••,70••]. Nevertheless, the exact mechanism of CO-directed resolution is unknown and requires experimental validation.

DNA cleavage at sites away from the branch points of the junctions does not *per se* separate the recombining DNA molecules. DNA segregation might ultimately require limited nucleolytic activity or DNA unwinding. Although the nuclease activity of Exo1 was found dispensable for meiotic CO resolution in yeast [48], it cannot be excluded, as it may be redundant with another process, such as degradation by another nuclease or DNA unwinding. The involvement of the EXO1 nuclease to degrade DNA from the nicking sites to the junctions would be consistent with MMR models [57]. Alternatively, a helicase could separate the DNA strands between the incision sites, allowing chromosomes to separate. The excellent candidate for this function is the BTR/STR complex. Indeed, Sgs1 was found to play a role in the Mlh3-dependent pro-CO pathway in genetic experiments [41], and footprints of extended branch migration were observed in the products of meiotic recombination in yeast and mice [22••,70••]. Similarly, BLM helicase localizes to sites of meiotic recombination in *C. elegans* and mice [42,68•,71].

Conclusion and perspectives

Mechanisms of Holliday junction dissolution and unbiased resolution are relatively well understood, and the reactions have been reconstituted with purified components. More recent research provided insights into the regulation of these pathways. In contrast, crossover-directed resolution in meiotic cells remains poorly understood. Recent genetic and biochemical studies allowed to propose the first models showing how biased crossing-over might be enforced, and are hinting at similarities with MMR. Elucidating the precise nature of meiotic recombination intermediates, their occupancy by MutLγ and co-factors and the underlying mechanism of DNA cleavage leading to COs remains a challenging goal for the future.

Conflict of interest statement

None declared.

Acknowledgements

Research in Cejka laboratory is supported by grants from the Swiss National Science Foundation (31003A_17544), European Research Council (681630) and Swiss Cancer League. The authors thank members of the Cejka laboratory for comments on the manuscript, and apologize to authors whose work could not be cited due to length limitation.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Kawale AS, Sung P: **Mechanism and significance of chromosome damage repair by homologous recombination**. *Essays Biochem* 2020, **64**:779-790.
2. Hicks WM, Kim M, Haber JE: **Increased mutagenesis and unique mutation signature associated with mitotic gene conversion**. *Science* 2010, **329**:82-85.
3. Hunter N: **Meiotic recombination: the essence of heredity**. *Cold Spring Harb Perspect Biol* 2015, **7**:a016618.
4. Sandhu R, Monge Neria F, Monge Neria J, Chen X, Hollingsworth NM, Borner GV: **DNA helicase Mph1(FANCM) ensures meiotic recombination between parental chromosomes by dissociating precocious displacement loops**. *Dev Cell* 2020, **53**:458-472 e455.
5. Cloud V, Chan YL, Grubb J, Budke J, Bishop DK: **Rad51 is an accessory factor for Dmc1-mediated joint molecule formation in meiosis**. *Science* 2012, **337**:1222-1225.
6. Szostak JW, Orr-Weaver TL, Rothstein RJ, Stahl FW: **The double-strand-break repair model for recombination**. *Cell* 1983, **33**:25-35.
7. Bizard AH, Hickson ID: **The many lives of type IA topoisomerases**. *J Biol Chem* 2020, **295**:7138-7153.
8. Wu L, Hickson ID: **The Bloom's syndrome helicase suppresses crossing over during homologous recombination**. *Nature* 2003, **426**:870-874.
9. Plank JL, Wu J, Hsieh TS: **Topoisomerase IIIalpha and Bloom's helicase can resolve a mobile double Holliday junction substrate through convergent branch migration**. *Proc Natl Acad Sci U S A* 2006, **103**:11118-11123.
10. 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**:1377-1382.
11. Chen SH, Plank JL, Willcox S, Griffith JD, Hsieh TS: **Top3alpha is required during the convergent migration step of double Holliday junction dissolution**. *PLoS One* 2014, **9**:e83582.
12. Bocquet N, Bizard AH, Abdulrahman W, Larsen NB, Faty M, Cavadini S, Bunker RD, Kowalczykowski SC, Cejka P, Hickson ID *et al.*: **Structural and mechanistic insight into Holliday-junction dissolution by topoisomerase IIIalpha and RMI1**. *Nat Struct Mol Biol* 2014, **21**:261-268.
13. Kasaciunaite K, Fettes F, Levikova M, Daldrop P, Anand R, Cejka P, Seidel R: **Competing interaction partners modulate the activity of Sgs1 helicase during DNA end resection**. *EMBO J* 2019, **38**:e101516.
14. Xue C, Wang W, Crickard JB, Moevus CJ, Kwon Y, Sung P, Greene EC: **Regulatory control of Sgs1 and Dna2 during eukaryotic DNA end resection**. *Proc Natl Acad Sci U S A* 2019, **116**:6091-6100.
15. Daley JM, Chiba T, Xue X, Niu H, Sung P: **Multifaceted role of the topo IIIalpha-RMI1-RMI2 complex and DNA2 in the BLM-dependent pathway of DNA break end resection**. *Nucleic Acids Res* 2014, **42**:11083-11091.
16. Martin CA, Sarlos K, Logan CV, Thakur RS, Parry DA, Bizard AH, Leitch A, Cleal L, Ali NS, Al-Owain MA *et al.*: **Mutations in TOP3A cause a bloom syndrome-like disorder**. *Am J Hum Genet* 2018, **103**:221-231.
17. Grigaitis R, Ranjha L, Wild P, Kasaciunaite K, Ceppi I, Kissling V, Henggeler A, Susperregui A, Peter M, Seidel R *et al.*: **Phosphorylation of the RecQ helicase Sgs1/BLM controls its**

DNA unwinding activity during meiosis and mitosis. *Dev Cell* 2020, **53**:706-723 e705.

18. Wang J, Chen J, Gong Z: **TopBP1 controls BLM protein level to maintain genome stability**. *Mol Cell* 2013, **52**:667-678.

19. Blackford AN, Nieminuszczy J, Schwab RA, Galanty Y, Jackson SP, Niedzwiedz W: **TopBP1 interacts with BLM to maintain genome stability but is dispensable for preventing BLM degradation**. *Mol Cell* 2015, **57**:1133-1141.

20. Kaur H, Gn K, Lichten M: **Unresolved recombination intermediates cause a RAD9-dependent cell cycle arrest in *Saccharomyces cerevisiae***. *Genetics* 2019, **213**:805-818.

21. Fasching CL, Cejka P, Kowalczykowski SC, Heyer WD: **Top3-Rmi1 dissolve Rad51-mediated D loops by a topoisomerase-based mechanism**. *Mol Cell* 2015, **57**:595-606.

22. •• Marsolier-Kergoat MC, Khan MM, Schott J, Zhu X, Llorente B: **Mechanistic view and genetic control of DNA recombination during meiosis**. *Mol Cell* 2018, **70**:9-20 e26

A study emplying next generation sequencing to monitor products of meiotic recombination. Exploiting the genetic divergence between two yeast strains, the authors monitor meiotic heteroduplex DNA patterns. The study finds footprints of extensive HJ branch migration. The authors propose that MLH1–MLH3 process meiotic joint molecules by nicking at sites some distance away from the HJs.

23. 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.

24. Matos J, West SC: **Holliday junction resolution: regulation in space and time**. *DNA Repair (Amst)* 2014, **19**:176-181.

25. Falquet B, Rass U: **Structure-specific endonucleases and the resolution of chromosome underreplication**. *Genes (Basel)* 2019, **10**:232.

26. Ip SC, Rass U, Blanco MG, Flynn HR, Skehel JM, West SC: **Identification of Holliday junction resolvases from humans and yeast**. *Nature* 2008, **456**:357-361.

27. Eissler CL, Mazon G, Powers BL, Savinov SN, Symington LS, Hall MC: **The Cdk/Cdc14 module controls activation of the Yen1 Holliday junction resolvase to promote genome stability**. *Mol Cell* 2014, **54**:80-93.

28. Blanco MG, Matos J, West SC: **Dual control of Yen1 nuclease activity and cellular localization by Cdk and Cdc14 prevents genome instability**. *Mol Cell* 2014, **54**:94-106.

29. Garcia-Luis J, Machin F: **Mus81-Mms4 and Yen1 resolve a novel anaphase bridge formed by noncanonical Holliday junctions**. *Nat Commun* 2014, **5**:5652.

30. Chan YW, West SC: **Spatial control of the GEN1 Holliday junction resolvase ensures genome stability**. *Nat Commun* 2014, **5**:4844.

31. • Arter M, Hurtado-Nieves V, Oke A, Zhuge T, Wettstein R, Fung JC, Blanco MG, Matos J: **Regulated crossing-over requires inactivation of Yen1/GEN1 resolvase during meiotic prophase I**. *Dev Cell* 2018, **45**:785-800 e786

The authors show that prematurely activated Yen1 can compensate for the lack of Mlh3 in yeast meiosis. As long as the ZMM function was intact, Yen1 could generate crossovers in a biased manner. Although the mechanism is not clear, the data suggest that the nature of the meiotic recombination intermediates can provide a signal for CO-biased processing in the absence of Mlh3.

32. 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**:158-172.

33. Princz LN, Wild P, Bittmann J, Aguado FJ, Blanco MG, Matos J, Pfander B: **Dbf4-dependent kinase and the Rtt107 scaffold promote Mus81-Mms4 resolvase activation during mitosis**. *EMBO J* 2017, **36**:664-678.

34. Gallo-Fernandez M, Saugar I, Ortiz-Bazan MA, Vazquez MV, Tercero JA: **Cell cycle-dependent regulation of the nuclease activity of Mus81-Eme1/Mms4**. *Nucleic Acids Res* 2012, **40**:8325-8335.

35. Szakal B, Branzei D: **Premature Cdk1/Cdc5/Mus81 pathway activation induces aberrant replication and deleterious crossover**. *EMBO J* 2013, **32**:1155-1167.

36. Waizenegger A, Urulangodi M, Lehmann CP, Reyes TAC, Saugar I, Tercero JA, Szakal B, Branzei D: **Mus81-Mms4 endonuclease is an Esc2-STUbL-Cullin8 mitotic substrate impacting on genome integrity**. *Nat Commun* 2020, **11**:5746.

37. Talhaoui I, Bernal M, Mullen JR, Dorison H, Palancade B, Brill SJ, Mazon G: **Slx5-Slx8 ubiquitin ligase targets active pools of the Yen1 nuclease to limit crossover formation**. *Nat Commun* 2018, **9**:5016.

38. Wyatt HD, Sarbajna S, Matos J, West SC: **Coordinated actions of SLX1-SLX4 and MUS81-EME1 for Holliday junction resolution in human cells**. *Mol Cell* 2013, **52**:234-247.

39. Wyatt HD, Laister RC, Martin SR, Arrowsmith CH, West SC: **The SMX DNA Repair Tri-nuclease**. *Mol Cell* 2017, **65**:848-860 e811.

40. Young SJ, Sebald M, Shah Punatar R, Larin M, Masino L, Rodrigo-Brenni MC, Liang CC, West SC: **MutSbeta stimulates Holliday junction resolution by the SMX complex**. *Cell Rep* 2020, **33**:108289.

41. Zakharyevich K, Tang S, Ma Y, Hunter N: **Delineation of joint molecule resolution pathways in meiosis identifies a crossover-specific resolvase**. *Cell* 2012, **149**:334-347.

42. Toledo M, Sun X, Brieno-Enriquez MA, Raghavan V, Gray S, Pea J, Milano CR, Venkatesh A, Patel L, Borst PL *et al.*: **A mutation in the endonuclease domain of mouse MLH3 reveals novel roles for MutLgamma during crossover formation in meiotic prophase I**. *PLoS Genet* 2019, **15**:e1008177.

43. Nambiar M, Chuang YC, Smith GR: **Distributing meiotic crossovers for optimal fertility and evolution**. *DNA Repair (Amst)* 2019, **81**:102648.

44. Otto SP, Payseur BA: **Crossover interference: shedding light on the evolution of recombination**. *Annu Rev Genet* 2019, **53**:19-44.

45. Pyatnitskaya A, Borde V, De Muyt A: **Crossing and zipping: molecular duties of the ZMM proteins in meiosis**. *Chromosoma* 2019, **128**:181-198.

46. 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.

47. He W, Rao H, Tang S, Bhagwat N, Kulkarni DS, Ma Y, Chang MAW, Hall C, Bragg JW, Manasca HS *et al.*: **Regulated proteolysis of MutSgamma controls meiotic crossing over**. *Mol Cell* 2020, **78**:168-183 e165.

48. Zakharyevich K, Ma Y, Tang S, Hwang PY, 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**:1001-1015.

49. Hayward BE, Steinbach PJ, Usdin K: **A point mutation in the nuclease domain of MLH3 eliminates repeat expansions in a mouse stem cell model of the fragile X-related disorders**. *Nucleic Acids Res* 2020, **48**:7856-7863.

50. Roy JCL, Vitalo A, Andrew MA, Mota-Silva E, Kovalenko M, Burch Z, Nhu AM, Cohen PE, Grabczyk E, Wheeler VC, Mouro Pinto R: **Somatic CAG expansion in Huntington's disease is dependent on the MLH3 endonuclease domain, which can be excluded via MLH3 splice redirection to suppress expansion**. *Nucleic Acid Res* 2021, **49**:3907-3918.

51. • Kadyrova LY, Gujar V, Burdett V, Modrich PL, Kadyrov FA: **Human MutLgamma, the MLH1-MLH3 heterodimer, is an endonuclease that promotes DNA expansion**. *Proc Natl Acad Sci U S A* 2020, **117**:3535-3542

First report showing that human MLH1–MLH3 is a nuclease. The authors focus on the function of MLH1–MLH3 nuclease to drive repeat expansion.

52. Viterbo D, Michoud G, Mosbach V, Dujon B, Richard GF: **Replication stalling and heteroduplex formation within CAG/**

CTG trinucleotide repeats by mismatch repair. *DNA Repair (Amst)* 2016, **42**:94-106.

53. Rogacheva MV, 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.

54. Rahman MM, Mohiuddin M, Shamima Keka I, Yamada K, Tsuda M, Sasanuma H, Andreani J, Guerois R, Borde V, Charbonnier JB *et al.*: **Genetic evidence for the involvement of mismatch repair proteins, PMS2 and MLH3, in a late step of homologous recombination**. *J Biol Chem* 2020, **295**:17460-17475.

55. Nawaz S, Ullah MI, Hamid BS, Nargis J, Nawaz M, Hussain S, Ahmad W: **A loss-of-function variant in DNA mismatch repair gene MLH3 underlies severe oligozoospermia**. *J Hum Genet* 2021, **66**:725-730.

56. Pashaiefar H, Sheikhha MH, Kalantar SM, Jahaninejad T, Zaimy MA, Ghasemi N: **Analysis of MLH3 C2531T polymorphism in Iranian women with unexplained infertility**. *Iran J Reprod Med* 2013, **11**:19-24.

57. Kadyrov FA, Dzantiev L, Constantin N, Modrich P: **Endonucleolytic function of MutLalpha in human mismatch repair**. *Cell* 2006, **126**:297-308.

58. Pluciennik A, Dzantiev L, Iyer RR, Constantin N, Kadyrov FA, Modrich P: **PCNA function in the activation and strand direction of MutLalpha endonuclease in mismatch repair**. *Proc Natl Acad Sci U S A* 2010, **107**:16066-16071.

59. •• Cannavo E, Sanchez A, Anand R, Ranjha L, Hugener J, Adam C, Acharya A, Weyland N, Aran-Guiu X, Charbonnier JB *et al.*: **Regulation of the MLH1-MLH3 endonuclease in meiosis**. *Nature* 2020, **586**:618-622

One of two studies that demonstrated that the nuclease activity of human MLH1–MLH3 is stimulated by MSH4–MSH5, EXO1 and RFC–PCNA. The data suggest that crossover-directed resolution in meiotic cells may employ a mechanism related to mismatch repair. The nuclease activity of the ensemble is supported by physical interactions between its components. The authors also identify prospective PCNA interaction motifs in MLH1 and MLH3, which are required for PCNA-mediated stimulation of MLH1–MLH3 nuclease *in vitro* and meiotic recombination in yeast cells.

60. •• Kulkarni DS, Owens SN, Honda M, Ito M, Yang Y, Corrigan MW, Chen L, Quan AL, Hunter N: **PCNA activates the MutLgamma endonuclease to promote meiotic crossing over**. *Nature* 2020, **586**:623-627

The second biochemical study that identified the function of RFC–PCNA in meiotic recombination. The authors show that MSH4–MSH5, EXO1, RFC–PCNA and MLH1–MLH3 form a functionally integrated complex, and propose first models how crossover-directed resolution might function. Using an elegant degron system, the authors show that RFC (and hence PCNA loading) is required for Mlh3-dependent crossing-over in yeast. Imaging experiments detected PCNA localized to prospective crossover sites along meiotic synapsed chromosomes.

61. 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**:5674-5686.

62. Claeys Bouuaert C, Keeney S: **Distinct DNA-binding surfaces in the ATPase and linker domains of MutLgamma determine its substrate specificities and exert separable functions in meiotic recombination and mismatch repair**. *PLoS Genet* 2017, **13**:e1006722.

63. Manhart CM, Ni X, White MA, Ortega J, Surtees JA, Alani E: **The mismatch repair and meiotic recombination endonuclease Mlh1-Mlh3 is activated by polymer formation and can cleave DNA substrates in trans**. *PLoS Biol* 2017, **15**:e2001164.

64. Schaetzlein S, Chahwan R, Avdievich E, Roa S, Wei K, Eoff RL, Sellers RS, Clark AB, Kunkel TA, Scharff MD *et al.*: **Mammalian Exo1 encodes both structural and catalytic functions that play distinct roles in essential biological processes**. *Proc Natl Acad Sci U S A* 2013, **110**:E2470-E2479.

65. Wild P, Susperregui A, Piazza I, Dorig C, Oke A, Arter M, Yamaguchi M, Hilditch AT, Vuina K, Chan KC *et al.*: **Network rewiring of homologous recombination enzymes during mitotic proliferation and meiosis**. *Mol Cell* 2019, **75**:859-874 e854.

66. Sanchez A, Adam C, Rauh F, Duroc Y, Ranjha L, Lombard B, Mu X, Loew D, Keeney S, Cejka P *et al.*: **Mechanism of in vivo activation of the MutLγ-Exo1 complex for meiotic crossover formation**. *Proc Natl Acad Sci U S A* 2021, **117**:30577-30588.

67. Gray S, Santiago ER, Chappie JS, Cohen PE: **Cyclin N-terminal domain-containing-1 coordinates meiotic crossover formation with cell-cycle progression in a cyclin-independent manner**. *Cell Rep* 2020, **32**:107858.

68. • Woglar A, Villeneuve AM: **Dynamic architecture of DNA repair complexes and the synaptonemal complex at sites of meiotic recombination**. *Cell* 2018, **173**:1678-1691 e1616

An elegant imaging study using structured-illumination superresolution microscopy to monitor DNA repair complexes at sites of meiotic recombination in *C. elegans*. The authors observed distinct populations of MSH4–MSH5 and BLM proteins at future crossover sites, showing that their localization is tighty controlled.

69. • Machin F: **Implications of metastable nicks and nicked Holliday junctions in processing joint molecules in mitosis and meiosis**. *Genes (Basel)* 2020, **11**

A theoretical concept paper discussing how strand discontinuities can affect subpathways of homologous recombination. The author introduces several models for crossover-biased resolution in meiotic cells.

70. •• Peterson SE, Keeney S, Jasin M: **Mechanistic insight into crossing over during mouse meiosis**. *Mol Cell* 2020, **78**:1252-1263 e1253

The authors monitor meiotic recombination products in mouse spermatocytes. Similar to the study in yeast (Ref. [22••]), the authors find evidence for branch migration during meiotic recombination. The authors observed a lack of heteroduplex DNA in a fraction of the recombined molecules, and propose that D-loops migrate in the direction of DNA synthesis before second end capture takes place.

71. Holloway JK, Morelli MA, Borst PL, Cohen PE: **Mammalian BLM helicase is critical for integrating multiple pathways of meiotic recombination**. *J Cell Biol* 2010, **188**:779-789.