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Branching out: meiotic recombination and its regulation

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Homologous recombination is a dynamic process by which DNA sequences and strands are exchanged. In meiosis, the reciprocal DNA recombination events called crossovers are central to the generation of genetic diversity in gametes and are required for homolog segregation in most organisms. Recent studies have shed light on how meiotic crossovers and other recombination products form, how their position and number are regulated and how the DNA molecules undergoing recombination are chosen. These studies indicate that the long-dominant, unifying model of recombination proposed by Szostak *et al.* applies, with modification, only to a subset of recombination events. Instead, crossover formation and its control involve multiple pathways, with considerable variation among model organisms. These observations force us to 'branch out' in our thinking about meiotic recombination.

Introduction

Homologous recombination is the process by which DNA loci of nearly identical nucleotide sequences interact and exchange DNA structure and sequence information. Recombination is an aspect of DNA metabolism conserved from viruses to humans and has two major roles. First, recombination is a universally important mechanism for the repair of DNA double-strand breaks (DSBs), which occur frequently because of DNA damage, such as replication-fork breakage. Second, in eukaryotes, recombination is central to meiosis, the process by which diploid cells give rise to haploid gametes, such as eggs, sperm and fungal spores.

A recombination event between two loci can result in one of two different outcomes: crossovers (COs) or non-crossovers (NCOs). In COs, the two strands of each homologous duplex are reciprocally broken and joined, causing exchange of alleles flanking the CO position; in NCOs, no such rearrangement occurs and flanking alleles maintain their original linkage (Figure 1). Both COs and NCOs can be accompanied by localized non-reciprocal 'donation' of sequence information from one homologous locus to another, termed 'gene conversion'.

In meiosis, COs occurring between homologous chromosomes are particularly important because they generate genetic diversity in the gametes and, after fertilization, in the offspring. In most organisms, interhomologue COs are also required for the unique mechanism of parental chromosome segregation during the first meiotic division. The COs link the homologous chromosomes physically so that they can be oriented correctly on the meiotic spindle. In the absence of COs, chromosomes often mis-segregate, resulting in aneuploid gametes and offspring.

Recent studies have advanced our understanding of how meiotic COs and NCOs form, how they are distributed across genomes, and how the pair of DNA molecules undergoing a CO is chosen. In this review, we focus on how advances in these three areas have challenged several core features of long-accepted models, revealing many new branches of the meiotic recombination 'pathway'. Most significantly, the mechanism of recombination associated with the well-known DSB repair model of Szostak *et al.* [1] (hereafter called the Szostak model), long believed to explain all meiotic recombination, applies only to the formation of COs and not to NCOs. A particular theme of recent advances is that a full understanding of recombination can be gained only by synthesizing insights from different model organisms.

The Szostak model of recombination

The modified Szostak model of recombination [1,2] has dominated modern thinking about the mechanism of meiotic recombination. It involves a single pathway of DNA intermediates that produces both COs and NCOs (Figure 1a), consistent with the existence of several mutations that impair both CO and NCO formation (but see later). The Szostak model predicts that recombination is initiated by DNA DSBs. Each DSB produces two duplex ends that are processed to give single-stranded overhangs. Single-end invasion (SEI) then occurs, in which one of the two processed ends of the DSB (the right end in Figure 1a) invades a homologous duplex, giving a displacement-loop (D-loop) structure. Next, the second processed end of the DSB (the left end in Figure 1a) anneals to the D-loop. The resulting joint molecule undergoes repair DNA synthesis to fill in single-strand gaps and is held together by two structures in which strands are exchanged between the interacting duplexes. These structures are called Holliday junctions (HJs) individually, together forming a double Holliday junction (dHJ) structure. Cleavage of pairs of strands in each HJ ('resolution') in one of two orientations (Figure 1a iii), chosen at random, produces COs or NCOs depending on which combination of cleavage orientations ('1' or '2' in Figure 1a iii) occurs at the two HJ sites. Gene conversions arise because of mismatch repair in regions of heteroduplex DNA in the joint molecule and so can be associated with either the CO or NCO outcome, as observed experimentally.

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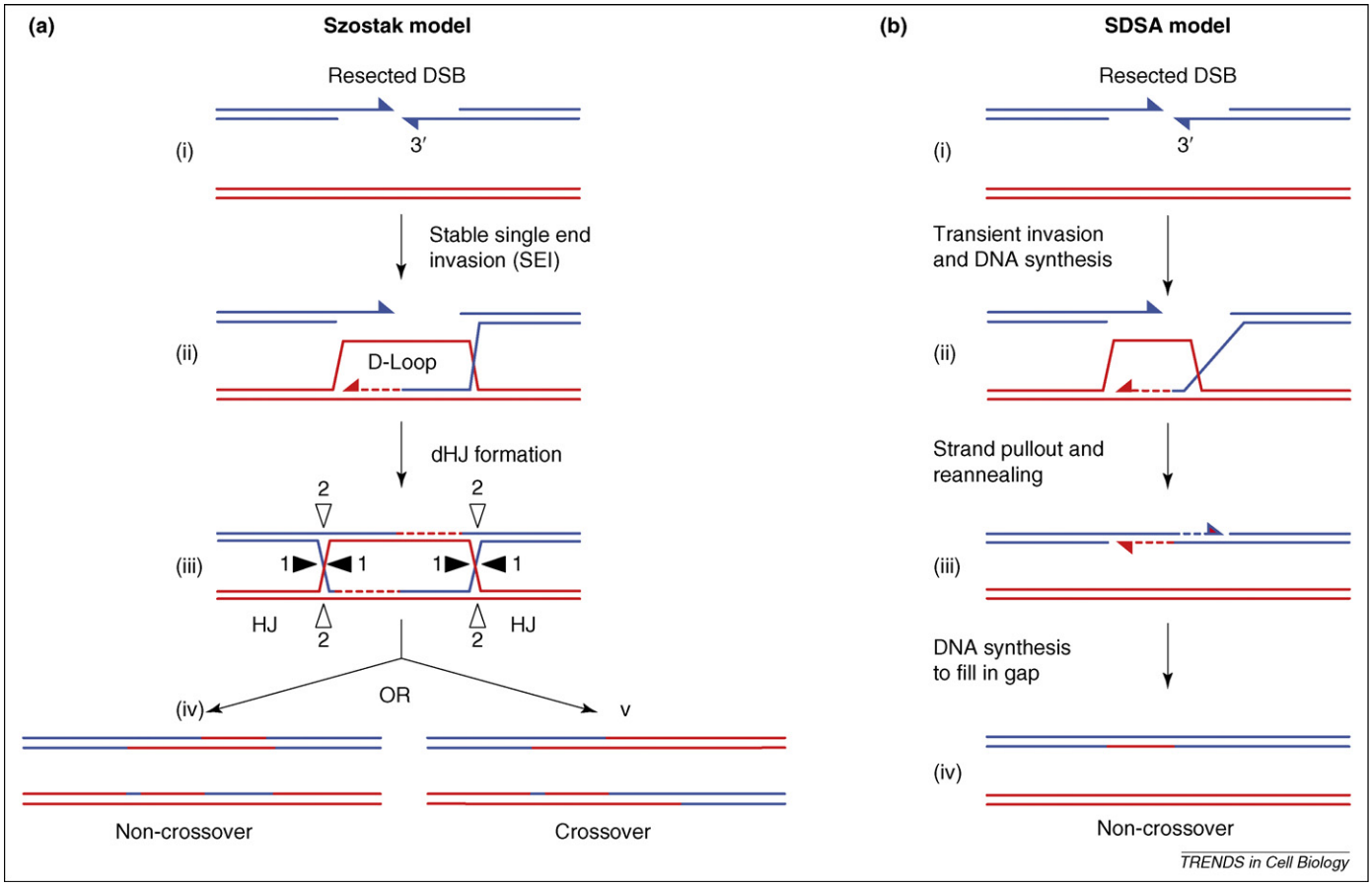

Figure 1. Szostak model of CO and NCO formation and the synthesis-dependent strand-annealing (SDSA) model of NCO formation. **(a)** In the Szostak model, a DSB occurs on one homologous chromosome (blue) and the two duplex ends of this DSB are processed to form single-strand 3′ overhangs (i). One end of the DSB invades another homologous chromosome (red) and sets up a SEI or displacement-loop (D-loop) structure (ii). DNA synthesis extends the D-loop and enables annealing of the second side of the DSB. Further DNA synthesis fills in the remaining gaps and a double HJ structure is formed (iii). Cleavage and re-annealing, or 'resolution', of either pair of like-oriented strands in each HJ ('1' or '2'), occurs at random. In the figure, the leftward HJ has been resolved in orientation '1'; resolution of the rightward HJ in orientation '1' then produces a NCO (iv) and in orientation '2' a CO (v). Note that, in the Szostak model, newly replicated DNA (broken lines) occurs on both homologous chromosomes, terminated at the DSB site on each (iii). **(b)** In the SDSA model, a DSB is formed and processed as in the Szostak model (i). One end of the DSB transiently invades another homologous chromosome and is extended by DNA synthesis (ii). This end then pulls out and the newly synthesized extension anneals with the other end of the DSB (iii). Further repair DNA synthesis occurs and a NCO is formed (iv). No HJ resolution has occurred. Note that, in the SDSA model, newly replicated DNA (broken lines) occurs only on one homologous chromosome and spans the DSB site (iii). In both models, gene conversions can occur by mismatch correction in any region of heteroduplex DNA, shown as duplexes containing one blue and one red strand.

Some predicted intermediates of the Szostak model, such as DSBs and dHJs, have been observed in the budding yeast *Saccharomyces cerevisiae* and DSBs have also been detected in the fission yeast *Schizosaccharomyces pombe* and inferred in mice [2–6]. However, recent work in different model organisms has led to the conclusion that the Szostak model is not a general model for meiotic recombination but applies, with revision, only to CO formation.

COs and NCOs arise through different branches of the recombination pathway

Mutations preventing DSB formation or processing eliminate both COs and NCOs, indicating that early steps in CO and NCO formation proceed by the same pathway. In the Szostak and previous models, the branching of the recombination pathway to produce COs or NCOs was hypothesized to occur at a very late stage. In these models, NCOs and COs both arise from the same HJ-containing intermediate, depending on the orientation of HJ resolution (Figure 1a). An important conclusion of recent studies is that this idea is incorrect and that, instead, the pathway branches soon after DSB formation and proceeds through different DNA intermediates to produce either COs or NCOs [7–10]. These new conclusions are based on the study of mutations affecting steps in recombination occurring after DSB formation and processing and on the study of physical recombination intermediates and products.

One prediction of the Szostak model is that COs and NCOs should occur at the same time. Using physical assays in budding yeast, a study of ectopic recombination events [7] showed that this is not true and that NCOs form before COs. Similarly, repair DNA synthesis associated with NCOs in budding yeast occurs earlier than does that associated with COs [9].

DSBs are precursors to both COs and NCOs, but the other observed intermediates of the Szostak model (D-loops and dHJs) appear to lie only on the CO pathway. In budding yeast, the timing of dHJ appearance and disappearance compared with the timing of CO and NCO formation suggests that dHJs are precursors only of COs [7]. Mutants with reduced SEI or dHJ formation have reduced CO but not NCO frequency [10]. In both fission [11,12] and budding [7] yeasts, mutations causing defects

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in HJ resolution reduce only CO frequency, arguing that COs but not NCOs arise from HJ resolution. Mutations specifically reducing CO but not NCO formation have also been identified in mice [8] and the fruit fly *Drosophila melanogaster* [13]. It therefore appears that the 'decision' to form COs or NCOs is early, after DSB formation but before HJ resolution or even SEI formation. To date, DNA intermediates specific to the NCO pathway have not been reported.

It has been suggested that NCOs occur by synthesis-dependent strand-annealing (SDSA), which is a mechanism involving strand invasion, DNA synthesis and strand 'pullout' that, crucially, does not involve HJs (Figure 1b). A recent study in budding yeast [9] used thymidine-analog incorporation to detect DNA synthesis occurring during meiotic recombination. This study determined the pattern of DNA synthesis at a DSB hotspot using DNA combing, in which individual DNA molecules are extended on a glass coverslip. The combed DNA was probed for thymidine-analog incorporation and position-defining loci and then examined by microscopy. Using this technique, it was observed that the pattern of DNA synthesis associated with COs is that expected from the Szostak model, whereas the pattern of DNA synthesis associated with NCOs is that expected from SDSA (Figure 1). Therefore, it appears that the SDSA model is valid for NCO recombination and the Szostak model, modified to assume strongly biased HJ resolution to COs, is essentially valid for CO recombination.

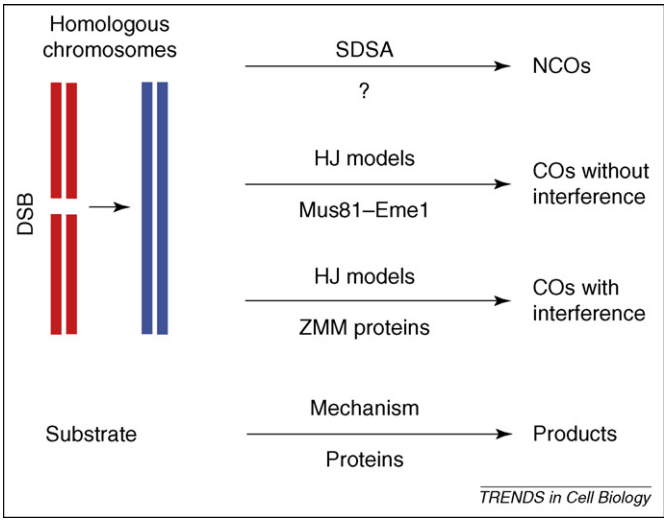

Figure 2. Three different pathways produce COs (with or without interference) and NCOs. All three pathways are initiated by DSBs. NCOs appear to be produced by the SDSA mechanism but the proteins specifically involved in this pathway are unknown. Work from budding yeast indicates that COs with interference are produced by the Szostak mechanism, progressing through dHJs and specifically using the ZMM proteins, including Msh4–Msh5. Work from fission yeast and budding yeast indicates that COs without interference are produced by a mechanism that might involve sHJs and specifically requires the Mus81–Eme1 proteins. This mechanism might be essentially the same as the Szostak model, modified to produce sHJs.

There is more than one pathway to crossing-over

As well as COs and NCOs arising by separate pathways, it is also becoming clear that there are at least two different pathways to forming COs, at least in budding yeast. One pathway depends on the Msh4–Msh5 complex and is subject to a form of spatial CO regulation called interference (discussed later), in which one CO prevents additional COs from occurring nearby. The other pathway depends on the Mus81–Eme1 complex and is not subject to interference. Both pathways, as well as the pathway to forming NCOs, are initiated by DSBs (Figure 2). Comparison of experimental results from different model organisms has been particularly important in deciphering this story.

In fission yeast, meiotic HJ resolution appears to be carried out solely by the Mus81–Eme1 endonuclease complex [11,12,14,15]. Mus81 and Eme1 (called Mms4 in budding yeast) are widely conserved and *in vitro* analysis of complexes from fission yeast, budding yeast and humans indicates that they all have HJ resolution activity [14,16–19]. However, *mus81*$^{-/-}$ mice are viable and fertile [20] and the effect of a *mus81* mutation in budding yeast is only a mild reduction in CO frequency [21].

The explanation for these phenotypic differences is that fission yeast and some other eukaryotes, including fruit flies [22], lack an alternative CO pathway that appears to be active in mammals and budding yeast. This pathway depends on a group of proteins called 'ZMM proteins' (see later) that include Msh4 and Msh5, so that, in budding yeast, mutations affecting both the Mus81 and Msh4–Msh5 pathways have a more severe reduction in CO frequency than do the individual mutations [21,23]. Msh4 and Msh5 are homologues of the bacterial MutS mismatch repair protein but, instead of acting in mismatch repair, they form a sliding clamp that recognizes HJs specifically [24]. Msh4 and Msh5 are apparently absent from fission yeast and fruit flies.

It appears that the Msh4–Msh5, but not the Mus81–Eme1, pathway is subject to CO interference. This affects the pattern of interference seen in each organism, depending on whether the organism has both pathways or only one (Figure 2). In the worm *Caenorhabditis elegans*, only the Msh4–Msh5 pathway appears to be active and COs are subject to exceptionally strong interference [21,25–27] (see later). By contrast, in fission yeast, where only the Mu81–Eme1 pathway is present, there is no interference [28]. In budding yeast, the Mus81–Eme1-dependent COs lack interference, whereas those dependent on Msh4–Msh5 are subject to interference [21]. Plants and mammals appear to have the proteins required for both CO pathways and, consistent with this, appear to have both interference-sensitive and -insensitive classes of COs [8,29–33]. In organisms in which both pathways are present, their relative importance can vary. Mutations affecting either pathway in budding yeast have similar, moderate defects in recombination [21,23] but *mus81*$^{-/-}$ mice are fertile [20], whereas *msh4*$^{-/-}$ or *msh5*$^{-/-}$ mice are sterile [34,35].

Single and double Holliday junctions

What is the relationship between the two CO pathways and the Szostak pathway of DNA intermediates? Both CO pathways are initiated by DSBs and two-dimensional gel electrophoretic studies in budding yeast, in which both CO pathways operate, identified the SEI and dHJ intermediates predicted by the Szostak model [3,36]. Budding yeast dHJs have also been seen by electron microscopy [15,37].

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By contrast, electron microscopic and two-dimensional gel electrophoretic studies in fission yeast, in which only the Mus81 pathway is active, observed mostly single HJs (sHJs), recombination intermediates not predicted by the Szostak model, and only a minority of dHJs [15]. The small number of fission yeast dHJs might represent closely spaced sHJs because CO interference is not active in that organism. Interestingly, a sizable minority of sHJs is also seen by electron microscopy in budding yeast. It is therefore possible that, although the two CO pathways are both initiated by DSBs, they might proceed through different DNA intermediates: sHJs for the Mus81 pathway and dHJs for the Msh4–Msh5 pathway (Figure 2). A sHJ might arise by cutting of the D-loop (Figure 1aii) before, rather than after, capture of the second end of the DSB. Similar proposals, that is, that Mus81–Eme1 acts on structures different to the dHJs of the Msh4–Msh5 pathway, specifically unligated or partial HJs, have been advanced based on the *in vitro* activities of the Mus81–Eme1 complex [11].

Regulation of CO number and position

CO control, that is, the regulation of CO position and number, is specified at several different levels. Most fundamentally, the DSBs that initiate recombination are distributed non-randomly. In many organisms, there is evidence that DSBs are concentrated at particular sites called hotspots [38]. In recent years, several studies have examined the genome-wide distribution of meiotic DSBs in budding yeast using Spo11-based chromatin immunoprecipitation (ChIP) and microarray analysis, a technique termed ChIP–chip. In these studies microarrays with a large number of oligonucleotide probes, giving genome-wide coverage, are used to determine which loci are enriched specifically during meiosis by ChIP of Spo11. Spo11 is the topoisomerase-like active-site protein that generates meiotic DSBs and, hence, the ChIP-enriched loci are inferred to be the sites at which DSBs have occurred. Such genome-wide studies in budding yeast indicate that DSB hotspots tend to occur in promoter regions and are somewhat suppressed in centromeric and telomeric domains [39–42]. This confirms previous conclusions drawn from Southern blot analysis of selected regions of the budding yeast genome [43]. By contrast, Southern blot and ChIP analyses of the fission yeast genome indicate that DSB sites are separated more widely [44,45].

COs and NCOs both arise from DSBs and the choice of which DSBs generate COs provides an additional level of CO control. In most, but not all, organisms, meiotic COs are distributed highly non-randomly. The total number of COs is low but even small chromosomes receive at least one. Two, possibly linked, effects contribute to this distribution. First, an obligate CO per chromosome occurs and, second, a gradient of suppression around each CO decreases the probability of additional COs but not DSBs or NCOs. The suppressive effect is termed CO interference (Figure 3).

For some years, it seemed likely that CO interference was mediated by the synaptonemal complex (SC). The only organisms known to lack interference, the fungi *S. pombe* and *Aspergillus nidulans*, also lack SCs, and a budding yeast mutation affecting Zip1, an SC component, abolishes both the SC and interference [46]. The SC is a proteinaceous structure that, in most organisms, links the cores of paired homologous chromosomes during meiosis I [47]. It consists of two lateral elements, one from each homologous chromosome, held together by a central element (Figure 3). Each lateral element is a proteinaceous structure forming a single core or axis encompassing both sister chromatids of one homologous chromosome. Before formation of the mature SC, lateral elements are called axial elements.

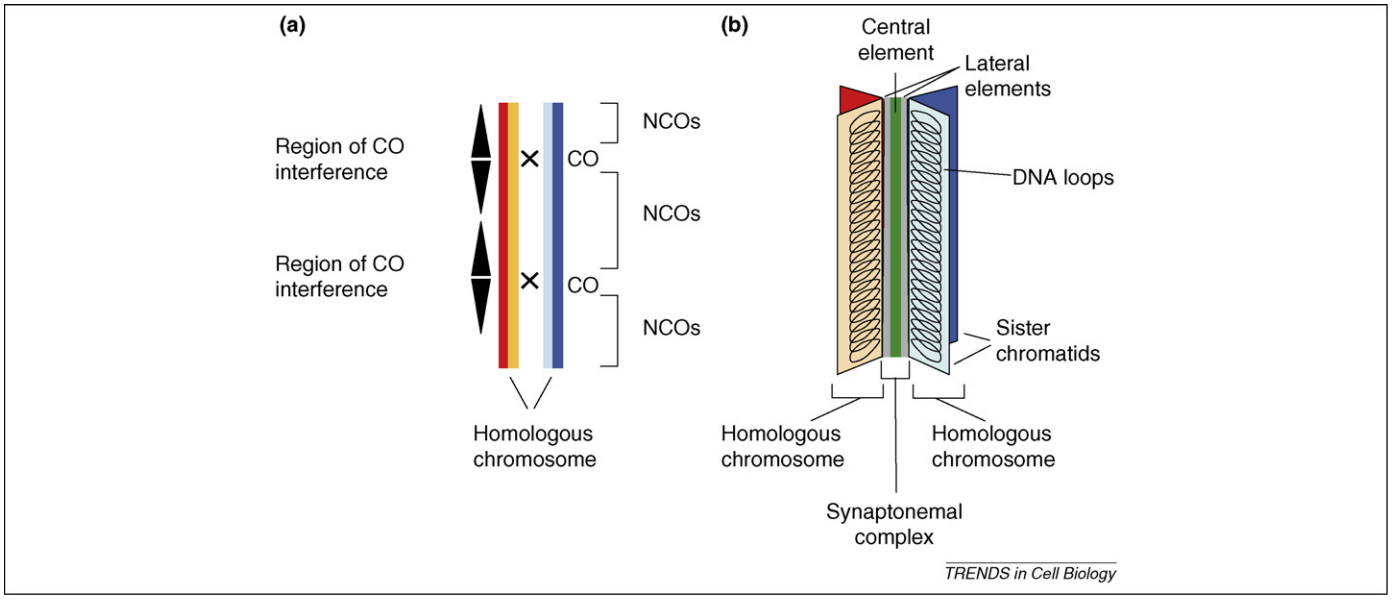

Figure 3. CO interference and the synaptonemal complex. **(a)** CO interference. Recombination occurs between homologous chromosomes, each consisting of two sister chromatids, as shown. A zone of interference (black elongated triangles) is centered at positions where DSBs are processed to give COs. In this zone, further COs are suppressed to varying degrees, with DSBs instead being processed to give more NCOs. The interference effect is strongest at the site of the CO and declines with distance. **(b)** Structure of meiotic chromosomes held together by the synaptonemal complex. Each chromosome, consisting of two sister chromatids, forms a single axis. The axial elements are held together by a central element in the mature SC, forming a tripartite proteinaceous structure, which joins the two homologous chromosomes together. In this structure, the descendents of the axial elements are called lateral elements. Loops of DNA are shown emerging from these lateral elements for two of the four chromatids (DNA loops in the other two are not shown).

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Recent research indicates that the pattern of interference is actually set up before the SC is formed. The Zip2 and Zip3 proteins, possibly components of a ubiquitin-conjugating complex [48], are members of the group of 'ZMM' proteins that are required specifically to produce COs that are subject to interference [10]. Msh4–Msh5 is a member of this group and colocalizes with Zip2–Zip3 [49]. Budding yeast Zip2–Zip3 foci appear to mark the sites where interference-sensitive COs will occur. These foci have a relative frequency and display a degree of interference matching those of the COs, even when the number of COs is increased or decreased by appropriate mutations [50–52]. Mouse Msh4–Msh5 foci also show interference [53]. Because Zip2–Zip3 foci are also the sites at which SC formation is nucleated [54,55], this indicates that at least this aspect of interference precedes formation of the SC. Instead, it appears that the SC is initiated at the sites of COs subject to interference.

If not through the SC, how is interference mediated? Recent evidence from the worm *C. elegans* suggests that the physical structure of the chromosomal axis can propagate interference. In *C. elegans*, only the Msh4–Msh5 pathway is active and an extreme form of interference ensures that essentially every chromosome has one and only one CO during meiosis [26]. However, when two or even three chromosomes are fused end-to-end, the resulting fusions, rather than experiencing two or three meiotic COs, respectively, still usually experience one and only one [27] (Figure 4). It appears that *C. elegans* CO control acts on chromosomes as a unit, independent of length, to ensure that each receives only one CO.

Continuity of chromosome axes is essential to the *C. elegans* mechanism of interference. When fused chromosomes undergo recombination with two unfused chromosomes, so that one of the two 'homologues' is 'broken' and therefore does not have a continuous axis, the number of COs increases (Figure 4). In addition, a mutation limiting the level of the meiosis-specific chromosome-axis component Him3 increases the frequency of chromosome pairs that experience double COs [56]. A current model suggests that stress forces in the axes of meiotic chromosomes promote meiotic COs, which, in turn, relieve axial stress locally, suppressing further COs, that is, generating interference [57]. However, how this would account for other aspects of interference, such as that of Zip2–Zip3 foci, is unclear.

Recently, an entirely new level of CO regulation in budding yeast has been reported: a phenomenon termed 'crossover homeostasis'. This work was carried out using hypomorphic alleles of Spo11, which reduce the DSB frequency to varying degrees. As the DSB frequency is reduced, the frequency of COs does not fall in tandem; rather, CO numbers are maintained and NCOs are reduced preferentially instead [58]. This supports models in which a fixed number of COs are derived from the 'pool' of all DSBs, with the remainder of the DSBs producing NCOs [57,58]. Whether CO homeostasis exists in organisms other than budding yeast has not yet been determined.

Several different non-random distributions can be observed in meiosis. These include the positions of DSBs, which subset of DSBs form COs, the number and locations of COs, and the positioning of protein complexes, such as Zip2–Zip3. Unifying models that explain multiple non-random distributions, seen across a range of model organisms, in terms of one or a small number of conserved, underlying processes are attractive [57]. However, it is also possible that more than one fundamental mechanism is involved and, hence, there might be more than one type of interference acting during meiosis. Fundamental

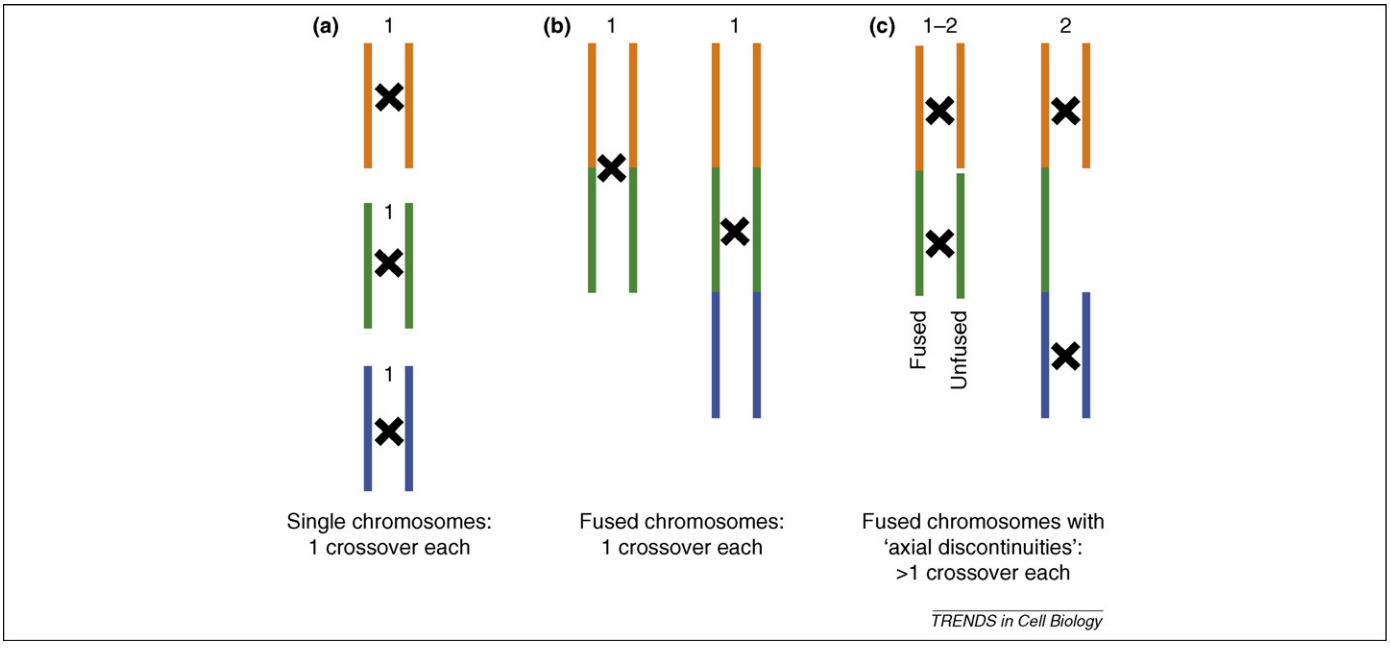

Figure 4. Intact chromosome axes are required for transmission of CO interference in the worm *C. elegans*. Each pair of homologous chromosomes usually undergoes one and only one CO per meiosis **(a)**. When two, or even three, chromosomes are fused, the resulting large chromosomes still usually undergo one and only one crossover per pair during meiosis **(b)**. This indicates that, for the purposes of interference, intact chromosomes are treated as a single unit, irrespective of size. By contrast, if a fusion of two or three chromosomes undergoes recombination with two unfused homologous chromosomes, more than one CO per meiosis occurs. This is equivalent to recombination between two fused chromosomes but where one has an axial discontinuity **(c)**. Therefore, an intact chromosome axis is necessary for transmission of interference [27].

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mechanistic differences might also exist among species. For example, in mice, protein complexes involved mostly in NCO or mostly in CO formation both show interference, of different strengths [53]. In addition, the protein complexes associated with the sites of COs in mice show interference in the absence of intact axial elements, as well as in the absence of intact SC [59].

Choice of partner-DNA molecule for crossing-over

An important function of meiotic recombination is the generation of COs between homologous chromosomes. However, when a DSB occurs, it does not have to be repaired against the homologous chromosome: recombination with the sister chromatid can also occur (Figure 5). In addition, if homology exists elsewhere in the genome, ectopic recombination with a non-allelic locus can take place. What determines the choice of partner in meiotic recombination?

Partner choice in meiotic recombination can be studied at DSB hotspots using homologous chromosomes with different patterns of restriction sites at a hotspot locus. This enables intersister recombination intermediates to be distinguished from interhomologue recombination intermediates. Such physical studies of DNA intermediates at a budding yeast meiotic recombination hotspot indicated that interhomologue recombination is greatly favored over intersister recombination, in contrast to the general bias for intersister events seen during mitosis [60]. Because this makes sense in terms of promoting productive interhomologue events, it was presumed to be a universal feature of meiosis. However, recent research in fission yeast has disproved the universality of this conclusion: intersister recombination is favored over interhomologue recombination at a meiotic recombination hotspot [15].

Mechanistically, the difference in partner choice between budding and fission yeast appears to be explained by the presence of a barrier to intersister recombination that is present in the former but not in the latter (Figure 5). This is seen most clearly when comparing the behavior of mutations affecting Dmc1, a meiosis-specific strand-exchange protein. In *dmc1* mutants of both yeasts, interhomologue recombination is reduced. However, in fission yeast, essentially every DSB is still repaired, presumably against a sister chromatid [61], whereas, in budding yeast, DSBs remain unrepaired, indicating the existence of a barrier to intersister repair [60]. Like fission yeast, plants appear to lack the intersister recombination barrier seen in budding yeast [62]. The nature of the budding yeast barrier is unclear but involves the Mek1 protein kinase and the Hop1 and Red1 axial-element components. Mutation of *mek1* or *red1* removes the barrier to intersister recombination in a budding yeast *dmc1* mutant [63–65].

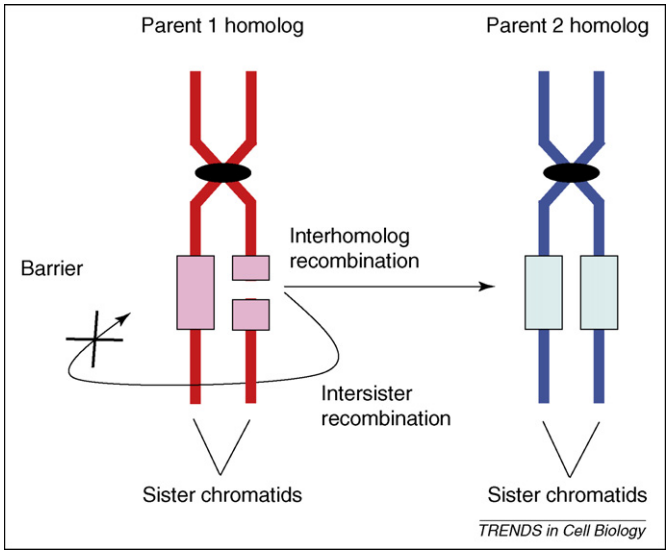

Figure 5. Partner choice during meiotic recombination. Recombination initiated by a DSB on one chromatid can occur with the homologous locus on the other (sister) chromatid of the same chromosome or with the homologous loci on either chromatid of the other homologous chromosome. In budding yeast, a strong bias to interhomologue recombination is seen in meiosis [60] but, by contrast, in fission yeast, a bias to intersister recombination is seen [15]. The bias to interhomologue events in budding yeast seems to be the result of a barrier to intersister recombination that is absent from fission yeast.

In addition to the intersister recombination barrier in budding yeast, interhomologue recombination might be upregulated specifically by the Hed1 protein in that organism. This protein promotes the use of Dmc1 by suppressing recombination carried out solely by the constitutively expressed Rad51 strand-exchange protein [66]. Hed1 has not yet been characterized in other model organisms.

As mentioned earlier, meiotic recombination can occur between homologous DNA sequences present at different places on the genome (ectopic recombination), as well as between the same locus on sister chromatids or homologous chromosomes (allelic recombination). Generally, ectopic events occur less frequently than allelic events, although, in budding yeast, some ectopic recombination rates approach allelic frequencies [67–69].

In general, budding yeast appears to have more forms of crossover control, such as interference and the barrier to intersister recombination, than does fission yeast. Why are these forms of regulation necessary when meiosis in fission yeast occurs successfully without them? CO control appears to exist to limit the total amount of recombination, implying that COs can be deleterious if occurring too frequently. Fission yeast, with an unusually small number of relatively large chromosomes, needs fewer total COs, in the absence of CO control, to ensure at least one CO per chromosome per meiosis. This number is presumably low enough to avoid deleterious effects and, hence, the necessity for CO control. However, these issues are far from settled and remain topics of debate.

Concluding remarks

Recent research has greatly expanded our understanding of how meiotic COs form, how their distribution and number are regulated and how CO partner choice is made. Novel pathways of CO and NCO formation have been identified, along with new forms of CO regulation. In particular, the single Szostak model for CO and NCO formation has been replaced by at least three branches leading from an initiating DSB to produce NCOs or interfering or non-interfering COs. The fuller picture now emerging highlights the mechanistic differences between meiotic recombination in different organisms and indicates that the idea of a single pathway of meiotic recombination is obsolete. Future studies will concentrate on exploring the new pathways that recent research has revealed and

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understanding the contrasting meiotic recombination mechanisms and their control in different organisms.

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