Making crossovers during meiosis

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Abstract
Homologous recombination (HR) is required to promote both correct chromosome segregation and genetic variation during meiosis. For this to be successful recombination intermediates must be resolved to generate reciprocal exchanges or ‘crossovers’ between the homologous chromosomes (homologues) during the first meiotic division. Crossover recombination promotes faithful chromosome segregation by establishing connections (chiasmata) between the homologues, which help guide their proper bipolar alignment on the meiotic spindle. Recent studies of meiotic recombination in both the budding and fission yeasts have established that there are at least two pathways for generating crossovers. One pathway involves the resolution of fully ligated four-way DNA junctions [HJs (Holliday junctions)] by an as yet unidentified endonuclease. The second pathway appears to involve the cleavage of the precursors of ligated HJs, namely displacement (D) loops and unligated/nicked HJs, by the Mus81-Eme1/Mms4 endonuclease.

The generation of gametes depends on a specialized form of cell division called meiosis, which reduces cellular chromosome content from diploid to haploid. This involves two consecutive cell divisions (called meiosis I and II) following a single round of DNA replication (Figure 1). Segregation of the homologous chromosomes (homologues) occurs during meiosis I, whereas sister chromatids segregate during meiosis II. In many ways, meiosis II resembles a normal mitotic cell division where cohesion between the sister chromatids is used to help guide their proper segregation. However, proper homologue segregation during meiosis I demands additional layers of complexity. Part of this complexity involves the stimulation of HR (homologous recombination) between the homologues. The function of this recombination in most organisms is threefold: (i) it promotes genetic diversity by creating new and potentially beneficial combinations of maternal and paternal alleles; (ii) it removes deleterious mutations that would otherwise result in the gradual decay of genetic information [1]; and (iii) it establishes physical connections (called chiasmata) between the homologues that are important for their proper bipolar attachment to the meiosis I spindle (Figure 1) (for a review, see [2]). Defects in this latter function can result in aneuploidy through homologue missegregation.

Much of our current understanding of meiotic recombination has come from studies of meiosis in fungi. Here, the four haploid products of a single meiosis can be analysed genetically, and studies that have employed this tractability have played a central role in the development of models for HR. Foremost among these is the DSB (double-strand break) repair model (Figure 2) [3]. This proposes that recombination is initiated by the formation of a DSB in one of the homologues. The broken DNA ends are then resected by degradation of their 5′-ended strands to generate single-stranded tails with 3′-OH termini (Figure 2, step 2). One of these tails then locates its equivalent sequence on the homologue and invades it to form a displacement loop (D-loop) [this is also referred to as an SEI (single end invasion)] (Figure 2, step 3). DNA synthesis primed by the invading strand extends the D-loop, enabling it to anneal to the single-stranded tail on the other side of the break (second end capture) (Figure 2, steps 4–5). Further DNA synthesis together with the ligation of strand breaks results in the homologues being connected by two four-way DNA junctions – a structure called the dHJ (double Holliday junction) (Figure 2, step 6). The dHJ can be resolved by cleavage of a pair of symmetrical strands at each junction. Cleavage of a different pair of strands at each junction splices together maternal DNA flanking one side of the dHJ with paternal DNA flanking the other side (Figure 2, steps 6 and 7). This type of recombinant product is called a crossover. It constitutes a new combination of alleles and establishes a chiasma. However, if the dHJ is resolved by cleavage of the same pair of strands at each junction, then crossing over does not occur (Figure 2, steps 6 and 7). Such non-crossover recombinants do not contribute to chiasma formation.

Since its proposal in 1983, many aspects of the DSB repair model have been substantiated by physical detection of intermediates, primarily in the budding yeast Saccharomyces cerevisiae. These include the DSBs, resected ends, SEIs and dHJs (see e.g. [4–8]). Many of the enzymes that catalyse its various stages have also been identified, and found to be evolutionarily conserved. These include a complex of proteins involving Spo11 that makes DSBs, the Mre11–Rad50–Xrs2 complex that is involved in strand resection, and Rad51, which, together with Dmc1 and various accessory proteins, drives the central reactions of homologous pairing and strand invasion that lead to the formation of the dHJ (for reviews,
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Recombination during meiosis I establishes connections (chiasmata) between the homologues that are mediated by sister chromatid cohesion. These connections help to guide the proper monopolar attachment of the homologues to the meiosis I spindle. Once all the chromosomes are correctly attached, cohesion along the chromosome arms is degraded, and the homologues are divided. Cohesion is maintained at the centromeres during the meiosis I division in order to guide correct bipolar attachment of the sister chromatids to the meiosis II spindle.

The Szostak et al. [3] DSB repair model

Dashed lines represent newly synthesized DNA. Note that non-crossovers are also formed by cleavage at A + B, and crossovers by cleavage at A + b. See main text for further details.

The DSB repair model predicts that both crossover and non-crossover recombinants derive from dHJ resolution [3]. This is consistent with the activities of known HJ resolvases that would cleave either pair of strands at each HJ with equal probability. However, in recent years, a number of mutations have been found in *S. cerevisiae* that reduce dHJ resolution and crossover formation without affecting the formation of DSBs and non-crossovers (reviewed in [12]). This indicates that crossovers and non-crossovers derive from independent pathways of DSB repair. In fact, it appears that dHJs are resolved exclusively as crossovers (Figure 3, steps 6a and 7a), whereas non-crossovers may stem from a mechanism called SDSA (synthesis-dependent strand annealing), in which dissociation of the SEI after DNA synthesis is followed by annealing of the two broken ends (Figure 3, steps 3b–6b) [13,14].

How does the cell direct some DSBs to be repaired by the crossover pathway and others by the non-crossover pathway? At least a part of the answer involves the recruitment of a set of meiosis-specific proteins (Zip1, Zip2, Zip3, Mer3, Msh4 and Msh5) to certain DSB sites [13,15]. These proteins, often referred to as the ZMM proteins, seem to promote SEI stability and dHJ formation, and ensure that resolution occurs with the appropriate bias to generate crossovers. Without them, SEIs appear to be transient and unstable structures, which cannot be detected by current physical analyses [13]. It is assumed that these unstable SEIs are channelled down alternative pathways such as SDSA [12].

Recent biochemical studies have shown how three of the ZMM proteins (Mer3, Msh4 and Msh5) could promote the stability of early strand invasion intermediates. Mer3 is a DNA helicase, which *in vitro* can extend the region of heteroduplex DNA formed during strand invasion catalysed by Rad51 (Figure 3, steps 3a and 4a) [16]. It is easy to see how this activity would promote SEI stability by increasing the number of base pairs linking the two homologues. Msh4 and Msh5 are homologues of the bacterial MMR (mismatch repair) protein MutS, which binds to mismatched DNA via one of two central holes in its homodimeric structure.

see [9,10]). However, the identity of the nuclease that cleaves the dHJ has remained elusive. In some ways, this is surprising because HJ resolvases that are involved in recombination reactions in bacteriophage, eubacteria, archaea, eukaryotic viruses and yeast mitochondria have been identified and characterized extensively [11].
Msh4 and Msh5 are not involved in MMR, and recent studies of the human homologues of these enzymes (hMSH4 and hMSH5) have shown that, instead of binding to mismatched DNA, they form a heterodimeric complex that binds specifically to HJs, including the half-HJs that are formed upon strand invasion (Figure 3, step 3a) [18]. The hMSH4–hMSH5 complex appears to encircle the HJ, with possibly each of its two central holes binding a different DNA duplex. Upon binding ATP, the complex releases from the HJ and slides along the adjacent duplex DNA. It is thought that this would allow further hMSH4–hMSH5 heterodimers to bind to the HJ (Figure 3, step 4a) [18]. The accumulation of these complexes encircling the recombination intermediate could help to stabilize it and direct the manner in which it is resolved, ensuring that only crossovers are formed (Figure 3, steps 5a–7a) [18,19].

During MMR in *Escherichia coli*, MutS interaction with MutL activates the latent endonuclease activity of MutH [17]. MutH then nicks a hemimethylated GATC site located within approx. 1 kb of the mismatch, and this allows entry of a DNA helicase and exonuclease to remove a stretch of single-stranded DNA containing the mismatch. In *S. cerevisiae*, homologues of MutL called Mlh1 and Mlh3 form a complex that functions downstream of Msh4–Msh5 to promote crossover formation [20–24]. This suggests that there is an endonuclease that is activated by the interaction of Msh4–Msh5 with Mlh1–Mlh3, which resolves dHJs. However, a homologue of MutH has not been found in eukaryotes and the identity of an endonuclease that might be controlled and/or activated by Msh4–Msh5 and Mlh1–Mlh3 remains elusive. An HJ resolvase activity, which depends on two Rad51-like proteins, Xrcc3 and Rad51C, has been detected in fractionated mammalian cell extracts [25]. Budding yeast does not contain direct homologues of Xrcc3 and Rad51C so it is unclear whether it uses a related resolvase for crossover formation. However, the plant *Arabidopsis thaliana* does contain these proteins, and moreover they seem to be critical for a late stage in meiotic recombination [26,27]. Like budding yeast, *Arabidopsis* has a crossover pathway that depends on MSH4 and MER3, but it is not known whether Xrcc3 and Rad51C are part of this pathway [28,29].

In *S. cerevisiae*, the majority of DSBs are repaired as non-crossovers, whilst comparatively few (∼90 in the entire genome) are repaired as crossovers. The distribution of these crossovers is non-random with both large and small chromosomes receiving at least one crossover and with crossovers tending not to be near one another on the same chromosome. This phenomenon of crossover interference ensures that crossovers are evenly spaced and depends on the recruitment of the ZMM proteins to particular DSB sites at an early stage in their repair [12,13,15]. The ZMM–dependent pathway is therefore synonymous with what is often referred to as the crossover interference pathway. It is not clear what underlies crossover interference (i.e. prevents ZMM proteins loading at neighbouring DSB sites). One simple idea is that consecutive loading of Msh4–Msh5 at a recombination intermediate at one site results in a localized depletion of these enzymes, preventing their loading at neighbouring sites [19].
Other models invoke concepts whereby the commitment of one site to become a crossover sends out a ‘signal’ to neighbouring sites, which inhibits crossover formation (discussed in [15]). Such a signal could involve the imposition and relief of stress being transmitted along each homologue axis [30].

The dependence on the ZMM proteins for crossover formation is not absolute in budding yeast. In fact, at 30°C, ZMM mutants typically retain approx. 50% of the normal observations here were that it could cleave HJs it was the main HJ resolvase in this organism [36]. The key analysis of Mus81–Eme1 in fission yeast had indicated that (reviewed in [35]). In fact, initial biochemical and genetic variety of branched DNA structures in vitro forms a complex with Mms4/Eme1 that can cleave a variant of the branched DNA family of structure-specific endonucleases and it being left to function without its normal activators and controllers. Although it might resolve some dHJs successfully, its being unfettered may allow it to cleave DNA that it is not subject to crossover interference. In a few organisms, such as the fission yeast Schizosaccharomyces pombe, crossover formation depends almost entirely on this Mus81-dependent pathway [32,33]. This correlates with the absence of ZMM proteins and the lack of crossover interference in these organisms [34].

Mus81 is related to the XPF (xeroderma pigmentum group F) family of structure-specific endonucleases and it forms a complex with Mms4/Eme1 that can cleave a variety of branched DNA structures in vitro, including HJs (reviewed in [35]). In fact, initial biochemical and genetic analysis of Mus81–Eme1 in fission yeast had indicated that it was the main HJ resolvase in this organism [36]. The key observations here were that it could cleave HJs in vitro and that mutation of mus81 dramatically reduced spore viability (an indicator of meiotic failure), which could be rescued by the heterologous expression of a bacterial HJ resolvase [36].

However, extensive analysis of the substrate specificity of Mus81–Eme1/Mms4 from both budding and fission yeast, as well as from humans, has shown that this enzyme is actually far more active on other kinds of branched DNA structures than it is on intact HJs [37–41]. In particular, it favours D-loops and nicked HJs [32,42]. This substrate specificity suggests that, instead of resolving dHJs, Mus81–Eme1/Mms4 might act earlier in the recombination reaction to cleave the D-loop that is formed by the SEI and the unligated HJ that is formed by second end capture (Figure 3, steps 3c and 5c) [32]. An appealing aspect of this model is that the way in which Mus81–Eme1/Mms4 cleaves each of these junctions would produce an obligate crossover in vivo (Figure 3, step 6c). This is consistent with genetic data showing that mutation of mus81 only affects crossover formation [32,33]. It also provides a simple explanation for why the overriding majority of DSBs in Schiz. pombe, unlike in S. cerevisiae, are repaired as crossovers [43,44].

In budding yeast, it is thought that the Mus81–dependent pathway is largely a backup for the ZMM-dependent pathway of crossover formation. Presumably, the loading of ZMM proteins at early recombination intermediates shields them from Mus81–Mms4 and allows the dHJ to form. Another MMR protein, Mlh2, may also be important here for preventing Mus81 from accessing the DNA [45]. Utilization of a ZMM or equivalent pathway, which is subject to crossover interference, appears to be the principal pathway of crossover formation in most organisms [34]. Like budding yeast, some organisms such as Arabidopsis may also employ a Mus81-dependent pathway for a proportion of their crossovers [28,29]. However, others, such as the worm Caenorhabditis elegans, appear to depend solely on a ZMM or ZMM-like pathway [34]. Recently, a Mus81 knockout mouse was made and found to be perfectly fertile and normal for gametogenesis [46]. In contrast, msh4−/− and msh5−/− mice are sterile and exhibit defects in chromosome pairing during meiosis I [47–49]. Likewise, both mlh1−/− and mlh3−/− mice are sterile and exhibit severe defects in crossing over [50,51]. These results suggest that mammals, like worms, may employ only the ‘ZMM’ type of pathway for crossover formation.

Are there other pathways of crossover formation? In budding yeast, a few crossovers are formed in an mlh1−/− mms4−/− double mutant, and this number actually increases by approx. 3-fold when MSH5 is also deleted [24]. These results suggest that there is a third pathway that is normally inhibited by Msh5. However, this pathway, rather than promoting the production of viable meiotic products (spores), is detrimental to it, since spore viability is much higher in the mlh1−/− mms4−/− double mutant than in the mlh1−/− mms4−/− msh5−/− triple mutant [24]. Possibly, this ‘third pathway’ is simply a product of the elusive HJ resolvase from the ZMM-dependent pathway being left to function without its normal activators and controllers. Although it might resolve some dHJs successfully, its being unfettered may allow it to cleave DNA that it is not supposed to, resulting in loss of viability.

The last few years have seen many exciting developments in the recombination field, which have given us a much clearer picture of how crossovers are formed during meiosis. However, before we can really get to grips with understanding the mechanics of crossing over, the elusive HJ resolvase from the ZMM pathway must be identified. Concerted efforts are being made, so hopefully we will not have to wait long.

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References
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