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New and old ways to control meiotic recombination

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Abstract

The unique segregation of homologs, rather than sister chromatids, at the first meiotic division requires in most species the formation of crossovers between homologs by meiotic recombination. Crossovers do not form at random along chromosomes. Rather, their formation is carefully controlled, both at the stage of formation of DNA double-strand breaks (DSBs) that can initiate crossovers and during the repair of these DSBs. We review control of DSB formation and two recently recognized controls of DSB repair: crossover homeostasis and crossover invariance. Crossover homeostasis maintains a constant number of crossovers per cell when the total number of DSBs in a cell is experimentally or stochastically reduced. Crossover invariance maintains a constant crossover density (crossovers per kb of DNA) across much of the genome in spite of strong DSB hotspots in some intervals. These recently uncovered phenomena show that crossover control is even more complex than previously suspected.

Multiple controls of meiotic recombination during gamete formation

The formation of haploid cells (gametes) from diploid precursor cells during meiosis is essential to maintain a constant number of chromosomes from generation to generation in sexually reproducing species. Haploids arise in meiosis because there are two nuclear divisions but only one round of replication. The major problem is to ensure that exactly one copy of each chromosome pair is inherited by each haploid cell. This requires that homologs, or more precisely homologous centromeres, segregate from each other at the first meiotic division and that sister centromeres segregate at the second meiotic division. In most species homolog segregation requires formation of a physical connection between homologs. This connection is detected genetically as a crossover or microscopically as a chiasma (pl., chiasmata). Meiotic recombination also forms new combinations of alleles, thereby speeding the evolution of species. Thus, recombination plays a dual role in meiosis, with both immediate and long-term consequences.

Almost from the time of their discovery a century ago, meiotic crossovers and chiasmata were known to be non-randomly distributed along chromosomes. Crossovers do not occur independently: a crossover in one interval decreases the likelihood of a crossover in a nearby interval, a phenomenon called crossover interference, the first recognized control (Box 1). Crossovers are rare in and around centromeres, because their occurrence there interferes with proper chromosome segregation. Crossovers too far from the centromere (*i.e.*, near the telomere) less effectively direct proper segregation, and in at least some species crossing over is reduced near the telomeres.

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One key to understanding these controls has come from studies of the mechanism of crossing over, which is initiated by the formation of lesions in one of the interacting DNA molecules. Double-strand breaks (DSBs) in DNA can initiate crossing over in two well-studied species, the budding yeast *Saccharomyces cerevisiae* and the very distantly related fission yeast *Schizosaccharomyces pombe*, although other lesions, such as single-strand breaks (nicks), have not been excluded (Box 2). DSBs are made by a meiosis-specific topoisomerase-like protein Spo11 (called Rec12 in *S. pombe*, Table 1) in conjunction with several “meiotic break proteins”, which, like Spo11, are essential for both DSB formation and meiotic recombination (Figure 1) [1, 2]. A Spo11 ortholog appears to be present in all species that undergo meiosis, making it likely that DSBs are important for meiotic recombination in all species.

At a DSB, the 5' ends are digested away (resected), and the resultant 3'-ended single-stranded (ss) DNA invades an intact duplex at a point of extensive nucleotide sequence identity. Base-pairing between the two interacting DNA molecules forms hybrid DNA (Figure 1). If the hybrid DNA contains one or more mismatches stemming from a genetic difference between the two parents, mismatch correction can produce three copies of one allele and only one of the other, a phenomenon called gene conversion or non-reciprocal recombination, an exception to Mendel's rule of 2:2 inheritance. Gene conversion can also arise from DNA synthesis that replaces the resected DNA. Resolution of the hybrid DNA intermediate can reciprocally recombine alleles flanking the hybrid DNA region to form a crossover. Alternatively, resolution can leave the flanking alleles in the parental configuration to form a non-crossover. Gene conversion can thus be accompanied by either a crossover or a non-crossover, both of which are forms of reciprocal recombination.

The formation of DSBs and their repair provide multiple levels for control of recombination. Gene conversion frequencies vary greatly along chromosomes, and not surprisingly DSBs were first detected at hotspots of gene conversion, sites that convert at a frequency higher than the genome average. Genome-wide studies have shown that DSB formation is far from random in both budding and fission yeasts, and DSB hotspots and gene conversion hotspots appear to be coincident. Since DSB formation occurs after replication, a DSB can be repaired by interaction with its sister chromatid or with either chromatid of the homolog. Until recently, it was assumed that DSB repair in meiosis occurred only with the homolog, because only crossovers between homologs can properly direct homolog segregation (Figure 2). Studies in both yeasts unexpectedly show, however, that DSBs can be repaired with either the sister or one of the two chromatids of the homolog [3, 4]. A further surprise is that resolution to crossover *vs.* non-crossover can be regulated in response to the total number of DSBs in the cell, as observed in budding yeast [5]. Here, we discuss factors that influence DSB formation and repair, including two recently described phenomena - crossover homeostasis [5] and crossover invariance [6].

Controlling DSB formation

Regulating the timing of DSB formation

In both yeasts, DSBs arise after pre-meiotic DNA replication [7–9]. There are two likely explanations for why this is so. First, sister chromatid cohesion distal to a crossover is essential to generate the tension needed for accurate chromosome segregation; sister cohesion is possible only after replication, as is crossing over between just two chromatids (Figure 2). Second, restricting DNA breaks until after DNA replication avoids errors that could arise by replicating across unrepaired breaks. So how are DSBs restricted to occur after replication?

Early data indicated that local DNA replication is a prerequisite for DNA breakage. In budding yeast when meiotic replication is inhibited by hydroxyurea treatment or by the absence of cyclins, DSBs are not observed [7, 10]. When replication timing is selectively delayed on a chromosome arm with inactive origins, DSBs appear later in that chromosomal region [7]. Later data, however, showed that when replication origin-firing is prevented in *S. pombe* or *S. cerevisiae* using pre-replicative complex mutants, meiotic DSBs are formed at wild-type levels, implying that DNA replication *per se* is not essential for DSB formation [11, 9].

Currently, the solution to this puzzle is unclear. Perhaps most DSBs depend on sister chromatid axis components and DNA replication, whereas others are independent. Alternatively, meiotic checkpoints may inhibit DSB formation when activated by stalled replication forks. If replication origins do not fire at all, these checkpoints are not activated and DSBs form normally (reviewed in [12, 13]). Further experiments are needed to test these scenarios.

DSBs may be formed post-replication because Spo11 or Rec12 and their partner proteins (Figure 1) are present only during this time. Break timing may also be regulated by replication fork-associated proteins that functionally modify these partner proteins [13]. For example, phosphorylation of the budding yeast Spo11 partner protein Mer2 [14–16] regulates the interaction of Mer2 with the DSB-forming complex. Phosphorylated Mer2 in turn recruits other break proteins to break sites. If the cyclin-activated protein kinases were active only at the replication fork, this would, in part, explain the temporal control of meiotic DNA breakage [13]. A similar control may also exist in *S. pombe*. The protein kinase Hsk1 is required for meiotic DNA breakage [17] and for binding of Rec12 to the *mbs1* DSB hotspot [15]. Hsk1 may phosphorylate a Rec12 partner protein.

Chromosomal region-specific requirements for chromosome axis and linear element proteins

Three classes of proteins differentially affect, across the genome, meiotic breakage and recombination: cohesins, condensins, and axial or linear element proteins. Cohesins are required for sister chromatid cohesion following replication, and condensins are required for compacting the chromatin and changing chromosome architecture to allow accurate chromosome segregation during cell division [18, 19]. Linear element or axial element proteins aid interactions between homologous chromosomes during meiosis [20, 21]. In *S. pombe* the absence of meiosis-specific cohesin subunits Rec8 and Rec11 strongly reduces meiotic DSB frequency and recombination in some chromosomal regions but much less so in others [22]. Absence of linear element protein Rec25 reduces intragenic recombination less than 2-fold at *ura1* but 135-fold at *ade6* [23]. Similar differential reductions are observed in *S. cerevisiae* lacking the axial element protein Red1 [24]. Why some regions are more dependent on these proteins than others remains a mystery.

In *C. elegans* condensins affect meiotic crossover distribution in specific regions of the genome [25]. Condensin I mutations (*dpy-28*) increase crossovers on the right end of the X chromosome, while condensin II mutations (*kle-2*) increase crossovers on the left end. In *dpy-28* mutants the crossover distribution is correlated with the Rad51 focus-distribution along the X chromosome, indicating that break distribution is affected in these mutants. Mutations in both *dpy-28* and *kle-2* alter the chromosome axis structure, indicating a role for chromosome structure in determining the position and frequency of meiotic breakage.

Requirements for transcription factors, local DNA sequence, and genomic context for DSB formation

Some hotspots require transcription factor-binding at a distinct local sequence for activity. One of the most thoroughly studied examples is the *M26* hotspot in *S. pombe*, created by a single base-pair mutation in *ade6* [26]. 5'-ATGACGT-3' or a closely related sequence is necessary for *M26* hotspot activity (*i.e.*, high frequency gene conversion) and DSB-formation specifically during meiosis [27–29]. The transcription factor Atf1-Pcr1, which binds this sequence, is essential for hotspot activity [30, 28]. Similarly, Rts2 and Php2, 3, 5 transcription factors activate their cognate sequences as hotspots when created in the *ade6* gene [31].

The chromosomal context of *M26* is crucial for its hotspot activity in cells. When 3–6 kb DNA fragments with *ade6-M26* centrally located are transplaced to distant chromosomal loci or onto a multi-copy plasmid, *M26* is in most but not all cases inactive [32, 33]. These results show that DNA more than 1.5 kb from *M26* can influence its activity. Optimal binding of Atf1-Pcr1 to naked DNA implicates an18 bp consensus sequence, and the *M26* hotspot requires appropriate base-pairs spread over at least 14 of these bp for full activity in cells [34]. When the consensus sequence was used to identify other potential break sites in the genome, 10 of 15 sites tested showed DSB hotspots within 1 kb of the *M26* sequence, and in the one case tested this sequence is required for hotspot activity [29]. Variable DSB frequency among these 10 sites implies additional, as-yet-unidentified factors that influence the intensity of *M26* hotspot activity.

Chromosomal context also affects budding yeast DSB hotspots. Insertion of foreign DNA can create a DSB hotspot at the insertion site while decreasing frequency of breakage at a site farther away [35, 36]. Deletions in the promoter region of *ARG4* can reduce, enhance or have no effect on gene conversion in *ARG4* [37]. Insertions of *ARG4* into hot and cold regions show chromosomal context-dependence of DSB propensity paralleling that of the native region [38].

It seems that transcription *per se* is not required for hotspot activity, since transcriptional strength does not correlate with hotspot activity. The budding yeast *HIS4* hotspot requires the transcription factors Bas1, Bas2 and Rap1 [39, 40], but reduction of transcription by deleting part of the promoter does not affect hotspot activity [39]. At both the *HIS4* hotspot and the *M26* hotspot in fission yeast no correlation has been observed between hotspot activity and transcript levels [39, 30].

Although individual transcription factor-dependent hotspots clearly exist, transcription factor-binding sites are not good indicators of DSBs on a genome-wide level. Early genome-wide analysis in budding yeast showed that of 20 DSB hotspots within intergenic regions, 13 are located between the 5' ends of two divergent genes, implying that a majority of intergenic hotspots require either transcription factor binding or divergent transcription for hotspot activity [41]. However, recent fine-scale mapping of oligonucleotides covalently linked to Spo11 (Figure 1) shows that transcription factor-binding sites have an equal probability of lying in a hotspot or not; indeed, some binding sites seem to obstruct Spo11 action [42]. Binding of transcription factors to their cognate sites may modify chromatin architecture and allow break proteins to act in some cases but not others.

Histone modifications affect meiotic breakage

Some chromosomal features, such as local structure and histone modifications, are correlated with break formation. In budding yeast meiotic DSB sites are associated with sensitivity to micrococcal nuclease (MNase) and DNase1, a defining feature of “open” chromatin [43, 44]. In fission yeast, nucleosome phasing and MNase sensitivity are altered

by *M26* [45]. In mice, the Eb recombination hotspot is DNase1-hypersensitive, but the Lmp2 hotspot is not [46].

Post-translational modifications of N-terminal tails of histones are associated with changes in the "openness" of chromatin. Thus, it is no surprise that chromatin modifying proteins affect meiotic DNA breakage, but the effects are complex. In budding yeast, absence of the histone deacetylase Sir2 affects meiotic breakage at ~12% of all genes, with increases at some sites and decreases at others [47]. Similarly, absence of Set1, which methylates histone H3-K4, a modification mostly associated with actively transcribed regions, reduces break formation at several hotspots [48]. Analysis of ssDNA that accumulates adjacent to DSBs in *dmc1* mutants shows that >80% of DSB hotspots genome-wide are dependent on Set1, but ~7% are repressed by Set1 [49]. *set1* mutants have delayed replication [48], which complicates the interpretation, since as noted above DSB formation is coupled to replication.

Histone modifications regulate DSB formation and meiotic recombination in *S. pombe* as well. The *M26* hotspot has hyper-acetylated histones H3 and H4, both marks of transcription activation [50]. Absence of the histone acetyltransferase Gcn5 and the chromatin remodeler Snf22 completely removes the chromatin remodeling associated with *M26* and reduces meiotic recombination at the *M26* hotspot.

Chromatin modifications have also been implicated in break formation in *C. elegans*. Partial deletion of *him-17*, encoding a chromatin-associated protein, reduces the level of H3-K9 methylation and causes almost complete absence of Rad51 DSB-repair foci in the meiotic cells in which recombination would normally occur [51]. Similarly, mutation in *xnd-1*, encoding a chromatin-binding factor, reduces meiotic Rad51 foci on the X chromosome and increases H2A-K5-Ac in meiotic cells [52].

A role for histone modifications in regulating meiotic breakage has been implicated in mammals. In mice, H3-K4 trimethylation and H4 acetylation are enriched at the *Psmb9* and *Hlx1* recombination hotspots [53]. The histone H3-K4 methyl transferase Prdm9 binds a degenerate 13-mer motif found in about 40% of human linkage disequilibrium-defined hotspots and also in the mouse *Psmb9* and *Hlx1* hotspots [54, 55]. Alleles of *Prdm9* correlate with hotspot activity, suggesting that Prdm9 binds to a hotspot and activates it.

Centromeres are transcriptionally and recombinationally silent regions of the genome. In fission yeast RNAi-mediated methylation of histone H3-K9 by Clr4 represses transcription at the centromeres [56]. Disrupting genes that encode RNAi factors or Clr4 dramatically increases meiotic DSBs at and around centromere 3 (*cen3*) and increases recombination between genetic markers flanking *cen3* ~100-fold [57]. Interestingly, disruption of some factors that increase transcription in the fission yeast centromeres does not affect meiotic recombination in *cen3*, suggesting that overlapping as well as distinct histone modifications dictate the accessibility of a region to transcription and recombination functions [57].

The studies mentioned above substantiate the correlations between meiotic DSB formation and histone modifications, chromosomal context, transcription factors and their cognate sites, and chromatin remodeling in numerous species. However, a clear and universal picture is not observed. Multiple factors affect DNA breakage variably, depending on the chromosomal site and the species studied. At some sites the transcription complex may localize to "open" promoters and recruit meiotic break proteins, while at other sites the transcription complex may occlude them. Modified chromatin may differentially control the accessibility of the transcription and the meiotic recombination machinery. In addition meiotic DSB proteins may recognize and bind to specific combinations of modified histones or to a "recruiting factor" that recognizes modified histones. Elucidating the role(s) of these factors in DSB-formation awaits further study.

Controlling DSB repair

There are more DSBs formed per meiosis, as measured by Rad51 DSB-repair foci, than crossovers in many organisms, including mice (~10:1) and *Arabidopsis thaliana* (~15:1) [58, 59]. A genome-wide study in *S. cerevisiae* estimated that about 60% of DSBs are repaired to generate crossovers (COs), with the remainder repaired as non-crossovers (NCOs) (Figure 2) [60], though some NCOs could be lost via mismatch correction and thus underestimated. The frequency of gene conversions (GCs) with an associated CO varies in different organisms, from an average of ~35% in *S. cerevisiae* to as high as 80% in some regions of the *S. pombe* genome [61, 62]. The restriction of COs is exceptionally strong in *C. elegans*: only one CO forms per homolog pair even when two chromosomes, each of which normally has one crossover, are fused end-to-end [63], a result of strong chromosome-wide crossover interference (Box 1).

It isn't as simple as one DSB–one crossover; so, what factors control how frequently a CO is formed during DSB repair? Crossover control during DSB repair can be viewed as two alternatives at each of two steps: whether repair is with the sister or with a homolog chromatid (Figure 2, steps 6 and 6a), and whether a CO or a NCO forms during repair (Figure 2, steps 7a and 9). Genetically observable COs and NCOs [*i.e.*, GCs] can be formed only by repair with a homolog chromatid as the template, as the sister chromatid is identical and repair with it would be genetically silent (Figure 2, step 6a). Repair with the homolog can result in either a CO or NCO by means discussed below.

Crossover vs. non-crossover repair of DSBs

The step of recombination at which COs are differentiated from NCOs has been the focus of substantial research. The canonical model of DSB repair proposes that COs and NCOs arise from alternative resolution of Holliday junctions (HJs), *i.e.*, at essentially the last step of DSB repair [64]. Enzymatic cleavage of one pair of strands generates a CO, and cleavage of the other a NCO (Figure 2, step 9). Electron microscopy and gel electrophoresis provided evidence for HJs in *S. cerevisiae*, in this case mostly, but not exclusively, double HJs (Figure 2, step 8) [65, 66], as well as mostly single HJs in *S. pombe* (Figure 2, step 7b) [3].

There is, however, growing evidence against such “late” control of CO *vs.* NCO formation during meiosis. In *S. cerevisiae* heteroduplex DNA (hDNA) generated during NCO formation does not show the expected symmetric structure predicted from double HJ resolution [67–69]. In the absence Zip1, 2, 3, and 4, Mer3, or Msh4, 5 (“ZMM” proteins, which act before HJ formation) (Figure 2, steps 3 and 6) COs and HJ intermediates are substantially reduced but NCOs are unaffected [70]. This result has been interpreted as differentiation of COs and NCOs before HJ resolution. Furthermore, hDNA from NCOs was detected earlier (at the same time as HJs) than hDNA from COs [71]. In strains deficient for the meiosis-specific transcription factor Ndt80, unresolved HJs accumulate and CO hDNA is reduced, but NCO hDNA is not reduced. It was proposed that NCOs are formed in a different, HJ-independent pathway via synthesis-dependent strand annealing (SDSA, Figure 2, step 7a), and that most or all HJs give rise only to COs. In support of this view, a temporal analysis of single-end invasions (Figure 2, step 6) suggested that the CO vs. NCO designation happens earlier than HJ resolution [72]. In *S. pombe*, mutants lacking the HJ-resolvase Mus81-Eme1 accumulate HJs, and COs are virtually eliminated, but gene conversions (NCOs) form with wild-type frequency [73, 74, 3].

To date, DNA intermediates specific to SDSA repair have not been detected, possibly due to their short half-life or high instability. A genetic assay for a class of NCOs most easily explained by SDSA, however, provided evidence that *S. cerevisiae* uses SDSA during

meiosis [75]. The structure of NCOs and COs formed during mitotic gap repair is also most consistent with NCOs being formed via SDSA, and COs via HJ resolution [76].

Although the results discussed above are consistent with COs and NCOs arising from different intermediates, they are also compatible with induction of a factor, perhaps Ndt80-dependent in *S. cerevisiae*, that directs HJ resolution to COs only late in meiosis. In the absence of this factor and HJ resolution, intermediates may be diverted into NCOs exclusively, perhaps via SDSA.

Homolog vs. sister chromatid for DSB repair

A DSB can be repaired using either the homolog or the sister chromatid as template, but what determines partner choice is unclear (Figure 2, step 6 and 6a). The most thorough examination of what influences the choice between interhomolog (IH) and intersister (IS) DNA repair has been done in *S. cerevisiae*. During meiosis in this yeast there is a strong preference for IH repair, at least by assay of HJs at one (artificial) hotspot via 2-D gel electrophoresis – IH HJs outnumber IS HJs 5 to 1 [77]. This homolog preference is dependent on Red1, Hop1, Mek1, and Rec8 [78–80]. In the absence of these chromosome axis-associated proteins, IH HJs and COs are reduced; IS HJs are increased in *mek1* mutants. Thus, IH repair is actively promoted by several proteins; IS repair may be actively suppressed.

In spite of these observations and conclusions, a recent study indicated that IS repair can be frequent during *S. cerevisiae* meiosis. This study used homologous chromosomes hemizygous for large (3.5 or 90 kb) deletions, and therefore DSBs on the intact homolog could be repaired only with the sister chromatid; the observed, high-level DSBs were efficiently repaired [4]. Additional observations suggest that a substantial fraction of DSBs on chromosomes with an intact homolog are also frequently repaired with the sister. IS repair may have been previously underestimated by measuring only HJ formation; a majority of IS repair could be via SDSA, or IS HJs may be less stable or actively destabilized.

Sister chromatid repair has also been implicated at *S. cerevisiae* centromeres, where recombination is reduced more than DSBs, relative to the genome average [81]. But unlike the rest of the genome, crossover homeostasis, described below, cannot account for this observation, as NCO and CO levels are equally suppressed; NCOs would be expected to increase in response to the DSB repair that does not proceed towards CO formation. In a *zip1* mutant, centromeric recombination is not suppressed, but DSBs are not increased, suggesting that Zip1 suppresses IH repair at the centromere in favor of IS repair [81]. The genome-wide CO reduction in an *msh4* mutant – in which DSBs are not reduced – is not accompanied by an increase in NCOs, suggesting IS repair as well [60]. Evidence of homolog-independent, and presumably intersister, DSB repair has been observed in *smc5*, *smc6*, and *brc1* mutants of *C. elegans* [82, 83]. IS repair during meiosis appears to be more prevalent than previously estimated and may partly account for Rad51 foci greatly outnumbering COs in some species.

Crossover homeostasis

The maintenance of a constant level of COs by adjusting the number of NCOs when DSB levels change has been termed crossover homeostasis, based on results from a study of *S. cerevisiae* non-null *spo11* mutants in which DSB formation was decreased [5]. In spite of DSBs being reduced to 80, 30, and 20% of wild type, the level of COs remained constant at numerous genetic loci, but NCOs were reduced in parallel with DSB reduction. Thus, COs were maintained at the expense of NCOs (Figure 3a). Genome-wide microarray analysis of

CO distribution in *S. cerevisiae* meiotic tetrads supports crossover homeostasis in wild type as well [81, 60]. COs and NCOs are not correlated, as a nearly constant level of COs is observed from cell to cell, while NCOs vary in parallel with DSBs per cell.

Crossover homeostasis is proposed to be part of the mechanism of crossover interference (Box 1). *S. cerevisiaezip2* and *zip4* mutants display reduced COs genome-wide, and there is a significant increase in the correlation between COs and NCOs [81]. Both homeostasis and interference are impaired, supporting a link between the two. The meiosis checkpoint protein Pch2 helps organize the meiotic axis components during synaptonemal complex formation and localizes to putative CO sites [84]. In the absence of Pch2, interference is reduced, gene conversions are elevated, and crossovers increase on large chromosomes (though not on smaller ones), but DSBs do not increase, suggesting the extra COs come from NCO or intersister events [84, 85]. While *pch2* mutants have no defect in spore viability alone, when combined with reduced DSBs in the *spo11* mutations noted above, spore viability is strongly reduced as the DSBs are reduced, due to chromosome missegregation events [84, 85]. Pch2 therefore appears necessary to establish interference and crossover homeostasis, suppressing excess COs in abundant DSB situations and ensuring proper CO distribution when DSBs are limiting, perhaps by balancing COs and NCOs and preventing excess IS repair. Organisms without interference, *S. pombe* for example, are predicted not to have homeostasis [5]; this prediction has yet to be tested.

How crossover homeostasis is maintained is still unclear, but it may be enforced during DSB repair. In *C. elegans*, the protein Rtel-1 was recently inferred to influence DSB repair towards NCOs, presumably via the SDSA pathway [86]. Purified human RTEL-1 disassembles D-loops, preferentially those with a 3' end invasion thought to be intermediates in DSB repair (Figure 2, step 6) [87, 86]. COs are increased in *C. elegans rtel-1* mutants, and increasing the number of DSBs by a *dpy-28* mutation [25] or ionizing irradiation further increases COs [86]. Interference was also impaired, as multiple COs per chromosome were observed, consistent with a role for Rtel-1 in crossover control by disrupting single-end invasions and preventing HJ and CO formation, sending repair down the SDSA pathway. Conversely, there might be proteins that push repair towards HJ and CO formation. D-loops formed by human DMC1, but not those formed by human RAD51, are resistant to dissociation by RAD54 [88]. This resistance may allow DMC1-promoted recombination intermediates to proceed to HJs and COs, but RAD51-promoted intermediates to SDSA and NCOs. Alternatively, homeostasis could be enforced early during the formation of DSBs.

Crossover invariance

The number of crossovers per unit of DNA in *S. pombe* is nearly uniform across much of the genome, even though the DSB distribution is highly variable [89, 90]. A recent study [6] explained this seeming discrepancy by partner choice differing at hotspots and in DSB-cold regions. Two DSB hotspots, the wild-type *mbs1* and the *M26*-like single base-pair mutation *ade6-3049*, were analyzed for HJ formation. At each hotspot IS HJs outnumber IH HJs ~4 to 1. These data provide direct evidence for IS repair being more frequent than IH repair, opposite of the result in *S. cerevisiae*. Both IS and IH HJs are completely dependent on Rad51 but independent of Dmc1, as is recombination at each hotspot. In contrast, HJs and recombination in regions distant from DSB hotspots are highly dependent on Dmc1 and Swi5, a mediator for both Dmc1 and Rad51 that is required only for IH HJ formation at hotspots. Dmc1 is also necessary specifically for IH DSB repair and recombination in *S. cerevisiae* [91, 78]. Deletion of the *mbs1* hotspot reduces COs less than DSBs, showing that substantial COs can be generated in DSB-cold regions, and these COs are strongly Dmc1-dependent. Although IS and IH HJs in DSB-cold regions were not directly measured due to their low levels, the dependence on Swi5 and Dmc1 implies that most HJs distant from DSB hotspots are IH (Figure 3b).

Genetic recombination data agree with this notion. Genetic intervals with weak DSB hotspots have about as many COs as comparable-size intervals with intense DSB hotspots. This phenomenon was termed crossover invariance and reflects a type of crossover control not previously reported: DSB hotspots primarily use IS repair and hence produce few COs per DSB, while regions with few DSBs predominantly use IH repair and may contribute as many COs as regions with DSB hotspots (Figure 3b). This feature keeps the level of crossing over nearly uniform across the genome, although for markers closely flanking *mbs1* this DSB hotspot is clearly a crossover hotspot [62].

Some organisms go to great lengths to promote IH repair and COs, so why is this IH preference not seen at DSB hotspots *S. pombe*? Non-disjunction of homologous chromosomes has serious consequences for all organisms, and IH connections are important to establish proper segregation, but not universally. In the fruit fly *Drosophila melanogaster* males do not recombine, but their chromosomes still segregate properly; single achiasmate chromosome pairs also segregate properly in females, though recombination is still vital for proper female meiosis [92]. Similarly, homolog segregation is nonrandom in *S. pombe* mutants lacking Rec12 (Spo11 homolog), properly occurring 63% of the time by a secondary system dependent on the dynein motor; spore viability is reduced to only ~20% of wild type in *rec12* null mutants [93]. Additionally, *S. pombe* has only three chromosomes that are relatively large (4 – 6 Mb) and have 10 – 20 COs each. Therefore, enforcement of a complicated IH-promoting system for crossing over isn't necessary. Rather, maintaining uniform COs across the genome increases the likelihood of advantageous haplotype rearrangements, and crossover invariance limits excess COs from forming at the same position, involving the same genes, generation after generation.

Concluding remarks and future perspectives

Multiple proteins are required to deliberately break DNA at distinct sites (hotspots) during meiosis. Hotspot determination is complex, involving multiple factors that activate Spo11 – break proteins and their modification, chromatin state, transcription factors, and chromosomal structural components. Although some hotspots are activated by transcription factors, it is not clear how wide-spread this control is, nor is it known how transcription factors act in DSB formation. The same is true for the other factors. Particularly puzzling is how chromosomal axis proteins, such as Rec8, impart their specificity on some but not other chromosomal regions. Correlating the binding of these factors with hotspots genome-wide should be informative.

Though crossover homeostasis and invariance can be seen as two distinct methods of achieving control via DSB repair, our understanding of both processes is still in its infancy. Whether homeostasis occurs in species other than *S. cerevisiae* and perhaps *C. elegans*, and invariance in species other than *S. pombe*, is unknown. These controls may arise from similar (or the same) mechanisms, since both maintain constancy of COs. IS repair of DSBs, as observed in *S. pombe*, *S. cerevisiae*, and *C. elegans*, could be as effective as NCO formation in preventing COs from occurring too close or too frequently in the same location. Indeed, the 50% association of COs with GCs in *S. cerevisiae* would allow DSBs to be reduced by only 2-fold while maintaining homeostasis, if NCO:CO were the only option; nevertheless, DSBs can be reduced by 5-fold and homeostasis is maintained [5]. Changing IS repair to IH repair [4] is another possible source of COs when DSBs are reduced. It will be interesting to see the effect of impeding IS repair on CO and NCO distribution and homeostasis; NCOs are expected to increase to prevent extra COs. Whether NCOs arise by SDSA would be substantiated by finding DNA intermediates specific to SDSA, thereby lending physical reality to this genetic concept. A genome-wide map of COs and NCOs in *S. pombe* meiotic tetrads using the methods established in *S. cerevisiae* might reveal

homeostasis in addition to invariance in that species and could illuminate their relation. Measuring in *S. pombe* the effect of fewer DSBs via hypomorphic Rec12 alleles on both the CO:NCO ratio and the IS:IH ratio at DSB hotspots could further help determine the relation. Ultimately, one would like to examine the DSB sites themselves to determine if they are designated, perhaps by chromatin modifications, for CO or NCO, and for IS or IH, repair prior to DSB formation. Finally, it will be exciting to see what layers of crossover control still remain to be discovered.

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Glossary

Meiotic recombination	Exchange of genetic information or DNA between homologous chromosomes during meiosis
Double-strand break (DSB)	A DNA molecule with both strands broken at the same position or closely spaced positions
Crossover	Recombination that results in the reciprocal exchange of genetic markers flanking the site of DNA interaction
Non-crossover	Recombination that results in local genetic exchange without exchange of flanking genetic markers
Gene conversion	Recombination that results in non-reciprocal transfer of genetic information from one DNA duplex to another; can occur by correction of base mismatches in heteroduplex DNA or by local DNA synthesis at the site of DNA interaction
Hotspots	Chromosomal sites with higher than genome average frequency of DSBs or of recombination
Hybrid DNA	A double-stranded DNA molecule with one strand from each of two interacting DNA duplexes
Heteroduplex DNA	Hybrid DNA with one or more base differences in the two interacting DNA duplexes
Synthesis-dependent single-strand annealing (SDSA)	A mechanism for DSB repair without crossing over but with potential gene conversion (see Figure 2, step 7a)
Meiotic checkpoint	A set of molecular pathways that control and regulate meiotic nuclear division progression
Haplotype blocks	A set of genetic alleles that are transmitted together through meiosis
Cohesins	Proteins required for holding sister chromatids together (cohesion). Rec8 is a meiosis-specific cohesin subunit
Condensins	Proteins involved in chromosome compaction during cell division

Linear elements	*S. pombe* nuclear structures replacing the SC and containing Rec10 (which has limited homology to *S. cerevisiae* Red1), Rec25, and Rec27
Synaptonemal complex (SC)	A proteinaceous structure formed during meiosis that aids chromosome pairing and recombination. The SC is made up of lateral elements connected by central regions. Each lateral element arises from an axial element, formed prior to SC formation along sister chromatids

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Box 1. Crossover interference

If the formation of COs during meiosis were random, a CO at one location would not affect the formation of a CO elsewhere in the genome. In most organisms studied, however, a CO decreases the likelihood that another forms nearby on the same chromosome. The mechanism of this phenomenon, called crossover interference, is not understood, but several models have been proposed. The polymerization model [94] suggests that, after a CO is initiated, an inhibitor is bi-directionally polymerized outward from the CO and prevents any sites nearby from also forming a CO. The mechanical stress model [95] is based on the chromosome being subjected to physical stress during meiosis; an initial DSB (or CO) relieves this stress and forms a CO, but the resulting lack of stress prevents other COs from forming nearby. The counting model [96] posits that after the first CO is formed a fixed number of NCOs must form along the chromosome before another CO is formed, though the observation of crossover homeostasis provides evidence against this model [5]. Recently, COs have been confirmed to interfere not just with other COs but with NCOs as well [60].

Interference can be measured quantitatively via the coefficient of coincidence, $S = R_D/(R_1*R_2)$, where R_1 and R_2 are the frequencies of crossovers in two adjacent genetic intervals and R_D is the frequency of double crossovers. Interference is then calculated as $I = 1 - S$; therefore, $S < 1$ indicates interference present, while $S > 1$ indicates negative interference.

Studies in *S. cerevisiae* indicate that not all COs are subject to interference [97]. The majority of COs are Msh4-Msh5-dependent and are subject to interference, whereas Mus81-Mms4-dependent COs are not. In *A. thaliana* and *M. musculus* most COs are Msh4-Msh5-dependent and manifest interference; in *C. elegans* all COs are Msh4-Msh5-dependent, and each bivalent forms only one CO (complete interference). On the other end of the spectrum, *S. pombe* has no CO interference; nearly all COs are dependent on Mus81-Eme1 [73, 74, 3].

Box 2. Detecting meiotic DSBs

In both budding and fission yeast DSBs are formed by a highly conserved, meiosis-specific protein Spo11 (Rec12 in *S. pombe*). Like a DNA topoisomerase, this protein becomes covalently linked to the DNA, but several "meiotic break proteins" are also essential (Figure 1). In these yeasts, DSBs are directly observed by Southern blot hybridization of meiotic DNA and are non-uniformly distributed across the genome – there are both "hotspots," with frequent DSBs, and "coldspots" or cold regions, with infrequent breaks. Examined carefully, hotspots are seen to be clusters of breaks generally spanning ~100–200 bp in *S. cerevisiae* [42] and up to 4 kb in *S. pombe* [90]. In mice, labeling of broken DNA ends using terminal deoxynucleotidyl transferase (TdT) revealed a DSB hotspot, although these could be single- rather than double-strand breaks [98]. To map DSBs genome-wide, meiotically broken DNA is enriched either by purifying ssDNA, naked or bound by Dmc1 or a single-strand binding protein, that accumulates on both sides of a DSB [99, 100] or by immunoprecipitation of Spo11 or Rec12 covalently bound to DSB ends [41, 90, 42] and hybridizing this DNA to whole genome microarrays. High-throughout sequencing of Spo11-bound oligonucleotides (Figure 1) has provided a nucleotide-level resolution of break sites [42]. The coincidence of break sites determined directly by Southern blot analyses and by the microarray and sequencing methods indicates that the latter two methods detect DSBs.

Since DSBs are thought to be prerequisites for recombination, DSB hotspots are inferred from exceptionally high local frequencies of gene conversion and crossovers or from linkage disequilibrium, taken to reflect historical recombination at high frequency between haplotype blocks, genetic markers between which there is little recombination during the evolution of a population [64, 101]. DSBs are also often inferred by fluorescence microscopy of meiotic recombination proteins, such as Rad51 or phosphorylated histone H2AX, that are recruited to break sites, but these low-resolution methods do not reveal hotspots. TdT labeling of DSB ends with fluorescently marked nucleotides (TUNEL staining) confirmed Rad51 foci as break sites in *C. elegans* [25].

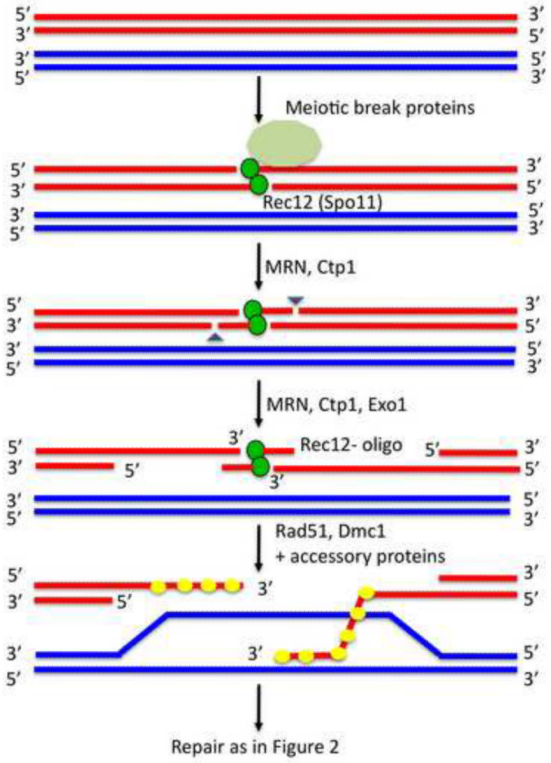

Figure 1. Meiotic recombination initiation in the fission yeast *S. pombe*

Programmed DNA double-strand breaks (DSBs) initiated by Rec12 (Spo11 in other species) during meiosis are efficiently repaired by homologous recombination with high fidelity (for simplicity only one chromatid from each homolog is depicted). Rec12 is aided by several meiotic break proteins to localize and form DSBs. In *S. cerevisiae* the MRX complex (Mre11, Rad50, Xrs2) is required for DNA breakage and repair, whereas in *S. pombe* MRN (Mre11, Rad50, Nbs1) is needed only for repair. Rec12, covalently linked to the 5' ends of the DSB, is clipped off attached to short oligonucleotides (~15–45 long) by MRN in conjunction with Ctp1 (Sae2 in *S. cerevisiae*). The 5' end is further resected by Ctp1 or Exo1 in conjunction with MRN, resulting in a free 3' DNA end. Rad51 and Dmc1, along with numerous accessory proteins, bind the ssDNA end and facilitate invasion of an intact duplex DNA with homology to the invading end. Synthesis of DNA from the end uses the invaded DNA as a template for repair. See Figure 2 for further reactions.

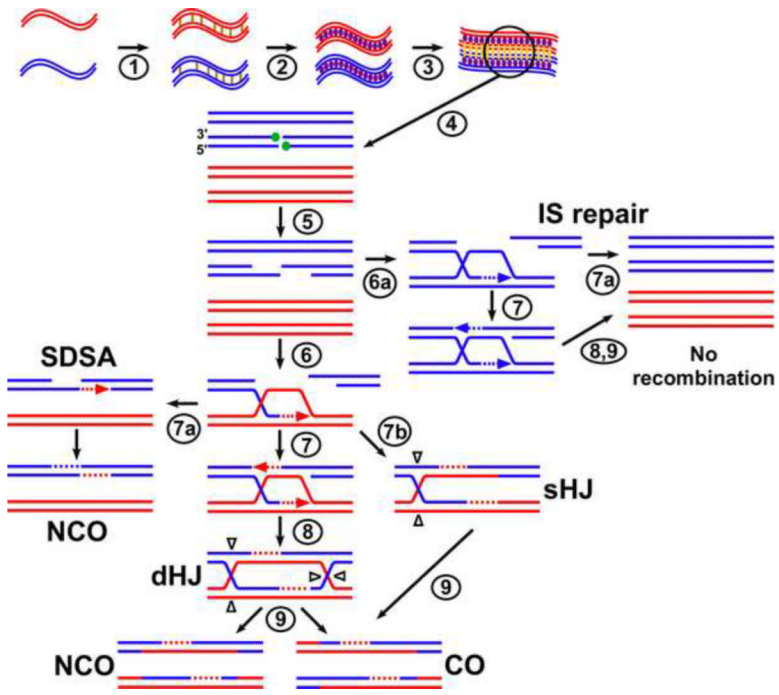

Figure 2. Pathways of meiotic DSB repair

During meiosis chromosomes are first replicated (step 1) and the identical sister chromatids (red and blue double lines indicate duplex DNA) are linked together by meiosis-specific cohesins (step 1, gold lines) and additional proteins that form axial elements (step 2, purple ovals). Pairing of homologs leads to formation of the synaptonemal complex (step 3, yellow bars); in *D. melanogaster*, for example, synapsis occurs independently of DSBs, while in *S. cerevisiae* synapsis would not occur until step 8. Recombination is initiated by programmed DSBs by Rec12 or Spo11 (Figure 1) and numerous partners (step 4, green circles). The now covalently bound Rec12 or Spo11 is removed and the DNA end is resected to create free 3' DNA ends (step 5). The 3' DNA ends invade either the homolog (step 6) or the sister chromatid (step 6a) to create a displacement loop (D-loop), which is extended by DNA synthesis primed by the invading 3' end (see Figure 1 for details). Rad51- or Dmc1-promoted annealing of the other 3' end ("second end capture," step 7) and ligation of ends forms a double Holliday junction (dHJ; step 8). A single HJ (sHJ; step 7b) is formed if the D-loop is cleaved before the second end anneals. HJ resolution yields a crossover (CO) or non-crossover (NCO), depending on the orientation of cleavage of the HJ(s) (white arrowheads, step 9). If, however, the D-loop is dissociated and the invading end, previously extended by DNA synthesis, anneals with the other DSB end (step 7a), a NCO is formed; this repair is called synthesis-dependent strand annealing (SDSA). Crossover control can act at steps 6, 7, or 9.

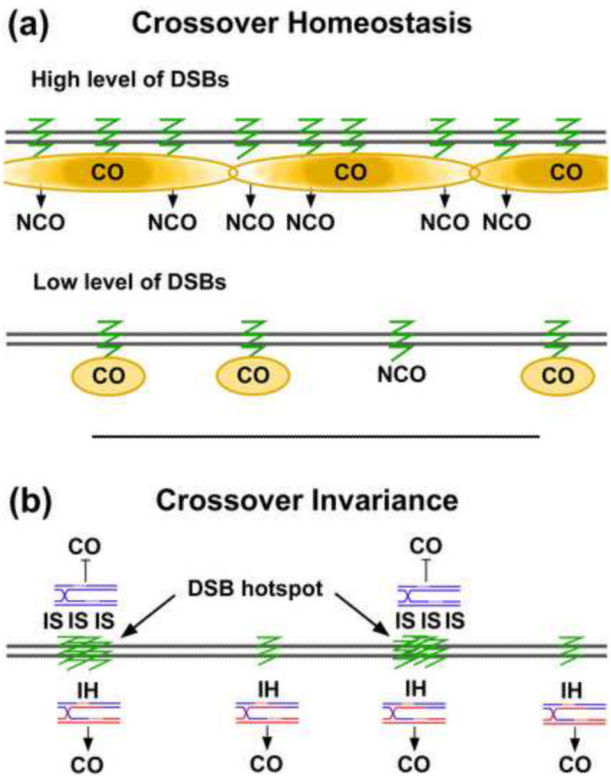

Figure 3. Two mechanisms to distribute crossovers across chromosomes

(a) Crossover homeostasis observed in *S. cerevisiae* [5, 81, 60]. A crossover (CO) generated at one DSB (green zig-zag) inhibits nearby DSBs from generating another CO, a phenomenon known as crossover interference (COI; Box 1) (represented by yellow clouds; deeper color representing greater interference). Instead, these adjacent DSBs are repaired as non-crossovers (NCOs). The amount of DSBs may vary from cell to cell in meiosis: one with abundant DSBs (top diagram) has more NCOs than one with few DSBs (lower diagram), but the overall level of COs remains constant. Homeostasis is thought to arise from the same mechanism as COI and to occur in other species. (b) A different mechanism of crossover control, crossover invariance, observed in *S. pombe* [6]. *S. pombe* has intense DSB hotspots that are widely space across the genome but a nearly constant level of COs per kb of DNA. In other words, a genetic interval with a DSB hotspot has about the same frequency of COs as one of similar size without a DSB hotspot. At these DSB hotspots, intersister (IS) repair is more frequent than interhomolog (IH) repair, but away from hotspots DSB repair is mostly or all IH. Since IS repair does not yield genetically observable COs, the amount of COs from hotspots is roughly equal to the COs generated away from hotspots, resulting in the observed crossover invariance.

Table 1

Proteins involved in meiotic recombination.

Protein	Function
Bas1, Bas2, Rap1	*S. cerevisiae* transcription factors that regulate meiotic recombination at some loci.
Brc-1	*C. elegans* DSB repair and recombination protein, homologous to mammalian BRCA1.
Ctp1	*S. pombe* protein involved in Rec12-oligonucleotide removal; called Sae2 in *S. cerevisiae.*
Clr4	*S. pombe* histone H3 methyltransferase specific for lysine 9. Methylated histone H3-K9 is associated with transcriptionally repressed (heterochromatic) regions.
Dmc1	Meiosis-specific DNA strand-exchange protein; paralog of Rad51.
Dpy-28	*C. elegans* condensin I complex protein that regulates crossover distribution in meiotic cells.
Exo1	5' to 3' exonuclease and flap endonuclease involved in DSB resection.
Hop1	*S. cerevisiae* protein present in axial elements; localization depends on Red1.
Him-17	*C. elegans* chromatin-associated protein
Hsk1	*S. pombe* protein kinase needed for meiotic DSB formation.
Kle-2	*C. elegans* condensin II complex protein that regulates crossover distribution in meiotic cells.
Mek1	*S. cerevisiae* meiosis-specific protein kinase that functions with Red1 and Hop1.
Mer2	*S. cerevisiae* protein required for DSB formation; a “meiotic break protein” in Figure 1.
MRN	*S. pombe* Mre11, Rad50, Nbs1 proteins involved in DSB repair. Known as MRX in budding yeast where Xrs2 replaces Nbs1.
Pch2	*S. cerevisiae* meiotic checkpoint protein that inhibits chromosome segregation when meiotic recombination is delayed or aberrant.
Rad51	DNA strand-exchange protein required for recombination; ortholog of bacterial RecA.
Rad54	ATP-dependent chromatin remodeling factor that stimulates DNA strand-exchange during recombination.
Rec8	Meiosis-specific subunits of sister chromatid cohesin; *S. pombe* Red 11 is another meiosis-specific subunit.
Rec25	*S. pombe* linear element protein; functions with Rec10 and Rec27.
Red1	*S. cerevisiae* protein component of axial elements and the lateral elements of the synaptonemal complex.
RTEL-1	Human DNA helicase; *C. elegans* homolog has anti-recombination activity.
Set1	*S. cerevisiae* histone H3 methyltransferase specific for lysine 4; mammalian Prdm9 also has a SET domain and methylates histone H3-K4. Methylated histone H3-K4 is mostly associated with actively transcribed (euchromatic) regions.
Sir2	*S. cerevisiae* histone deacetylase
Smc5, Smc6	Structural maintenance of chromosomes proteins; present in a complex important for DNA repair.
Spo11	DNA topoisomerase II-like protein that makes meiotic DSBs; called Rec12 in *S. pombe* and Mei-W68 in Drosophila.
Swi5	Mitotic and meiotic DNA repair protein; an “accessory protein” in Figure 1.
Xnd-1	*C. elegans* chromatin protein that affects crossover distribution
ZMM	*S. cerevisiae* Zip1, 2, 3, 4, Msh4, 5 and Mer3 proteins in the synaptonemal complex.