

Meiotic and Mitotic Recombination in Meiosis

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ABSTRACT Meiotic crossovers facilitate the segregation of homologous chromosomes and increase genetic diversity. The formation of meiotic crossovers was previously posited to occur via two pathways, with the relative use of each pathway varying between organisms; however, this paradigm could not explain all crossovers, and many of the key proteins involved were unidentified. Recent studies that identify some of these proteins reinforce and expand the model of two meiotic crossover pathways. The results provide novel insights into the evolutionary origins of the pathways, suggesting that one is similar to a mitotic DNA repair pathway and the other evolved to incorporate special features unique to meiosis.

MEIOSIS is essential to maintaining the proper complement of chromosomes in sexually reproducing organisms. By following one round of DNA replication with two rounds of cellular division, meiosis effectively halves the chromosome content of participating cells. Prior to the first meiotic division, homologous chromosomes pair and, in many organisms, undergo recombination. Both crossovers, characterized by the reciprocal exchange of flanking markers, and noncrossovers, in which flanking DNA remains unchanged, result from these recombination events. Crossovers can also occur in mitotically proliferating cells during repair of certain types of DNA damage, especially double-strand breaks. Meiotic crossovers likewise are initiated from double-strand breaks, and many of the proteins used in mitotic repair are also used in meiotic recombination. This has led to the suggestion that meiotic recombination evolved from mitotic recombination (Marcon and Moens 2005). However, several modifications were necessary to give rise to meiotic recombination in its current form (reviewed in Villeneuve and Hillers 2001). First, a mechanism of generating programmed double-strand breaks to initiate recombination was needed. This was achieved through the use of *Spo11*, a conserved protein that generates regulated meiotic double-strand breaks (Keeney *et al.* 1997). Second, whereas crossovers are avoided in mitotic cells to prevent loss of heterozygosity and chromosome rearrangement, crossover formation is emphasized in meiotic recombination to

facilitate the segregation of homologous chromosomes and to increase genetic diversity. Third, the preferred repair template was changed from the sister chromatid in mitotic cells to the homologous chromosome in meiotic cells, since only crossovers between homologs give the aforementioned benefits. Finally, exquisite crossover control mechanisms arose to regulate the number and distribution of crossovers across the genome and relative to one another. In particular, every chromosome pair receives at least one crossover, sometimes called an obligate crossover (Jones 1984). Also, if additional crossovers occur, they tend not to be near one another, a phenomenon called crossover interference (reviewed in Berchowitz and Copenhagen 2010).

A complication obscuring the relationship between the mitotic and meiotic recombination pathways has been the apparent existence of two meiotic crossover pathways—one pathway that produces crossovers subject to interference and another that produces noninterfering crossovers. Recent studies suggest that the interfering crossover pathway fits the scenario described above—*i.e.*, it is a derivative of the mitotic double-strand break repair pathway that contains numerous meiosis-specific embellishments. The noninterfering pathway, however, shares striking similarities to mitotic double-strand break repair in its original form. Additional discoveries reveal functions that are essential for generating meiotic crossovers and can be carried out by different proteins in different species. These findings provide a new framework through which meiotic recombination pathways can be viewed and allow organisms previously thought to use disparate crossover pathways to be brought under the same umbrella.

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Crossovers and Noncrossovers in Meiotic Recombination Models

In 1964, Holliday proposed a novel molecular model to explain how meiotic recombination could produce both crossovers and noncrossovers (Holliday 1964). The central intermediate in his model is a structure in which strands from two homologous duplexes swap pairing partners across a short region, yielding a four-stranded intermediate now known as the Holliday junction. Holliday proposed that these junctions are cleaved by DNA repair enzymes, now known as resolvases, to reestablish two separate duplexes. Depending on which strands are nicked, this process, now called resolution, could result in crossover or noncrossover products. The equally likely outcomes of resolution fit with fungal recombination studies that suggested that crossovers and noncrossovers occur in equal numbers.

In Holliday's model, meiotic recombination is initiated by symmetric nicks on homologous chromosomes, but this mechanism did not fit with subsequent observations (reviewed in Stahl 1994). To accommodate the new data, Szostak *et al.* (1983) proposed that meiotic recombination is initiated by a double-strand break on one chromatid. In the double-strand break repair model they proposed (Figure 1A), based largely on observations of double-strand gap repair in mitotic cells, the precrossover intermediate has two Holliday junctions. Each junction in this double-Holliday junction intermediate is proposed to be resolved independently, but the outcome is similar to Holliday's model: crossovers and noncrossovers are produced in equal numbers. Strong support for the double-strand break repair model came from physical studies of meiotic recombination intermediates and products in *Saccharomyces cerevisiae*. These studies identified joint molecules that form between homologous chromosomes (Collins and Newlon 1994; Schwacha and Kleckner 1994). These joint molecules have many of the properties expected of double-Holliday junctions, the key intermediate in the double-strand break repair model (Schwacha and Kleckner 1995), and are widely considered to be double-Holliday junctions. In this review, we use "joint molecule" to refer to the structure detected in experiments, and "double-Holliday junction" to refer to the intermediate predicted in models.

Subsequent studies of joint molecules also led to a major challenge to the double-strand break repair model. Allers and Lichten (2001) discovered that noncrossovers arose at the same time as joint molecules and prior to crossovers, a finding incompatible with the Szostak *et al.* (1983) double-strand break repair model. In light of this finding, Allers and Lichten (2001) suggested that noncrossovers do not come from double-Holliday junctions, as in the double-strand break repair model, but from an earlier intermediate in the pathway, the extended displacement loop (D-loop) (Figure 1B). A D-loop is formed when a single-stranded DNA end invades a homologous duplex, annealing to one strand and displacing the other. Allers and Lichten (2001) suggested that meiotic noncrossovers arise via synthesis-dependent strand annealing, a process proposed to be a major mechanism through which crossovers are

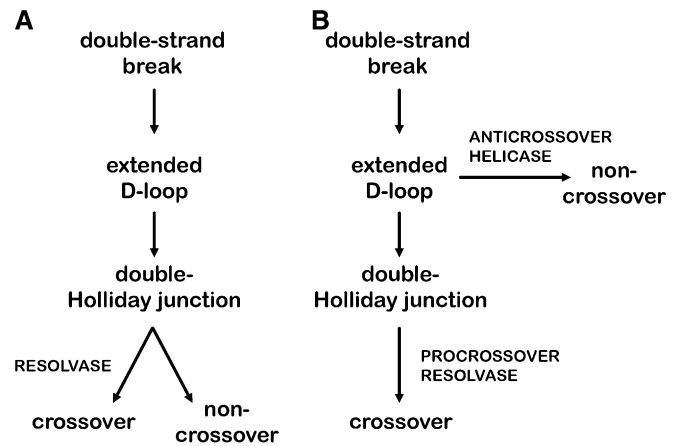


Figure 1 Models of meiotic double-strand break repair. (A) In the Szostak *et al.* (1983) model, recombination initiates with a double-strand break that is processed into an extended displacement loop (D-loop) and then a double Holliday junction structure. The double-Holliday junction is resolved into either a crossover or noncrossover with equal probability. (B) In the revised model of Allers and Lichten (2001), some extended D-loops are unwound by an anticrossover helicase to produce noncrossovers, and double-Holliday junctions are resolved by a procrossover resolvase into crossovers.

avoided in mitotic double-strand break repair (reviewed in Pâques and Haber 1999). In synthesis-dependent strand annealing, after the invading strand is extended by DNA synthesis, helicases can disrupt the D-loop, freeing the nascent strand to anneal to the other end of the double-strand break.

Allers and Lichten (2001) noted another departure from the original double-strand break repair model: Most joint molecules are processed into crossovers (Figure 1B). Although this discovery opposes the notion that resolution of a double-Holliday junction can produce a crossover or a noncrossover with equal probability, it more readily accommodates the finding that noncrossovers outnumber crossovers, sometimes by a factor of 10 or more (reviewed in Cole *et al.* 2012b). Thus, in the revised model of Allers and Lichten (2001), the backbone of the original double-strand break repair model is intact, but double-Holliday junctions are now preferentially repaired as crossovers, and noncrossovers arise via synthesis-dependent strand annealing instead of double-Holliday junction resolution. In this revised model, helicases that promote synthesis-dependent strand annealing act as anticrossover factors and Holliday junction resolvases become procrossover factors rather than proteins that produce both crossovers and noncrossovers.

Rise of the Two-Pathway Paradigm

Another major impact on meiotic recombination models came from studies of the *S. cerevisiae* ZMM (*Zip1–Zip4*, *Msh4–Msh5*, *Mer3*) proteins. *Msh4* and *Msh5* are two widely conserved ZMM proteins that form a meiosis-specific complex (Pochart *et al.* 1997). Notably, loss of *Msh4* or *Msh5*, like loss of other ZMM proteins, does not eliminate crossovers,

but merely reduces them by ~50–70% (Ross-Macdonald and Roeder 1994; Hollingsworth *et al.* 1995). In *Caenorhabditis elegans*, however, Msh4 and Msh5 seem to be essential for all meiotic crossovers (Zalevsky *et al.* 1999; Kelly *et al.* 2000). To reconcile these organismal differences and explain the remaining crossovers in *S. cerevisiae* *msh4* and *msh5* mutants, Zalevsky *et al.* (1999) proposed that there are two different pathways for meiotic crossover formation (Table 1). The first pathway, which requires Msh4–Msh5, is responsible for a majority of crossovers in *S. cerevisiae* and all crossovers in *C. elegans*; the second, independent of Msh4–Msh5, produces the remaining crossovers in *S. cerevisiae* *msh4* and *msh5* mutants.

The idea of two meiotic crossover pathways helped explain additional seemingly disparate findings in other organisms. The fission yeast *Schizosaccharomyces pombe* lacks orthologs of Msh4 and Msh5 (Villeneuve and Hillers 2001). Instead, most or all crossovers are dependent on the Mus81–Mms4 resolvase (the ortholog of Mms4 is called Eme1 in *S. pombe* and many other organisms; for simplicity, we use the *S. cerevisiae* protein name here) (Boddy *et al.* 2001; Smith *et al.* 2003). In budding yeast, loss of Mus81–Mms4 causes only ~20% reduction in meiotic crossovers (Argueso *et al.* 2004). In light of the two-pathway paradigm, these results suggested that Mus81–Mms4 functions in one of the two meiotic crossover pathways, and that this pathway is responsible for all meiotic crossovers in *S. pombe* but only a subset of crossovers in *S. cerevisiae*. This begged the question of whether Mus81–Mms4 and Msh4–Msh5 function in the same meiotic crossover pathway or in two different pathways. In both *S. cerevisiae* and *Arabidopsis thaliana*, double mutants that lack both the Msh4–Msh5 and Mus81–Mms4 complexes have more severely reduced crossover levels than mutants lacking either one, strongly supporting the existence of two pathways—one dependent on Msh4–Msh5 (class I) and another on Mus81–Mms4 (class II) (de los Santos *et al.* 2003; Berchowitz *et al.* 2007).

The nature of the crossovers produced by the two pathways was also in question: If there are two meiotic crossover pathways, do the crossovers produced by them have different properties? A clue to the answer came from mathematical modeling of crossover interference. Copenhagen *et al.* (2002) were able to fit *Arabidopsis* data to a counting model for interference (Foss *et al.* 1993) if they assumed two types of crossovers: some that participate in interference and some that do not. Consistent with this prediction, experimental studies demonstrated that the residual crossovers in *Arabidopsis* and budding yeast *msh4* and *msh5* mutants do not display interference (Novak *et al.* 2001; Argueso *et al.* 2004; Higgins *et al.* 2004; Lu *et al.* 2008), as predicted by Zalevsky *et al.* (1999). Conversely, Mus81–Mms4-independent crossovers in *S. cerevisiae* and *Arabidopsis* do exhibit interference (de los Santos *et al.* 2003; Berchowitz *et al.* 2007). These results suggested that the Msh4–Msh5-dependent pathway produces crossovers subject to interference, whereas the Mus81–Mms4-dependent pathway produces noninterfering crossovers (Table 1). This formulation explains the finding

Table 1 Percentage of crossovers attributed to each pathway in the early two-pathway paradigm

Class	Type of crossover	Defining proteins	Percentage of crossovers				
			Sc	Sp	Ce	At	Dm
I	Interfering	Msh4–Msh5	50–70	0	100	75–85	0 ^a
II	Noninterfering	Mus81–Mms4	20	100	0	9–12	<10

Sc, *Saccharomyces cerevisiae*; Sp, *Schizosaccharomyces pombe*; Ce, *Caenorhabditis elegans*; At, *Arabidopsis thaliana*; Dm, *Drosophila melanogaster*. See text for references.

^a A recent study shows that at least 90% of crossovers in *Drosophila melanogaster* require the mei-MCM complex, which appears to perform a function similar to that of Msh4–Msh5 (Kohl *et al.* 2012).

that crossovers are noninterfering in *S. pombe* (Munz 1994), as these crossovers are produced from the Mus81–Mms4 pathway, and the strong interference of all crossovers in *C. elegans* (Meneely *et al.* 2002), as these are all produced by the Msh4–Msh5 pathway.

Although the paradigm of two meiotic crossover pathways helped to explain many observations, this model also had some weaknesses. First, not all meiotic crossovers fit into these two pathways. *S. cerevisiae* mutants lacking both Msh4–Msh5 and Mus81–Mms4 still have some residual crossovers (de los Santos *et al.* 2003). In addition, though mathematical models of recombination in *Drosophila* fit best if most or all crossovers are interfering (Copenhagen *et al.* 2002), the *Drosophila* genome lacks Msh4 and Msh5 (Sekelsky *et al.* 2000), raising the possibility that another pathway generates interfering crossovers in this species.

Another shortcoming of the two-pathway paradigm is that the proteins used to define these pathways have very different functions: Mus81–Mms4 is a Holliday junction resolvase whose activity presumably directly produces crossover products (Boddy *et al.* 2001) (*i.e.*, it is a pro-crossover resolvase). Msh4–Msh5, however, does not directly produce crossovers, but instead stabilizes crossover-specific intermediates, thus blocking unwinding by anticrossover helicases (*i.e.*, it is an anti-anticrossover complex; see discussion below). Notably, the pro-crossover resolvase that acts in the Msh4–Msh5-dependent pathway was unknown. Furthermore, the relationship between these pathways and the revised model of Allers and Lichten (2001) for meiotic crossovers was unclear. Does the model fit both class I and class II pathways, with different proteins used for each, or is a second model necessary? These apparent weaknesses in the two-pathway paradigm for meiotic crossovers have largely been solved in the past year, as studies in a number of laboratories using different model organisms have clarified the roles and identities of pro-crossover resolvases, anticrossover helicases, and anti-anticrossover complexes.

Anticrossover and Pro-crossover Activities of Sgs1

Studies of anticrossover helicases have been particularly illuminating. Crossovers are a beneficial product of meiotic recombination, but they are avoided during mitotic recombination because they can cause genome instability. Double-strand breaks

in nonmeiotic cells are preferentially repaired into noncrossovers, largely through the action of various anticrossover helicases. One key anticrossover protein is the Bloom syndrome helicase, BLM (reviewed in Andersen and Sekelsky 2010). Although BLM likely has many anticrossover functions, two activities are relevant to double-strand break repair. First, studies in *Drosophila* suggested that BLM promotes synthesis-dependent strand annealing, probably by disrupting D-loops after repair DNA synthesis (Adams *et al.* 2003; McVey *et al.* 2004), an activity human BLM has been shown to have *in vitro* (van Brabant *et al.* 2000; Bachrati *et al.* 2006). Second, *in vitro* studies demonstrated that BLM, together with topoisomerase III α and other proteins, can catalyze double-Holliday junction dissolution, a process in which the two Holliday junctions are migrated toward one another and then decatenated (Wu and Hickson 2003). Unlike resolution of double-Holliday junctions by cleavage, dissolution generates only noncrossovers.

Genetic studies suggested a similar anticrossover role for the *S. cerevisiae* BLM ortholog *Sgs1* in meiosis. Crossovers are reduced in mutants lacking ZMM proteins, including *Msh4–Msh5*, but, remarkably, crossovers are restored in double mutants that also lack *Sgs1* (Jessop *et al.* 2006; Oh *et al.* 2007). An attractive interpretation of these results is that one function of ZMMs is to antagonize the anticrossover activity of *Sgs1*. Thus, *Msh4–Msh5* is an anti-anticrossover protein.

Although these experiments with ZMMs and *Sgs1* are consistent with the known mitotic anticrossover functions of *Sgs1*, *sgs1* mutants have only a modest increase in meiotic crossovers, much less than would be expected if all double-strand breaks were processed through a pathway in which double-Holliday junctions were produced and resolved into crossovers (Rockmill *et al.* 2003; Jessop *et al.* 2006). Novel insights into the solution to this apparent paradox came again from physical measurements of recombination intermediates and products. De Muyt *et al.* (2012) found that noncrossovers are still produced in *sgs1* mutants, but, unlike the case in wild-type cells, these noncrossovers arise as joint molecules disappear and crossovers appear. This suggests that when *Sgs1* is absent, double-Holliday junctions are resolved into crossovers and noncrossovers, as in the original double-strand break repair model.

Additional insights came from physical studies of recombination in mutants lacking the known Holliday junction resolvases. Three proteins, *Mus81–Mms4*, *Yen1*, and *Slx1–Slx4*, possess resolvase activity *in vitro* (Boddy *et al.* 2001; Ip *et al.* 2008; Fekairi *et al.* 2009). *Mus81–Mms4* was shown to be important in generating mitotic crossovers, with *Yen1* playing a compensatory or partially redundant role (Ho *et al.* 2010). Experiments by De Muyt *et al.* (2012) and Zakharyevich *et al.* (2012) found that single mutants lacking any one of these enzymes were still able to resolve most joint molecules and produce approximately normal numbers of crossovers. Even triple mutants lacking all three resolvases showed only a modest reduction in joint molecule resolution and crossover formation. These results suggest that the known resolvases

collectively process only a small fraction of joint molecules. If these are joint molecules from the class II pathway, then most joint molecules must be generated in the class I pathway and be resolved by an unidentified resolvase.

Yet another surprise came when the same experiments were done in the absence of *Sgs1*. In this case, removing all three resolvases resulted in most joint molecules being left unresolved. Again, this result indicates that joint molecules produced in the absence of *Sgs1* are different from those produced in the presence of *Sgs1*. In the absence of *Sgs1*, joint molecules are acted on by the known resolvases to produce both crossovers and noncrossovers, much like in the original double-strand break repair model. The known resolvases, functioning in the class II pathway, are therefore neither pro-crossover nor anti-crossover, since they generate both outcomes. Conversely, joint molecules produced in the presence of *Sgs1* (class I pathway) are cut by an unknown, pro-crossover resolvase to produce exclusively crossovers.

What is the identity of the pro-crossover resolvase that functions in the class I pathway? It had previously been suggested that the mismatch repair proteins *Mlh1–Mlh3* (MutL γ complex) and *Exo1* might act in double-Holliday junction resolution (Nishant *et al.* 2008; Zakharyevich *et al.* 2010). Crossovers are reduced in *mlh3* mutants, but removal of *Sgs1* restores crossovers, suggesting that *Mlh3*, like ZMMs, functions in the class I pathway (Oh *et al.* 2007). Consistent with this hypothesis, Zakharyevich *et al.* (2012) found that when all three known resolvases were removed, eliminating *Mlh3* resulted in a similar reduction in crossovers as eliminating *Sgs1*. A parallel set of experiments suggested that *Exo1* functions in a different pathway than *Mus81–Mms4*, putting *Exo1* also in the class I pathway.

These results are consistent with *Sgs1* having the expected anticrossover functions: It promotes synthesis-dependent strand annealing (in wild-type cells) and double-Holliday junction dissolution (when the three known resolvases and the putative pro-crossover resolvase are all missing). Unexpectedly, the results also reveal a pro-crossover role of *Sgs1*. This pro-crossover role may be in influencing pathway choice: In the presence of *Sgs1*, the ZMM-dependent class I crossover pathway can be used, but in the absence of *Sgs1*, the alternative class II pathway gives rise to both crossovers and noncrossovers from double-Holliday junction resolution.

Extending the Two-Pathway Paradigm

The results discussed above provided substantial support for and clarification of the two-pathway paradigm for meiotic crossovers in *S. cerevisiae*. Other recent results reveal the applicability of this paradigm to other model organisms. *Drosophila* was sometimes believed to generate interfering crossovers through a different pathway, since *Msh4* and *Msh5* are absent and a different resolvase may be used (see below) (e.g., Schwartz and Heyer 2011). However, Kohl *et al.* (2012) showed that a complex of meiotic mini-chromosome maintenance proteins (the mei-MCM complex) functionally replaces *Msh4–Msh5* in

Drosophila. Mutants that lack mei-MCM components have greatly reduced crossovers, but removal of the BLM/*Sgs1* ortholog restores crossover formation, similar to the restoration of crossovers by *Sgs1* elimination in *S. cerevisiae* *msh4* and *msh5* mutants (Jessop *et al.* 2006; Oh *et al.* 2007). Kohl *et al.* (2012) hypothesized that evolution of the mei-MCM complex allowed *Msh4*–*Msh5* to be lost from *Drosophila* and other higher flies.

In addition to using a different anti-anticrossover complex, flies appear to use a different nuclease as their primary Holliday junction resolvase. The *Drosophila* *Rad1*/XPF endonuclease ortholog *MEI-9* acts in the same pathway as the mei-MCMs and is responsible for ~90% of meiotic crossovers (Baker and Carpenter 1972; Sekelsky *et al.* 1995). Thus, in *Drosophila* most crossovers are generated through the class I pathway, with a unique anti-anticrossover complex (mei-MCM) and a different procrossover resolvase (*MEI-9*).

This theme of swapping in different proteins to accomplish the same function may hold true in other organisms. Crismani *et al.* (2012) found that *Arabidopsis* mutants lacking the FANCM helicase had elevated crossovers. The additional crossovers were interference-insensitive, arose from a ZMM-independent pathway, and relied on *Mus81* for formation. These findings parallel results seen in *S. cerevisiae*, where the absence of *Sgs1* leads to crossovers being formed in an alternative, *Mus81*-dependent pathway. Furthermore, loss of FANCM rescues the meiotic defects of *Arabidopsis* *zmm* mutants, just like the removal of *Sgs1* in *S. cerevisiae* *zmm* mutants (Crismani *et al.* 2012; Knoll *et al.* 2012). These findings strongly suggest that *Arabidopsis* FANCM functions as a meiotic anticrossover protein in a role that is antagonized by the ZMM proteins, similar to the role of *Sgs1* in budding yeast. Thus, it appears that organisms can exchange proteins that occupy the same functional niche (in this case, swapping two anticrossover helicases) and still follow the framework of the two-pathway paradigm.

Meiotic and Mitotic Recombination in Meiosis

These recent findings have added much to our understanding of the two crossover pathways used in meiosis and suggest a unified model that describes the relationship between the two pathways (Figure 2). By understanding that organisms use each pathway to varying degrees and use different proteins to accomplish the same tasks, this unified model appears to be applicable to a more diverse set of model organisms than previously recognized. Furthermore, it is now apparent that the class II pathway is strikingly similar to mitotic double-strand break repair in many respects (De Muyt *et al.* 2012). First, noncrossovers—not crossovers—are the predominant product. This outcome is achieved through synthesis-dependent strand annealing, mediated by one or more anticrossover helicases. In instances where synthesis-dependent strand annealing does not occur and a double-Holliday junction is generated, this intermediate can be resolved in an unbiased manner by “mitotic” resolvases to give either a noncrossover or a crossover, but these crossovers are noninterfering. Despite

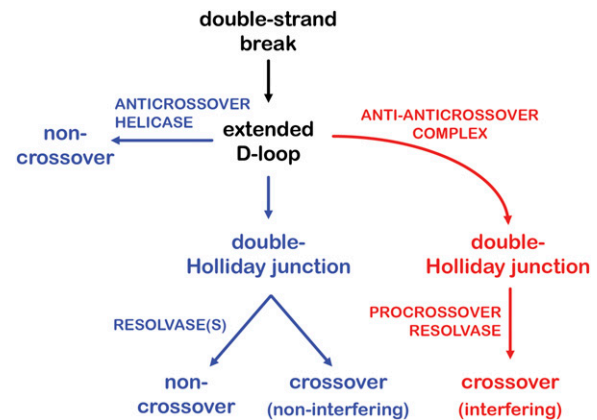


Figure 2 Two meiotic crossover pathways. In this unified model synthesizing ideas from several sources (Börner *et al.* 2004; De Muyt *et al.* 2012; Zakharyevich *et al.* 2012), a double-strand break is processed into an extended displacement loop (D-loop). In the “mitotic-like” pathway (blue, class II), the extended D-loop can be unwound by an anticrossover helicase to produce noncrossovers. In some cases a double-Holliday junction is generated and then resolved by unbiased resolvases into either crossover or noncrossover products; the crossovers that are formed are noninterfering. Another possible fate of this intermediate is dissolution by a helicase and topoisomerase, to produce noncrossover products (not shown). In the meiosis-specific crossover pathway (red, class I), an anti-anticrossover complex blocks the action of anticrossover helicases to promote formation of a double-Holliday junction intermediate, which is then acted upon by a procrossover resolvase to form interfering crossovers. A double-Holliday junction is presented as a key intermediate to fit the original models (Figure 1) and the detection of joint molecules with properties of double-Holliday junctions in physical assays (Collins and Newlon 1994; Schwacha and Kleckner 1994; Schwacha and Kleckner 1995). However, there are other models that posit additional or alternative intermediates, including single Holliday junctions and multichromatid joint molecules (Osman *et al.* 2003; De Muyt *et al.* 2012; Zakharyevich *et al.* 2012). Variations on the two-pathway model as presented here can accommodate other intermediates and less-common fates of double-strand breaks.

these similarities, it should be noted that there are features of the class II pathway that are unique to meiosis. For example, double-strand breaks are generated by meiosis-specific *Spo11* complexes, and engagement of DNA strands from the broken chromosome to the homologous chromosome is mediated in most species by meiosis-specific strand exchange proteins like *Dmc1* (reviewed in Neale and Keeney 2006). These events, however, may occur prior to the split between the class I and class II pathways (Figure 2).

While the class II pathway is similar to mitotic double-strand break repair, the class I pathway is a meiosis-specific double-strand break repair mechanism with embellishments to favor the formation of interfering crossovers (Börner *et al.* 2004; De Muyt *et al.* 2012; Zakharyevich *et al.* 2012). To ensure that double-Holliday junctions are generated, anti-crossover activities of helicases are blocked by meiosis-specific anti-anticrossover proteins. These double-Holliday junctions are resolved mostly or exclusively into crossover products by a procrossover, possibly meiosis-specific, resolvase. Finally, class I crossovers exert and are sensitive to crossover interference, perhaps as a consequence of functional connections between this pathway and structural components of meiotic chromosomes, including the synaptonemal complex and

meiosis-specific cohesins (Zickler and Kleckner 1999; de Boer and Heyting 2006).

The findings described above provide new insights into the evolution of meiotic recombination. Meiosis has long been thought to have evolved from mitosis (Cavalier-Smith 1981; Wilkins and Holliday 2009), and the evolution of meiotic double-strand break repair from mitotic double-strand break repair has been suggested previously (Marcon and Moens 2005). We hypothesize that early in the evolution of meiosis, meiotic recombination occurred only through the class II pathway, a method of double-strand break repair already in use in somatic cells. Over time, the class I pathway evolved to place additional constraints on meiotic recombination to promote the optimal placement of crossovers. To ensure crossover formation, additional regulation of anticrossover helicases active during mitotic double-strand break repair was developed. This functional niche was filled by meiosis-specific anti-anticrossover proteins like *Msh4-Msh5* and the mei-MCMs. The class I pathway also evolved so that double-Holliday junctions are resolved in a biased way to produce crossovers but not noncrossovers. The class II pathway remained available, perhaps as a failsafe to ensure that all double-strand breaks are repaired.

Many questions about meiotic recombination remain unsolved. One key question is how the crossover/noncrossover decision (*i.e.*, whether a given double-strand break is repaired as a crossover or a noncrossover) is made. Studies in *S. cerevisiae* suggest that this decision is made early in the recombination process (reviewed in Bishop and Zickler 2004), while studies in mice and *C. elegans* suggest that crossover/noncrossover differentiation occurs via a two-step process, wherein sites are marked early as potential crossovers but only a subset of these are subsequently designated as future crossovers (Cole *et al.* 2012a; Yokoo *et al.* 2012). In light of the two-pathway paradigm, the crossover/noncrossover decision must be enforced at or prior to divergence of the class I and class II pathways. Since *Sgs1* appears to control pathway choice (De Muyt *et al.* 2012; Zakharyevich *et al.* 2012), it stands to reason that this protein may play a role in the crossover/noncrossover decision. The crossover/noncrossover decision must also be intertwined with the mechanism that mediates crossover interference. In the two-pathway model presented in Figure 2, interference could be mediated by crossovers themselves or by any precrossover intermediate specific to the class I pathway, such as D-loops or double-Holliday junctions loaded with the anti-anticrossover complex. Feedback from these precrossover intermediates or class I crossovers would have to impact the crossover/noncrossover decision of nearby double-strand break repair events to ensure they go down the noncrossover pathway. How the crossover/noncrossover decision is made, how *Sgs1* mediates this decision, and how crossover interference works are important areas for future research in the meiotic recombination field. The unified view of recombination pathways depicted in Figure 2 may help to guide some of these studies.

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