Crossover promotion and prevention

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Abstract Homologous recombination is an important mechanism for the repair of double-strand breaks in DNA. One possible outcome of such repair is the reciprocal exchange or crossing over of DNA between chromosomes. Crossovers are beneficial during meiosis because, as well as generating genetic diversity, they promote proper chromosome segregation through the establishment of chiasmata. However, crossing over in vegetative cells can potentially result in loss of heterozygosity and chromosome rearrangements, which can be deleterious. Consequently, cells have evolved mechanisms to limit crossing over during vegetative growth while promoting it during meiosis. Here, we provide a brief review of how some of these mechanisms are thought to work.

Key words chromosome segregation, crossover, DNA helicase, Holliday junction, homologous recombination, meiosis

Abbreviations used D-loop, displacement loop; DSB, double-strand break; HJ, Holliday junction; dHJ, double HJ; HR, homologous recombination; SCF, Skp, Cullin, F-box; SDSA, synthesis-dependent strand annealing; SF1, superfamily I; ZMM, Zip1, Zip2, Zip3, Msh4, Msh5 and Mer3

Meiotic crossover mechanisms

Meiosis is a specialized cell division in which two consecutive rounds of chromosome segregation take place without intervening DNA replication. This reduces the chromosome set from diploid to haploid, which is necessary in order to compensate for the chromosome doubling during zygote formation. During the first meiotic division, the homologous chromosomes (or homologues) are segregated. To ensure correct homologue segregation, most organisms need to establish connections called chiasmata between the homologues. These connections are mediated by cohesion between the sister chromatids and are established through the repair of programmed DSBs (double-strand breaks) by HR (homologous recombination) that results in crossovers between the homologues [1]. In 1983, Szostak et al. [2] proposed a model for how DSBs might be repaired by HR (Figure 1). They envisaged that the DSB is resected by an exonuclease to expose 3′-ended single-stranded DNA tails. One tail would then invade the homologue (single-end invasion) to generate a D-loop (displacement loop). DNA synthesis, primed by the end of the invading strand, would then extend the D-loop, enabling it to base-pair to the other end of the break (second end capture). Following further DNA synthesis, and the ligation of strand discontinuities, two four-way DNA junctions are formed – a structure called the double Holliday junction (dHJ). The dHJ is then resolved by the cleavage of pairs of strands at each junction, with the relative orientation of cleavage determining whether crossing over occurs.

Many of the tenets of the DSB repair model have been upheld by the physical detection (mainly in *Saccharomyces cerevisiae*) of key intermediates of the process (e.g. DSBs, resected DSBs, single-end invasions and dHJs) [3–5]. Enzymes capable of catalysing the various steps in the reaction have also been identified [6]. Some of these, such as Spo11, which makes the DSB, are meiosis-specific, whereas others, such as Rad51, which catalyses the central reactions of homologous pairing and strand exchange, promote HR in both meiotic and vegetative cells. However, the resolution of the dHJ is one step that is still poorly characterized, and this is mainly due to the fact that the nuclear HJ (Holliday junction) resolvase has not been identified. Nevertheless, some things have become apparent; for example, contrary to the DSB repair model, it has been shown that, in *S. cerevisiae*, crossovers and non-crossovers stem from quite distinct pathways [4,7,8]. Crossovers appear to be formed by the biased resolution of dHJs, whereas

non-crossovers are thought to be formed by a mechanism called synthesis-dependent strand annealing (SDSA) (Figure 1). SDSA follows a similar path as the DSB repair model except that the invading DNA strand is unwound prior to second end capture and then simply anneals to the other end of the break.

Since the enzyme that resolves dHJs during meiosis has yet to be identified, it is unclear how resolution is biased in favour of crossing over. After all, the dHJ is essentially a symmetrical structure and therefore its resolution should generate crossovers and non-crossovers with equal frequency as predicted by the DSB repair model. Presumably, biased resolution of dHJs depends on other proteins that direct the way in which the HJ resolvase binds and then cleaves each HJ. An example of how this can be achieved is seen in the bacterium *Escherichia coli*, where the RuvAB branch migration enzyme directs the orientation of cleavage by the RuvC HJ resolvase [9,10]. In *S. cerevisiae*, crossover formation by biased dHJ resolution depends on the so-called ZMM (Zip1, Zip2, Zip3, Msh4, Msh5 and Mer3) proteins [7]. It is possible that some or all of these proteins direct HJ cleavage by the unidentified resolvase. Indeed, human Msh4 and Msh5 form a heterodimer that binds to HJs *in vitro* [11], and therefore could conceivably influence the direction of resolution *in vivo*.

The ZMM-dependent pathway is the major mechanism of crossover formation in *S. cerevisiae* and is subject to crossover interference – a poorly understood mechanism that prevents crossovers from being close together and which ensures that each chromosome receives at least one crossover [12]. Crossovers are also formed by a second 'back-up' pathway, which depends on Mus81–Mms4 (the orthologue of Mms4 in *Schizosaccharomyces pombe* and mammals is called Eme1) and is not subject to crossover interference [13]. Mus81–Mms4/Eme1, which we will refer to as Mus81*, is a structure-specific endonuclease that is thought to generate crossovers by cutting the D-loops and nicked HJs that precede dHJ formation [14] (Figure 1). Mus81* cleaves these inherently asymmetrical early recombination intermediates to generate exclusively crossovers [15]. In other words crossover formation might be guaranteed without the necessity for additional guiding factors.

A number of organisms, including *S. cerevisiae* and *Arabidopsis thaliana*, appear to utilize both ZMM- and Mus81*-dependent pathways for crossover formation [7,13,16]. This may also be true of mammals [17]. However, there are organisms that utilize only one pathway. In the nematode *Caenorhabditis elegans*, crossover interference is strongly enforced, suggesting that it depends solely on the ZMM-dependent pathway despite containing a Mus81 orthologue [14,18,19]. In contrast, the archiascomycetous fungus *Schizosaccharomyces pombe* lacks the ZMM proteins, displays no crossover interference and relies on Mus81* for making crossovers during meiosis [15,20,21]. Intriguingly, even within the Hemiascomycetes (of which *S. cerevisiae* is a member), there are organisms, such as *Debaryomyces hansenii* and *Yarrowia lipolytica*, that contain Mus81 but lack key ZMM proteins, suggesting that crossover formation may depend solely on the Mus81 pathway [22].

Limiting crossovers in vegetative cells

During vegetative growth, DSB repair by HR occurs mainly between sister chromatids. Here, crossing over generates sister chromatid exchanges, which are genetically silent. However, occasionally, recombination occurs between homologues or repeated DNA elements, and here crossing over can be deleterious by causing loss of heterozygosity and/or gross chromosome rearrangements. A high rate of this kind of genome instability in mammals is associated with diseases such as cancer. It is probably for this reason that there are mechanisms in place to avoid making crossovers in vegetative cells.

DNA helicases play important roles in crossover avoidance. This has been documented in *S. cerevisiae* for the Sgs1 and Srs2 DNA helicases that limit crossing over in an interchromosomal recombination assay system, where the HO endonuclease is used to make the initiating DSB [23]. Sgs1 is a member of the RecQ subfamily of DNA helicases [24,25]. These helicases are conserved from bacteria to mammals, and play important roles in preserving genome stability; so much so that, in humans, defects in the RecQ helicases BLM, WRN and RecQL4 cause the cancer-prone diseases Bloom's, Werner's and Rothmund–Thomson syndromes respectively [24]. Intriguingly, Bloom's syndrome is associated with a high incidence of sister chromatid exchange, and Werner's syndrome with increased rates of gross chromosomal rearrangement,

indicating that, like Sgs1, BLM and WRN limit crossover formation. RecQ helicases generally have the ability to unwind branched DNA structures so they could limit crossovers by unwinding D-loops to promote SDSA as has been suggested for the orthologue of BLM in *Drosophila melanogaster* (which is encoded by *mus309*) [26]. Furthermore, RecQ helicases can function together with topoisomerases and, in the case of BLM and topoisomerase IIIα, have been shown to 'dissolve' dHJs into non-crossover products *in vitro* [27]. dHJ dissolution results from a combination of BLM-driven HJ branch migration to generate a hemicatenane, followed by strand disentanglement by topoisomerase IIIα (Figure 1).

Srs2 is an SF1 (superfamily I) DNA helicase and, *in vitro*, can strip the Rad51 recombinase from DNA (a similar activity has been observed for a related bacterial helicase called UvrD) [28–30]. This activity is thought to limit HR at stalled replication forks and single-strand gaps, enabling post-replicative repair mechanisms to operate. Srs2 is also needed for DSB repair in *S. cerevisiae*, where it is believed to promote SDSA. One way that it could do this is by limiting the extent of Rad51 nucleofilament assembly, which would presumably reduce D-loop stability, making them more susceptible to being unwound.

In contrast with Sgs1, there are no obvious orthologues of Srs2 in humans. However, humans do contain a closely related SF1 helicase called Fbh1 [31]. Fbh1 is unique among DNA helicases in that it contains an F-box. F-box proteins are substrate recognition components of SCF (Skp, Cullin, F-box) ubiquitin ligase complexes that catalyse the polyubiquitination of proteins to target them for degradation. Human Fbh1 is known to form an SCF complex but its target(s) for ubiquitination have not been identified [32]. Fbh1 is absent from *S. cerevisiae* but present in *Schizosaccharomyces pombe* [33,34], which also contains a RecQ helicase (Rqh1) and an orthologue of Srs2. Intriguingly, deletion of *fbh1* results in a dependence on both Srs2 and Rqh1 for viability, which is remedied by removing Rad51. A similar interaction is seen between Srs2 and Rqh1 [35]. These results indicate that Fbh1, Rqh1 and Srs2 share overlapping functions in suppressing inappropriate recombination and/or in processing toxic recombination intermediates. It is currently unknown whether Fbh1 limits crossover formation, but experiments are under way in our laboratory to test this possibility. Nevertheless, the results for *Schizosaccharomyces pombe* are sufficient to suggest that Fbh1 might be fulfilling an Srs2-like role in humans, possibly with the added ability to target recombination proteins for degradation.

Mus81 and crossover formation in vegetative cells

Mus81*, which is able to produce crossovers from HJ-like intermediates during meiosis (see above), is also active in vegetative cells. However, here it is dispensable for DSB repair induced by γ irradiation or the HO endonuclease [19,36]. Although there is evidence that it can still promote crossover formation in vegetative cells based on results from a plasmid gap repair assay in *Schizosaccharomyces pombe* [15] (W. Sun and M.C. Whitby, unpublished work), Mus81*'s vegetative role appears to be mainly in the repair of interstrand cross-links, broken replication forks, and possibly lesion-containing single-strand gaps left behind after impeded DNA replication [37,38].

Mus81* is essential in the absence of the RecQ helicase in both *S. cerevisiae* and *Schizosaccharomyces pombe* [37,39]. This synthetic interaction is suppressed by deleting *RAD51* in *S. cerevisiae*, which is consistent with the idea that Sgs1 and Mus81* provide alternative ways of processing recombination intermediates [40]. Based on such results, it has been suggested that Mus81* might account for the elevated levels of crossing over in RecQ family mutants. However, *MUS81* mutant mouse embryonic stem cells show elevated levels of mitomycin C-induced sister chromatid exchange, demonstrating that, for some types of damage, Mus81* can act to suppress crossing over [41]. Furthermore, in human cell lines, Mus81 can co-immunoprecipitate with BLM and, *in vitro*, BLM can enhance the cleavage activity of Mus81* on nicked HJs, suggesting that, in some instances, Mus81* and BLM might act together [42].

Roles of Srs2, Fbh1 and RecQ helicases during meiosis

In *Schizosaccharomyces pombe*, where there is no crossover interference, it appears that most DSBs, which are detectable by physical assays, are repaired as crossovers [43]. In contrast, in *S. cerevisiae* and other organisms, which exhibit crossover

interference, the majority of DSBs are repaired as non-crossovers [6]. As mentioned above, it is believed that these non-crossovers stem from SDSA. One might expect therefore a requirement for the same DNA helicases that promote SDSA in vegetative cells. Certainly, RecQ helicases do play roles during meiosis. This is indicated in humans by the impaired fertility of Bloom's, Werner's and Rothmund–Thomson syndrome patients [24] and by the fact that BLM and Rad51 co-localize in mouse spermatocytes during meiotic prophase [44]. However, it is worth noting that effects on meiotic crossover frequency have not been observed in any of the various '*recQ*' mutant mice [24]. In contrast, mutation of *mus309* results in an increased frequency of meiotic crossovers in *Drosophila* [45]. The same is also true for Sgs1 mutation in *S. cerevisiae*, but only in certain mutant strain backgrounds [46]. However, in *C. elegans*, mutation of *HIM-6* (which encodes a BLM orthologue) decreases crossover frequency and results in Rad51 foci persisting into late pachytene [47]. Here it would appear that a RecQ helicase is actually needed to process recombination intermediates into crossover products. The same may also be true in *Schizosaccharomyces pombe* where deletion of *rqh1* results in a reduction in crossing over during meiosis (F. Osman and M.C. Whitby, unpublished work).

In *S. cerevisiae*, Srs2 also has a role during meiosis, and accordingly exhibits increased expression levels concomitant with the commitment to meiotic recombination [48]. Without Srs2, meiotic progression is delayed and spore viability is reduced [49]. However, the poor spore viability of an *srs2-101* mutant cannot be rescued by mutation of *SPO13* and *MEI4*, which should bypass meiosis I and the need for DSB repair [49]. It would seem therefore that Srs2 is needed during pre-meiotic S-phase, and we are unaware of any documented effect on crossing over. Certainly, in *Schizosaccharomyces pombe*, deletion of *srs2* has no effect on spore viability and crossover formation (F. Osman and M.C. Whitby, unpublished work). In contrast, deletion of *fbh1* has a dramatic effect on spore viability, indicating that Fbh1 plays an important role during meiosis (W. Sun and M.C. Whitby, unpublished work). Studies are ongoing in our laboratory to assess what this critical function is.

Conclusion

Enzymes that promote and prevent crossover formation are present in both vegetative and meiotic cells. It would seem therefore that the dichotomy between the paucity of crossovers during vegetative growth and their relative abundance during meiosis must be explained by state-specific factors that selectively activate and/or attenuate specific crossover controlling enzymes. This is clearly the case in *S. cerevisiae* where the meiosis-specific ZMM proteins drive crossover formation. However, in *Schizosaccharomyces pombe*, Mus81* is responsible for essentially all meiotic crossovers, yet it is somehow prevented from promoting crossovers in vegetative cells. Possibly, without certain meiosis-specific factors, Mus81* is simply outmanoeuvred by the enzymes that promote SDSA. Alternatively, Mus81*'s activity might be attenuated during vegetative growth. Certainly it is known that *Schizosaccharomyces pombe* Mus81 is prevented from cleaving replication forks, which are stalled by hydroxyurea-mediated dNTP depletion, by a Cds1-dependent phosphorylation that delocalizes it from chromatin [50]. Perhaps a similar mechanism acts to attenuate Mus81 during vegetative DSB repair. Similarly, the DNA helicases that promote SDSA in vegetative cells may be attenuated during meiosis. In this regard, it is interesting to note that, in some organisms, RecQ helicases are needed to promote crossover formation rather than to prevent it.

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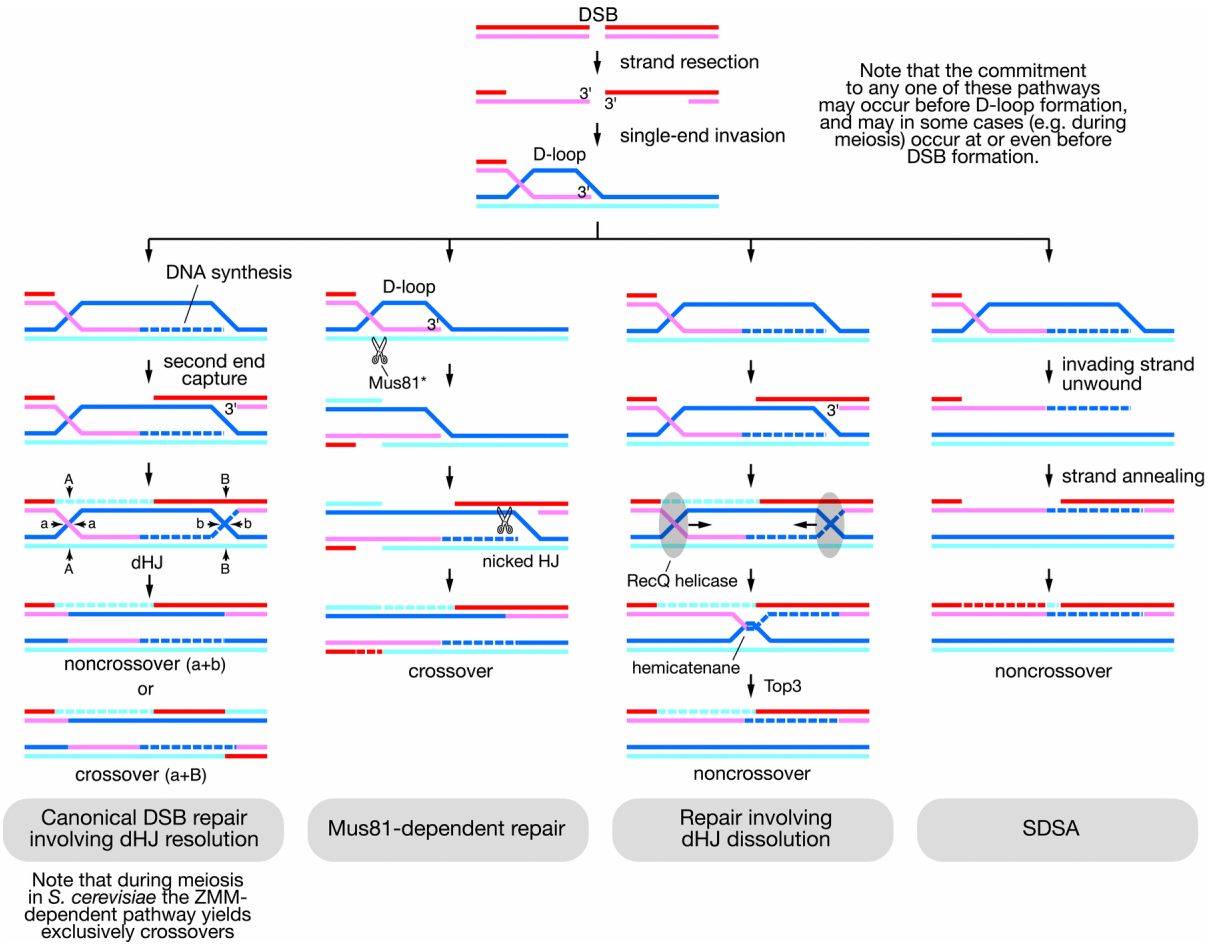

Figure 1. Pathways of DSB repair by HR (see main text for details).