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REVIEW

SUBJECT COLLECTION: EXPLORING THE NUCLEUS

Let's get physical – mechanisms of crossover interference

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ABSTRACT

The formation of crossovers between homologous chromosomes is key to sexual reproduction. In most species, crossovers are spaced further apart than would be expected if they formed independently, a phenomenon termed crossover interference. Despite more than a century of study, the molecular mechanisms implementing crossover interference remain a subject of active debate. Recent findings of how signaling proteins control the formation of crossovers and about the interchromosomal interface in which crossovers form offer new insights into this process. In this Review, we present a cell biological and biophysical perspective on crossover interference, summarizing the evidence that links interference to the spatial, dynamic, mechanical and molecular properties of meiotic chromosomes. We synthesize this physical understanding in the context of prevailing mechanistic models that aim to explain how crossover interference is implemented.

KEY WORDS: Chromosomes, Meiosis, Phase separation, Crossover interference

Introduction

Meiosis forms haploid gametes from diploid germline cells, an essential step in sexual reproduction. Although the meiotic program has much in common with mitotic division, it differs in two main respects: meiosis results in a reduction of chromosome number, and it is non-conservative. In virtually all organisms, meiosis yields genetically recombinant gametes by exchanging information between the two parental homologous chromosomes (homologs). This exchange is performed by the crossover repair pathway, which breaks then joins homologs together before the first meiotic division (Fig. 1A) (Gray and Cohen, 2016; Hunter, 2015). In addition to generating genetic diversity, the mechanical linkages formed by crossovers are necessary to correctly partition chromosomes between gametes. In humans, errors in chromosome segregation are the leading cause of infertility, pregnancy loss and conditions such as Down and Edwards syndromes (Gruhn et al., 2019; Wang et al., 2017).

While crossovers are formed at random locations in any given meiosis, the number of crossovers is tightly regulated to ensure healthy progeny. For instance, because crossovers are required for reliable chromosome segregation at meiotic anaphase I, multiple mechanisms assure the presence of at least one 'obligate' crossover on each chromosome. Furthermore, inhibitory mechanisms limit excessive crossovers, resulting in a tight distribution of total crossover number (Cole et al., 2012; Zickler and Kleckner, 2015). Here, we focus on an intriguing form of regulation called crossover interference, in which closely spaced crossovers are conspicuously underrepresented, and hence the remaining crossovers are separated by larger intervals than would be expected for independent events (Koszul et al., 2012). Crossover interference can be incredibly robust and exert inhibition over whole chromosomes. For instance, the roundworm *Caenorhabditis elegans* undergoes exactly one crossover per chromosome in wild-type animals, and only sometimes achieves two (well-separated) crossovers in 35 Mb fusion chromosomes (Hillers and Villeneuve, 2003; Libuda et al., 2013; Yokoo et al., 2012). This spatial anticorrelation of crossovers, first observed more than a century ago (Muller, 1916; Sturtevant, 1913), has since been characterized and quantified in a wide variety of organisms and appears to be a ubiquitous feature of meiosis.

Failure to form the obligate crossover results in chromosome mis-segregation and aneuploidy, and the implications of these events on reproductive health are well documented (Gruhn et al., 2019; Wang et al., 2017). Crossovers are also necessary to allow purifying selection of linked alleles (McDonald et al., 2016; Otto and Payseur, 2019). However, the functional and evolutionary advantages of crossover interference per se are not fully obvious. Crossover formation entails a risk of genome rearrangement, suggesting that crossovers carry a reproductive cost that would limit their overall number. In addition, nearby crossovers have been postulated to impair chromosome segregation, and while evidence for this idea is emerging (Hollis et al., 2020; Oh et al., 2007), much work remains to be done to confirm this hypothesis. Alternatively, the answer to the 'why' of interference's robustness might be that it is integral to how crossovers are formed, or to the surveillance system that ensures formation of the obligate crossover (Chen et al., 2008). Given these possible interrelationships, working to understand the molecular mechanism by which crossover interference is implemented promises to improve our understanding of the meiotic nucleus as a whole.

In this Review, we contextualize crossover interference in our current understanding of meiotic progression, chromosome structure and DNA repair, building on earlier comprehensive reviews of these topics (Crickard and Greene, 2018; Gray and Cohen, 2016; Page and Hawley, 2004; Pâques and Haber, 1999; Zickler and Kleckner, 1999). We start by defining the environment in which crossovers form, highlighting the role a phase-separated interface between the homologs plays in the regulation of crossovers. We then briefly survey how pathway choice during DNA repair controls crossover formation, with particular attention to how the reversibility of some repair intermediates supports crossover interference. We conclude by discussing the commonalities and differences between mechanistic models that have been proposed to implement crossover interference. We focus on insights obtained using cell biological and biophysical approaches, and on the interplay between nucleus-wide and per-chromosome regulation of crossovers.

Anatomy of the interhomolog environment

Chromosomes in the prolonged prophase that precedes the meiotic divisions are defined by the interactions between three constituents (Zickler and Kleckner, 1999) (Fig. 1A,B): the chromatin of each homolog, the axis (or axial elements) of the synaptonemal complex (SC) and the central region (CR) of the SC. Although the axis and

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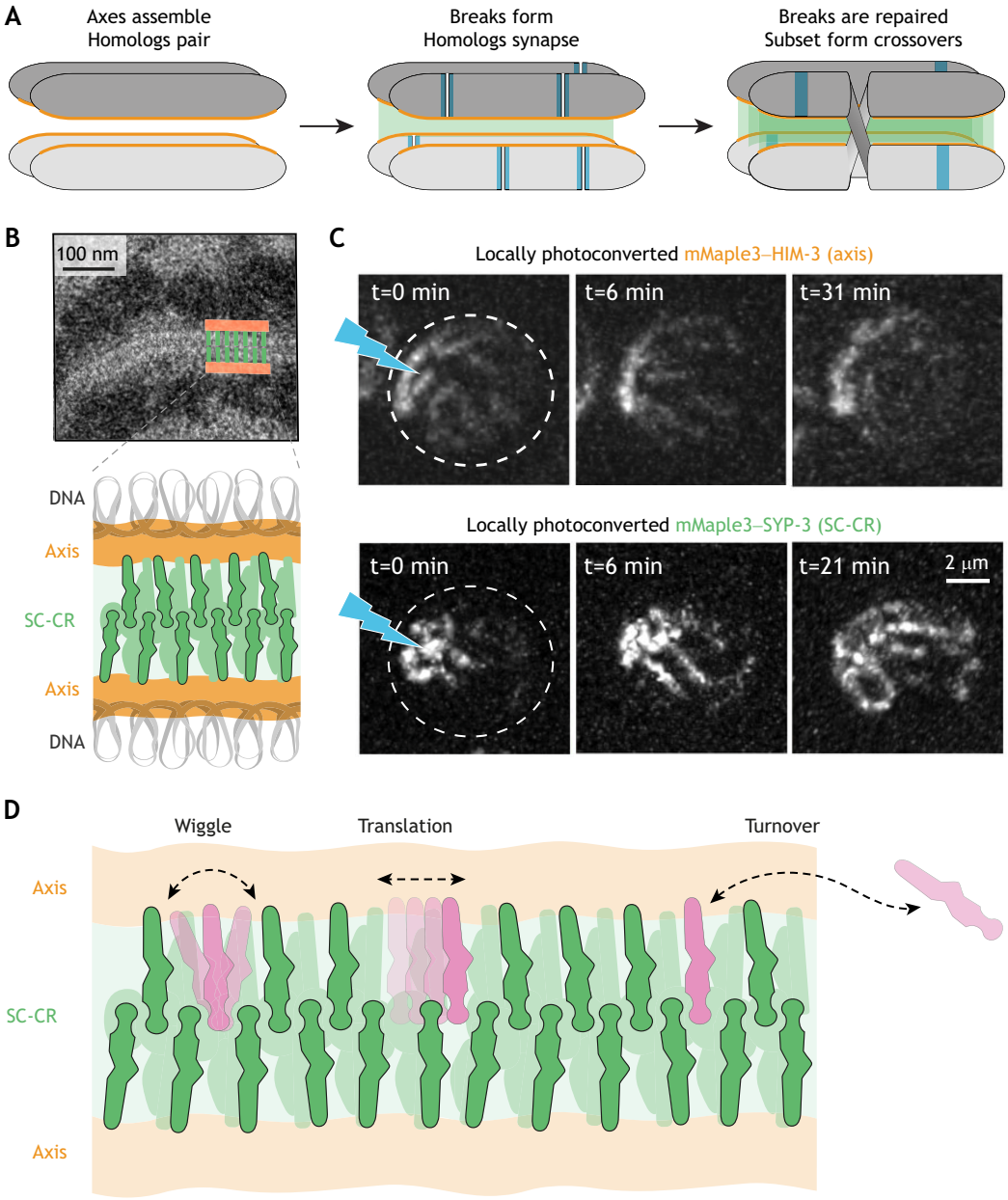

Fig. 1. The chromosomal environment during crossover formation. (A) Left: the axis (orange) assembles onto the parental chromosomes (dark and light gray pairs of sister chromatids). Middle: the homologs are brought into close physical proximity by the SC-CR (teal), and breaks (blue) are induced at random positions. Right: a tightly regulated subset of these breaks form crossovers between homologous chromatids, while all other breaks are repaired without forming crossovers. (B) Top: electron micrograph of *C. elegans* chromosomes during meiosis, with ladder-like structure of the SC-CR superimposed. Bottom: schematic of chromatin (gray), axis (orange) and SC-CR (teal). (C) Dynamics of the axis (HIM-3, top) and the SC-CR (SYP-3, bottom) as detected by photoconversion of mMaple3 fluorescent protein fusions in live *C. elegans* worms during meiosis. A local pool of fluorescent protein within the nucleus (dashed line) is photoconverted with a spatially defined pulse of UV light (blue lightning bolt). The axis protein HIM-3 does not significantly redistribute, whereas the SC-CR protein SYP-3 does, indicating the liquid-like nature of the SC-CR. (D) Schematic illustration of possible liquid-like dynamics within the SC-CR. At the smallest scale, individual proteins may rearrange locally ('wiggle'). As implied by the data shown in C, at larger scales, proteins might also exhibit translational motion within the SC-CR and/or turnover (i.e. exchange with the nucleoplasm). Panels B and C adapted from Rog et al. (2017), where they were published under a CC BY 4.0 license.

CR of the SC have historically been considered part of the same structure, they assemble at different stages of meiosis and play distinct functional roles in crossover formation. To clearly differentiate them, we refer to them as the axis and SC-CR, respectively.

Upon meiotic entry, the axis assembles onto chromatin and transforms each chromosome from a globular mass into an array of loops, each tens to hundreds of kilobases in size, by binding each loop at its base and stacking those binding sites into a linear array (Borde and de Massy, 2013; Zickler and Kleckner, 1999). The packaging of chromatin into similarly-sized loops ensures a broad agreement between the genetic and physical location of crossovers, such that crossovers between genetic markers that are far apart are also spaced apart along the axis (Zickler and Kleckner, 2015). The axis plays a structural or regulatory role in almost all aspects of meiosis, including the regulation of meiotic progression and multiple steps in crossover formation (Zickler and Kleckner, 1999). The protein components that make up the axis have

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conserved functions between between organisms and typically include one or more proteins containing a HORMA domain (present in a variety of chromosome-associated proteins, for example, HIM-3 in worms), as well as meiosis-specific variants of the cohesin complex (Láscarez-Lagunas et al., 2020; Page and Hawley, 2004).

The SC-CR forms the interface that assembles between the axes of the two homologs in meiotic prophase and extends localized pairing interactions to bring homologs in close juxtaposition and align them from end to end. The SC-CR is present in all but a few eukaryotes and exhibits a conserved ultrastructure, resembling a ladder or train tracks in electron micrographs, that spans 100–150 nm between the homologs, with regularly spaced 'rungs' every 10–20 nm (Zickler and Kleckner, 1999). The SC-CR is generally composed of several proteins in each organism, for example SYP-1 through SYP-6 in worms (Láscarez-Lagunas et al., 2020). SC-CR proteins of different organisms exhibit little primary sequence conservation, but tend to harbor extensive domains predicted to form coiled coils (Cahoon and Hawley, 2016; Page and Hawley, 2004). In addition, SC-CR proteins have been mostly refractory to *in vitro* structural analysis. However, immuno-electron microscopy and super-resolution microscopy studies have demonstrated that each SC-CR component is present in a specific location and orientation relative to the axes (Anderson et al., 2005; Dong and Roeder, 2000; Köhler et al., 2020 preprint; Schild-Prüfert et al., 2011; Schmekel and Daneholt, 1995; Schücker et al., 2015).

The functions of the SC-CR in homolog alignment, and its appearance as an ordered ladder, spawned a longstanding analogy to a zipper, and with it the implication that the SC-CR is rigid and mostly static. Yet early evidence also indicated that the SC-CR exhibits more plasticity than typical structural elements. For example, the SC-CR undergoes post-assembly remodeling (synaptic adjustment) after initial assembly onto chromosomes (Zickler and Kleckner, 1999); this is particularly dramatic for chromosomes carrying rearrangements, such as inversions or translocations (Henzel et al., 2011). More recent studies have shown that the SC-CR is a laminar phase-separated compartment with liquid-like characteristics, in which subunits are internally rearranged and added (Fig. 1C,D; Box 1) (Nadarajan et al., 2017; Pattabiraman et al., 2017; Rog et al., 2017; Voelkel-Meiman et al., 2012). The molecular interactions that underlie the liquid dynamics of the SC-CR are not known; however, the SC-CR in yeast, worms and flies is dissolved by aliphatic alcohols (Rog et al., 2017), suggesting a significant role for hydrophobic interactions. In addition, SC-CR proteins from distant phyla include intrinsically disordered regions that contain abundant charged residues, suggesting that they might also play a role in driving liquid-like behaviors (Gao and Colaiácovo, 2018; Zhang et al., 2020). Although liquid-like behavior may be a universal feature of the SC, its functional significance has not been demonstrated. As explored below, however, certain emergent behaviors of phase-separated compartments – such as recruitment and local concentration of interacting proteins – are perfectly suited to regulate crossovers.

Box 1. Biomolecular phase separation

Under certain conditions, macromolecules (for example, proteins or RNA) can phase separate out of solution while maintaining liquid-like organization (Alberti, 2017; Banani et al., 2017; Shin and Brangwynne, 2017). The distinct compartments formed by this process are known as membraneless organelles or biomolecular condensates, and include the nucleolus, centrosomes and at least some DNA-damage foci. Common hallmarks of condensates include having a deformable shape, being composed of multiple molecular species without fixed stoichiometry, and exhibiting dynamic turnover of components between the condensate and its surroundings. Phase-separated liquids at equilibrium tend towards forming a single large droplet (a process termed Ostwald ripening), but they can be regulated to form a defined number of structures.

Typically, condensates are experimentally shown to exhibit signatures of phase separation both *in vivo* and by using *in vitro* reconstitution, which allows for the precise measurement of biophysical parameters (Alberti et al., 2019). Although it has been difficult to reconstitute the SC-CR *in vitro*, *in vivo* evidence, such as the dynamic rearrangement of SC-CR subunits (Fig. 1C,D) (Nadarajan et al., 2017; Pattabiraman et al., 2017; Rog et al., 2017) and the ability of chromosome-free assemblies of SC-CR subunits to deform and fuse (Hughes and Hawley, 2020; Rog et al., 2017), are compelling evidence that the SC-CR is indeed a liquid condensate. The biophysical properties of phase-separated liquids, combined with the SC-CR-mediated formation of an interhomolog interface, may allow SC-CR-localized signaling molecules to efficiently regulate the assembly of repair sites along chromosomes.

While most liquids are isotropic, some liquids have a large degree of internal order. Perhaps the most well-characterized are lipid membranes, which exhibit long-range orientational order, making them phase-separated liquid crystals (Levental and Veatch, 2016). The SC-CR's liquid properties and its organized appearance in electron micrographs (Rog et al., 2017) (Fig. 1B) suggest it is also a liquid crystal. Liquid crystals are known to change state in response to external stimuli (Ruff et al., 2018; Yoo et al., 2019), a property used in technological applications such as liquid-crystal displays that respond to changes in electrical current. Although state changes of the SC-CR are yet to be observed, their quick propagation over large distances would supply an appealing model for conveying information along meiotic chromosomes.

Biochemistry and cell biology of crossover formation

Whereas meiosis varies in different organisms, including in the number of crossovers and the strength of crossover interference, the biochemical steps of DNA repair and the enzymes responsible are broadly conserved, as determined by analysis of repair outcomes in a broad array of experimental systems (including fungi, such as *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe* and *Sordaria macrospora*; the nematode *C. elegans*; *Drosophila melanogaster* flies; mice; and *Arabidopsis thaliana* plants), as well as by physical assays in yeast, which have defined the identities of many DNA repair intermediates (Crickard and Greene, 2018; de Massy, 2013; Page and Hawley, 2004; Pâques and Haber, 1999; Szostak et al., 1983). Thus, despite other variations in the meiotic program between organisms, crossover interference can be understood using a shared framework: whether and how specific repair intermediates are allowed to give rise to crossovers.

In brief, meiosis begins with programmed double-strand DNA breaks (which we refer to here as 'breaks'), followed by competition between several repair pathways (Fig. 2, middle). These breaks can, in principle, be 'pasted' back together by nonhomologous end joining or repaired by single-strand annealing. However, in meiosis, breaks are generally repaired by relying on a homologous repair template, and nonhomologous pathways are mostly inactive (Joyce et al., 2012; Lemmens et al., 2013; Macaisne et al., 2018). Homologous repair pathways initiate with resection of DNA on each side of the break to expose a single strand capable of interacting with a repair template (Cao et al., 1990; Mimitou et al., 2017; Sun et al., 1989; Symington, 2014; White and Haber, 1990). Recombinases then catalyze the invasion of one of the free DNA strands into the homologous template (Hunter and Kleckner, 2001) (Fig. 2; Box 2). Capture of the exposed template strand by the other end of the break results in a double Holliday junction (dHJ). Resolution of the dHJ can create crossover products that exchange flanking sequences and

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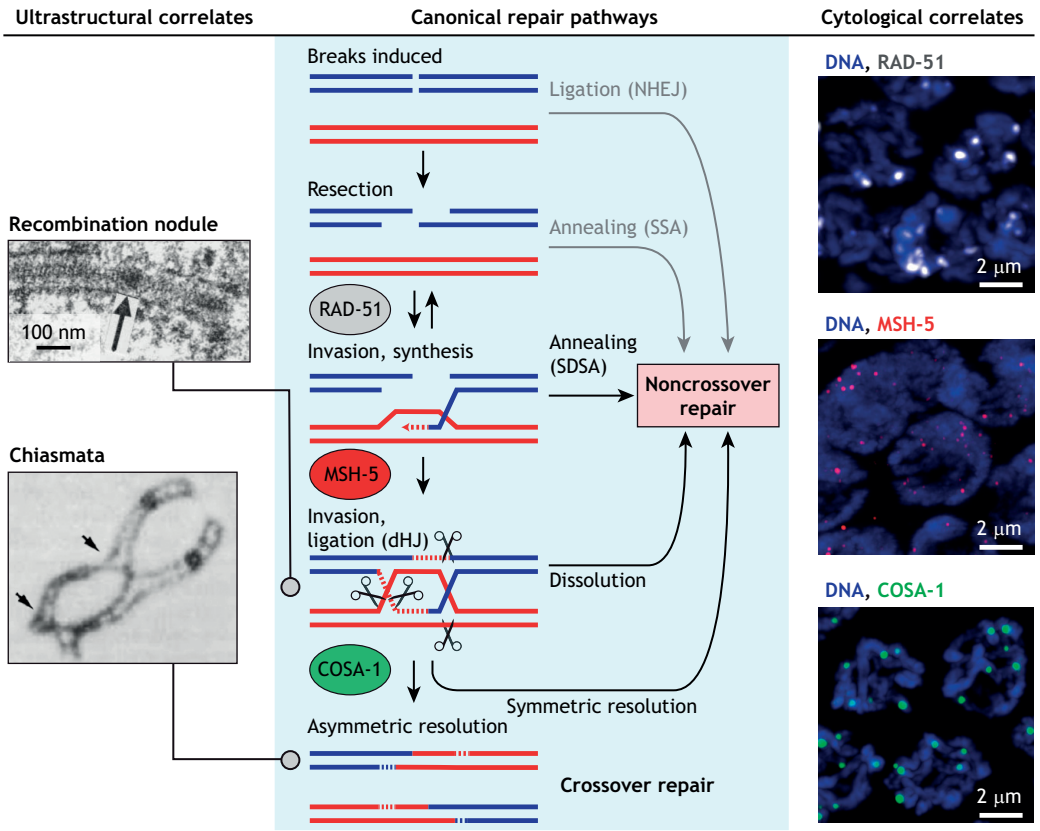

Fig. 2. Illustration of crossover repair. Middle: repair pathways that generate crossover and noncrossover products. Proteins from *C. elegans* are shown as representative examples. Blue and red lines indicate double-stranded DNA from each of the two homologous chromosomes participating in repair. Breaks are resected to allow binding of the recombinase RAD-51, which mediates strand invasion into the homolog. Breaks can be repaired before resection (by nonhomologous end joining, NHEJ) or afterwards (single-strand annealing, SSA), but these pathways are generally inactive in meiosis. After invasion, the free strand anneals, and DNA is copied using the homolog as a template (dashed line). This intermediate can be repaired by synthesis-dependent strand annealing (SDSA) to form a noncrossover product or undergo reciprocal invasion and ligation by the homolog to form a dHJ, which is bound by proteins including the MutSγ component MSH-5 in worms. The dHJ intermediate can be dissolved or, alternatively, cut by nucleases (scissors) in two patterns: either asymmetrically (as shown) or symmetrically. Symmetric resolution leads to a noncrossover product, whereas asymmetric resolution leads to a crossover. Intermediates destined to become crossovers are marked in the worm by the dHJ designating factor COSA-1. Left: representative electron micrographs showing a recombination nodule in *Drosphila* (top, marked with an arrow) and chiasmata in salamander (bottom, marked with arrows). Top image adapted from Carpenter (2003) with permission. Copyright (2003) Genetics Society of America. Bottom image reproduced from Koszul et al. (2012) with permission. Copyright (2012) Genetics Society of America. Right: confocal microscopy images of break intermediates in *C. elegans* marked by RAD-51, MSH-5 or COSA-1, with DNA labeled in blue (six pairs of homologous chromosomes per nucleus). Note the presence of six COSA-1 foci per nucleus, indicating the one designated dHJ per each of the six chromosome pairs. The original images shown were generated using DAPI staining and protein immunolabeling by Spencer Gordon and Lisa Kursel in the Rog laboratory.

manifest as the chiasmata required for proper chromosome segregation (Kowalczykowski, 2015; Morrical, 2015). By contrast, repair intermediates that depart from this pathway form noncrossover products (Fig. 2, middle) (Hatkevich and Sekelsky, 2017; Page and Hawley, 2004; Pâques and Haber, 1999).

Depending on the organism in question, each chromosome may receive an average of two- to thirty-fold more breaks than crossovers, with the remainder repaired to form noncrossover products (Zickler and Kleckner, 2015). Quantitative analysis indicates that DNA intermediates are reduced in number as they progress along the repair pathway en route to becoming crossovers (Gray and Cohen, 2016). However, recent data suggest that intermediates do not proceed smoothly down any one pathway until they are stably repaired. Instead, repair intermediates can reverse their progress and recommit to a different repair fate. One mechanism for this reversibility is the unwinding of strand-invasion intermediates by helicases, as elimination of these helicases has been shown to affect the proportion of crossover outcomes (Hatkevich and Sekelsky, 2017; De Muyt et al., 2012; Zakharyevich et al., 2012). Unwound single-stranded ends can also switch to using a different template (e.g. from the sister chromatid to a homologous chromosome; see Box 2), and recent evidence indicates that template switching is quite common in yeast (Marsolier-Kergoat et al., 2018). Such reversibility implies that robust crossover interference does not rely on one-off decisions, but that pathway choices might have to be reinforced or reselected many times over.

dHJs have emerged as a crucial regulatory hub in the processing of repair intermediates. Unlike strand-invasion intermediates, dHJs are not reversible and cannot engage with alternative templates. They are dynamic, and as demonstrated in yeast, can migrate short distances along the chromosome (Marsolier-Kergoat et al., 2018). They are also not necessarily destined to become crossovers. When the two Holliday junctions are driven into each other, dHJs dissolve, yielding a noncrossover product (Hatkevich and Sekelsky, 2017). Even when they are resolved, dHJs produce a crossover only when the junctions are cleaved asymmetrically, that is, cleaving the invading strands on one side and their complements on the other

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Box 2. Homolog-directed repair

Meiotic break repair and crossover formation occur in the context of four juxtaposed DNA molecules with a high degree of local sequence homology: two sisters from each of the two homologs. Although sister chromatids are competent to serve as repair templates (Goldfarb and Lichten, 2010), meiotic break repair is heavily biased toward the homolog. This is apparent, for example, in analysis of repair intermediates in budding yeast (Kim et al., 2010; Schwacha and Kleckner, 1994). Crucially, the repeated cycles of strand invasion and unwinding during template switching suggest that homolog bias might have to be implemented repeatedly even in the course of a single repair event (Marsolier-Kergoat et al., 2018). Thus, the selection of the homolog over the sister represents another key layer of crossover regulation in addition to those discussed in the main text.

Homolog bias supports the goals of meiosis, since interhomolog crossovers are required for robust chromosome segregation and generate new genetic combinations. Accordingly, the mechanism and extent of homolog bias is an area of considerable interest. The mechanisms conferring this bias likely involve both the meiotic axis and the SC-CR, as well as the activity of helicases that unwind recombination intermediates (Almanzar et al., 2021; Kim et al., 2010; Oh et al., 2007; Sandhu et al., 2020; Toraason et al., 2021.

(Fig. 2). Yet despite this complex set of possibilities, in most species, a subset of dHJs are designated to eventually achieve a crossover fate (Allers and Lichten, 2001): they are stabilized, preventing them from collapsing, and they almost invariably are resolved asymmetrically to yield crossovers (Pyatnitskaya et al., 2019). Under some circumstances, dHJs are resolved to form crossovers without being so designated, and such 'class II' crossovers appear to form by a separate mechanism, are rare (representing less than 10% of all crossovers) or absent in most organisms, and do not exhibit interference (Gray and Cohen, 2016). As would be expected, designated dHJs indeed exhibit interference, whereas earlier repair intermediates tend to exhibit only weak or no interference.

The localization of specific repair intermediates and proteins has been instrumental to our understanding of dHJ designation and repair progression. Early observations using electron microscopy revealed what was likely the first known physical signature of designated dHJs: so-called recombination nodules of ∼100 nm diameter (Fig. 2, left) (Carpenter, 1975a; Zickler and Kleckner, 1999). Recombination nodules appear late in meiotic prophase, correlate with the expected number of crossovers and, tellingly, exhibit interference along the length of the chromosomes. More recently, fluorescence microscopy has defined the molecular components that designate dHJs to form crossovers, and these efforts have been facilitated by the ability to analyze mutant conditions where genes necessary for gamete formation are disrupted. Some of the key factors defined by these studies include the conserved MutSγ complex, which binds and stabilizes dHJs (Snowden et al., 2004); the Zip3 family of proteins (discussed below), which play a role in dHJ designation (Agarwal and Roeder, 2000); COSA-1 and CNTD1, which accumulate at designated dHJs in worms and mammals, respectively (Gray et al., 2020; Holloway et al., 2014; Yokoo et al., 2012); and the MutLγ complex, which resolves dHJs (Cannavo et al., 2020; Kulkarni et al., 2020; Zakharyevich et al., 2012). Complementing genetic studies, these cytological analyses map the development of distinct classes of repair foci and illustrate how they are pruned down in later stages of meiosis (Fig. 2, right) (Gray and Cohen, 2016). Analysis of dHJ-designating factors has also documented the robustness of crossover regulation, showing that the number of total designated dHJs is tightly regulated even if the number of breaks is perturbed (Cole et al., 2012; Rosu et al., 2011; Yokoo et al., 2012), demonstrating the cytological basis for a genetically defined phenomenon (Martini et al., 2006).

The findings described above have allowed integration of the temporal, spatial and molecular aspects of crossover formation and regulation. This understanding of crossover regulation narrows down the mechanistic question of crossover interference to a specific pathway choice: how are designated dHJs regulated to be well spaced, while other intermediates are channeled into other repair outcomes? Despite that specificity, the mechanism implementing crossover interference and the molecular players responsible are still unclear. To date, genetic screens and directed mutagenesis have not delivered a clear candidate that both (1) specifically affects interference when removed, while still allowing dHJ designation and crossover formation at typical levels, and (2) localizes in a manner that is consistent with it being the signal for interference.

Mechanistic models of crossover designation and interference

An enduring mystery of meiosis is the mechanism that enables the impressive range of crossover interference; that is, how does the local designation of a dHJ affect the fate of repair intermediates micrometers away on the chromosomes? After decades of elaboration, theories of crossover interference have converged on two main assumptions (Berchowitz and Copenhaver, 2010; Otto and Payseur, 2019; Zhang et al., 2014a). First, each recombination intermediate has an initially identical structure, regardless of position on the chromosome or order of appearance, and second, the designation of crossover intermediates is competitive, such that designated intermediates exert an inhibitory effect on other intermediates that is reduced with increased distance along the chromosome (Fig. 3A). These assumptions are agnostic to the nature of inhibition and the identity of interfering intermediates, and are sufficient to account for observed crossover distributions (Fujitani et al., 2002; King and Mortimer, 1990; Zhang et al., 2014a). Here, we focus on two classes of models, mechanical and diffusion-based, that propose contrasting molecular mechanisms for how meiotic chromosomes regulate, relay and respond to crossover formation.

Mechanical models

Mechanical models propose that information about dHJ designation is propagated by physical constraints acting on meiotic chromosomes. For example, some of the earliest studies of crossover interference speculated that the rigidity of chromatin in metaphase might prevent multiple chiasmata from forming close to each other, as this would require excessive twisting of the chromosome (Muller, 1916; historical review in Koszul et al., 2012). In light of the evidence discussed above that interference is implemented in late prophase – much earlier than metaphase – more recent work has considered the interplay of force with repair intermediates in what has been termed the beam-film model (Kleckner et al., 2004; elaborated in Zhang et al., 2014a). This model proposes that crossover formation requires mechanical strain and that the relief of strain is the inhibitory signal (Fig. 3B). The namesake formulation stems from a physical analogy. An elastic metal beam coated with a brittle film is heated; the metal expands with heat, but the film does not, instead cracking at one of many defect sites under the strain of the metal's expansion. Cracks

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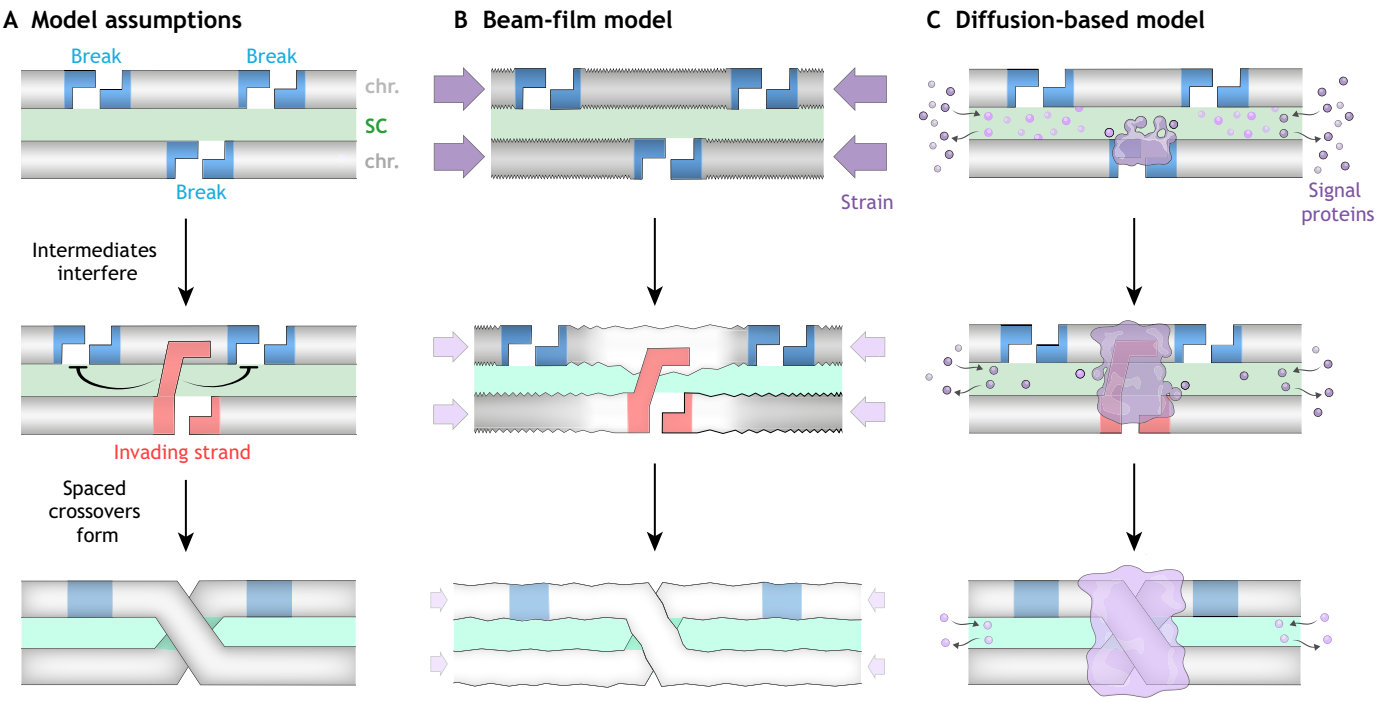

Fig. 3. Models of crossover interference. Interference occurs within the space of the chromosome (chr., gray) and the SC-CR (SC, teal). (A) Model assumptions. Top: breaks initially form randomly (blue). Middle: at an advanced stage of repair (shown here as strand invasion, red), intermediates are differentiated from each other, and the repair intermediate that is destined to become a crossover inhibits crossover formation in nearby breaks (black inhibition arrows), resulting in well-spaced crossovers (bottom). (B) Beam-film model. High strain (inward-pointing mauve arrows and chromosome 'accordion') promotes progression of break repair along the crossover pathway. As intermediates form at a random subset of sites (red), strain is relieved, limiting the formation of further crossover-designated intermediates. (C) Diffusion-based model. Crossover-designating proteins (signal proteins, mauve) diffuse along the chromosome. Nucleation of a protein focus at a random subset of break sites (red) competitively sequesters additional crossover-designating proteins and depletes them from nearby breaks. Only nucleation sites with a critical concentration of crossover-designating proteins progress to a crossover fate, resulting in well-spaced crossovers.

immediately relieve strain in the film, preventing further cracks from forming nearby. In the case of meiosis, early repair intermediates play the role of defects, and designated dHJs act as the cracks. DNA repair enzymes are presumed to be regulated downstream from strain and its relief, with pro-crossover proteins accumulating (or being otherwise activated) at sites where strain is high and alternate pathways acting where it has been relieved.

Because the beam-film model does not make any assumptions regarding a specific strain-bearing structure, experiments to test it have had to first define the structure that experiences strain and link it to crossover formation (Hillers and Villeneuve, 2003; Libuda et al., 2013; Sym and Roeder, 1994). The SC-CR was initially favored based on its localization as a continuous interface between the homologs and its assembly at the time when interference is implemented (Sym and Roeder, 1994). Further hints came from phylogenetic observations, such as that several fungi that lack the SC-CR also do not exhibit crossover interference (Egel-Mitani et al., 1982), and from mutational analysis, such as how null mutants of SC-CR components in budding yeast produce crossovers and viable spores but lose interference (Sym and Roeder, 1994). More recently, data from *C. elegans* has lent further support to the role of the SC-CR by showing that perturbation of the SC-CR affects crossover interference but not crossover formation or meiotic progression (Gordon et al., 2021; Hurlock et al., 2020; Köhler et al., 2020 preprint; Libuda et al., 2013). Furthermore, in line with the predictions of the beam-film model, in worms, crossovers are accompanied by local, longitudinal expansion of the SC-CR (Libuda et al., 2013). However, evidence from budding yeast argues against a role for the SC-CR in mediating interference. Sites at which SC-CR assembly initiates are also decorated by dHJ-designating factors, and these sites exhibit interference even in the absence of a continuous tract of SC-CR (Fung et al., 2004). Moreover, analysis of mutations affecting different steps of crossover repair also found evidence for an implementation of interference prior to complete SC-CR assembly (Börner et al., 2004). Accordingly, attention has turned to chromatin as a possible conduit of the interference signal. The condensation of meiotic chromatin is a significant source of strain (Liang et al., 2015), and perturbation of topoisomerase II, which relieves strain in chromatin, reduces crossover interference in budding yeast (Zhang et al., 2014b). However, the difficulty of monitoring or perturbing mechanical strain *in vivo* has impeded further testing of this model.

The beam-film model makes additional testable predictions. For instance, telomeres are attached to the nuclear envelope during meiosis (Hiraoka and Dernburg, 2009), 'clamping' them and potentially maintaining a higher level of strain (Zhang et al., 2014a). Whereas some higher eukaryotes have a higher rate of crossovers near telomeres, this does not hold for fungi (Haenel et al., 2018), and worms and flies exhibit a depletion of crossovers at telomeres (Lindsley and Sandler, 1977; Rockman and Kruglyak, 2009). Future work directly measuring strain at telomeres may help to link high levels of mechanical strain to crossover interference. Similarly, as studied extensively in *Drosophila*, crossovers are dramatically less common near the centromere (Lindsley and Sandler, 1977). This effect may be due in part to the differential chromosome compaction of the centromeric region, which is likely to impact the

propagation of mechanical strain. While less well understood, the reduction of crossovers near the breakpoints in heterozygous inversions in *Drosophila* may also result from a local reduction of mechanical strain (Gong et al., 2005). Another prediction is that heterozygous chromosomal rearrangements would have an impact on crossover interference, since alignment of wild-type and rearranged chromosomes could result in discontinuities in the SC-CR that affect the propagation of strain. Although the mechanical impacts of heterozygosity remain poorly understood, it has been shown that heterozygosity for chromosomal fusions weakens crossover interference in worms (Hillers and Villeneuve, 2003). Finally, a prediction of the beam-film model that clearly distinguishes it from other models is that relief of mechanical strain, and thus the transduction of the interference signal, would be virtually instantaneous. While the speed of strain relief in meiotic chromatin has not been directly measured, other biophysical studies, such as of the relief of mechanical stress in the bacterial cell wall by cracking, have observed mechanical propagation at velocities of ∼1 µm/ms (Theriot et al., 2015), a thousandfold faster than some of the fastest-propagating reaction–diffusion signals (Chang and Ferrell, 2013). Although the currently available data on the progression of repair intermediates lacks the temporal resolution to discriminate between these rates, an observation of faster signal propagation than is possible by diffusion would provide compelling evidence for the beam-film model.

Diffusion-based models

An alternative to the above-discussed mechanical models are diffusion-based models, which posit that the localization and concentration of signaling molecules drives crossover designation and interference (Fig. 3C). While no single formulation such as the beam-film model has been proposed, a number of hypothesized reaction–diffusion models (Fujitani et al., 2002; King and Mortimer, 1990; Sym and Roeder, 1994; Zhang et al., 2018) suggest mechanisms in which crossover-designating molecules form foci that interfere with the formation of nearby foci, thus resulting in crossover interference. In such competitive sequestration models, repair intermediates would compete to bind and sequester crossover-designating proteins, mature along the repair pathway and progressively recruit more diffusing molecules from their surroundings. In the process, diffusing molecules would be locally depleted along the chromosome, thus inhibiting nearby nucleation and maturation of other recombination events and, consequently, crossovers. Analogous to the spontaneous formation of a single crystal in a saturated solution, homo-oligomerization of dHJ-designating proteins could provide an economical mechanism to limit the number of foci that form designated dHJs. As an additional layer of regulation, molecules bound at designated dHJs could be protected from degradation (Ahuja et al., 2017; He et al., 2020) or other inhibitory signals, thereby increasing the robustness of the designation.

To provide evidence for such a mechanism, it is necessary to identify pro-crossover signals that exhibit the characteristics detailed above. The model predicts that perturbation of these factors would not only abolish interference but would also abolish crossovers (or at least interfering crossovers). Cytologically, we expect these signals to distribute and diffuse throughout meiotic chromosomes prior to formation of breaks or early recombination intermediates, before eventually localizing exclusively to designated dHJs. A group of crossover-promoting proteins, referred to as the Zip3 family (Lake et al., 2015; Zhang et al., 2018), behave in a way that supports a model of competitive sequestration. Family members have been found in yeast (Zip3, also known as Cst9; Agarwal and Roeder, 2000; Ouspenski et al., 1999), worms (ZHP-3 and ZHP-4; Bhalla et al., 2008; Jantsch et al., 2004; Nguyen et al., 2018; Zhang et al., 2018), flies (Vilya; Lake et al., 2015), *Sordaria* (Hei10; De Muyt et al., 2014), plants (HEI10; Ziolkowski et al., 2017) and mammals (RNF212; Reynolds et al., 2013). These proteins all localize to and are necessary for forming designated dHJs. Most family members [with the exception of Zip3 itself, which forms foci prior to synapsis initiation (Agarwal and Roeder, 2000)] initially localize throughout the SC-CR, before concentrating at designated dHJ. In mice and plants, crossover control depends on the expression levels of Zip3 family proteins, underscoring the importance of reaching a threshold of concentration to promote crossover formation (Reynolds et al., 2013; Ziolkowski et al., 2017). Although these observations do not conclusively link Zip3 family proteins to crossover interference, they support the testable hypothesis that an experimentally directed concentration of these pro-crossover proteins at specific dHJs would promote their designation.

The molecular biology of Zip3 family members provides a hint about their ability to act as signaling molecules. All members contain RING domains, a signature of SUMO and ubiquitin E3 ligases, and Zip3 has been confirmed to be a SUMO E3 ligase (Cheng et al., 2006). In addition to promoting SC-CR assembly in budding yeast (Leung et al., 2015), these proteins appear to stabilize proteins necessary for dHJ formation and designation, as has been shown in mice for the pro-crossover factors MSH4 and MSH5 (Reynolds et al., 2013). Furthermore, SUMOylation of components at DNA repair foci in mammals has been shown to promote their homo-oligomerization (Lallemand-Breitenbach and de Thé, 2018), supporting this possible mechanism for the formation of a limited number of protein foci. Finally, anti-crossover proteins that limit the number of designated dHJs have been found in mice (HEI10, also known as CCNB1IP1 in humans; Rao et al., 2017; Qiao et al., 2014) and worms (ZHP-1 and ZHP-2; Zhang et al., 2018). These factors may increase the difference in stability between designated dHJs and other repair intermediates, potentially augmenting the robustness of crossover interference.

A crucial aspect of the diffusion-based model is the physical dimensions over which sequestration acts. Local concentration of break-promoting factors has been implicated in the spatial regulation of meiotic break formation in fission yeast (Fowler et al., 2018). However, competitive sequestration that acts throughout the nucleus is unlikely to account for crossover interference, because in most organisms, dHJ designation occurs when the chromosomes are wrapped around each other in a small volume within the nucleus, and the distance along each pair of homologs over which interference acts is much larger than the three-dimensional distance between adjacent chromosomes (Carpenter, 1975a,b).

Recent insights that nuclear proteins are often organized into phase-separated compartments (Box 1) (Banani et al., 2017) support another possibility. Phase-separated liquids allow for diffusion of constituent and recruited ‘client’ molecules throughout their extent while limiting their exchange with their surroundings (Woodruff et al., 2017). The identification of the SC-CR as a liquid-like compartment (Rog et al., 2017) supports the possibility that a pro-crossover signal, such as a member of the Zip3 family, could be specifically propagated along the SC-CR rather than freely throughout the entire nucleoplasm. In this way, each pair of chromosomes could locally compartmentalize diffusion of crossover-designating molecules to control the spatial distribution of crossovers. Intriguingly, budding yeast provide a potential

exception to this mechanism, as well-spaced repair intermediates form prior to complete SC-CR assembly (Börner et al., 2004; Fung et al., 2004). However, budding yeast exhibits weak crossover interference, which acts only over ∼150 kb (Fung et al., 2004) versus ∼15 Mb in worms (Libuda et al., 2013), and SC-CR-based competitive sequestration may thus only have a relatively minor role, with other mechanisms such as break regulation providing the residual amount of crossover interference (Box 3).

The localization pattern of Zip3-family proteins – first to whole chromosomes and then to designated dHJs – could in principle be a consequence, rather than a cause, of dHJ designation; however, there is evidence supporting the latter option. When the SC-CR is prevented from loading onto chromosomes, it forms chromatin-free assemblies, called polycomplexes, that maintain many of the structural features of the SC-CR (Hughes and Hawley, 2020; Rog et al., 2017; Roth, 1966; Zickler and Kleckner, 1999). ZHP-3 and ZHP-4 exhibit dynamic localization on polycomplexes, first localizing throughout polycomplexes before being restricted into one or two foci at their edges (Rog et al., 2017; Zhang et al., 2018). In budding yeast, Zip3 (along with other crossover-designating factors) also forms foci abutting polycomplexes, although their dynamics have not been examined (Shinohara et al., 2015; Voelkel-Meiman et al., 2019). This localization pattern reflects innate properties of Zip3 family proteins and their interaction with the SC-CR, since polycomplexes lack DNA and, therefore, repair intermediates.

Crossover interference at the nuclear level

Crossover interference acts at the level of a single chromosome, but crossovers are also regulated by nucleus-wide interchromosomal effects. When crossovers do not form on a subset of chromosomes, crossovers on the remaining chromosomes are upregulated (Crown et al., 2018; Schultz and Redfield, 1951) or differently distributed (Carlton et al., 2006). A potential mechanism to explain this effect is the existence of a checkpoint that is activated by lack of an obligate crossover on each chromosome. In worms, for example, the break-inducing factors DSB-1 and DSB-2 persist in nuclei that harbor even a single homolog pair that lacks a crossover (Rosu et al., 2013; Stamper et al., 2013). A potentially related phenomenon is the increased break formation on shorter chromosomes in budding yeast (Murakami et al., 2020; Thacker et al., 2014). The SC-CR has been implicated in nucleus-wide surveillance. For instance, in worms, the turnover of SC-CR proteins slows during late meiotic prophase, and the SC-CR preferentially redistributes to crossover-bearing chromosomes (Machovina et al., 2016; Pattabiraman et al., 2017). Further highlighting the coordination of meiotic repair events across the nucleus, in the gametes of mammals, plants and fungi, crossover number is up- or down-regulated on all chromosomes rather than exhibiting a per-chromosome variability (Wang et al., 2019).

Although the mechanistic underpinnings of interchromosomal effects are not well understood, interpreting them alongside mechanisms for crossover interference has the potential to improve our understanding of both. Explaining interchromosomal effects within the framework of diffusion-based models of interference extends the relevant scope of diffusion and sequestration. Rather than focusing solely on interference signals compartmentalized within each SC-CR compartment, such a model would consider transport of signals between all chromosomes and the nucleoplasm. For instance, if a hypothetical signal were sequestered at designated crossovers, crossover completion could be monitored by the degree of depletion of this signal from the nucleoplasm. In that case, surveillance of the obligate crossover and crossover interference would be achieved by the same mechanism. By contrast, as mechanical coupling is unlikely to extend beyond each pair of homologs, mechanical models of interference would require another layer of regulation in the form of sentinels that sense strain on each chromosome and relay signals to nucleoplasmic effectors. As this comparison illustrates, extending mechanistic models to the entire nucleus is a natural extension of studies of crossover interference, both as a means of validation and a springboard for further work.

Box 3. Double-strand break interference

Although each double-strand break represents a random event, most genomes harbor preferred sites for endogenous break formation (termed 'hotspots') (Borde and de Massy, 2013). At the population level, the distribution of crossovers generally corresponds to the distribution of breaks (Lange et al., 2016; Pan et al., 2011; Yu et al., 2016), indicating that despite the dramatic paring down of intermediates over the course of meiosis, the availability of breaks plays a key role in determining crossover distribution.

In addition to their population-level distribution, breaks also exhibit interference, as they are widely separated on each chromatid. In yeast, the distribution of breaks is regulated by the conserved DNA-damage kinase Tel1 (known as ATM in metazoans), which prevents breaks from occurring near each other (Anderson et al., 2015; Fowler et al., 2018; Garcia et al., 2015). Although break interference has not been demonstrated in metazoans, ATM/Tel1 has been shown to negatively regulate meiotic breaks in mammals and worms, suggesting that local inhibition might be a conserved phenomenon (Checchi et al., 2014; Lange et al., 2011). The ATM/Tel1-mediated response to breaks appears to spread outwards in three dimensions, rather than exclusively along the chromosome (Collins et al., 2020; Li et al., 2020). This is consistent with the length scale over which break interference acts and with the fact that ATM/Tel1 exerts break interference both within the chromosome that carries the break and on its homolog (Zhang et al., 2011).

Conceptually, inhibition of nearby crossovers might result from the inhibition of adjacent breaks. However, crossovers still exhibit interference when breaks are randomly distributed, such as when they are formed by ionizing radiation (Yokoo et al., 2012), or when ATM/Tel1 is missing (Zhang et al., 2014b), indicating that crossover interference is not solely based on the regulation of breaks and that these regulatory layers are likely independent of each other. This idea is further supported by the vastly different length scale over which the processes appear to act in some species, for instance *C. elegans*, in which crossover interference acts on the scale of entire chromosomes (Libuda et al., 2013). However, in other species, such as budding yeast, the length scale over which break interference and crossover interference act is similar (∼150 kb) (Anderson et al., 2015; Fung et al., 2004; Garcia et al., 2015), complicating interpretations of certain mutant scenarios.

Conclusion and outlook

Here, we have surveyed recent work on crossover regulation and meiotic chromosome structure, and placed it in the context of two prevailing models that propose molecular mechanisms for generating crossover interference. The first relies on the mechanical properties of meiotic chromosomes and their ability to both bear strain and be regulated by it. The second relies on phase-separated compartments that assemble along meiotic chromosomes and that could serve as one-dimensional conduits of diffusion-based information. Although these models differ in their assumptions and implications, they are not mutually exclusive. For instance, the initial nucleation or later accumulation of pro-crossover factors might be driven in part by the mechanical conditions near repair intermediates (Shin et al., 2018). Conversely, condensation-driven accumulation of repair factors might deform the axis and the SC-CR

(Woglar and Villeneuve, 2018), thus affecting their mechanical characteristics.

Mapping the identity of repair intermediates along the path to crossover designation has been instrumental in defining the progression of crossover repair, as well as the layers of regulation acting at each stage of the process. New cytological approaches promise to better define the 'ground truth' dynamics of break repair upon which our models of crossover interference are built. For instance, one crucial parameter is the time window during which a recombination intermediate is designated to become a crossover, as this has direct implications for how fast the interference signal must spread. Further breakthroughs in our understanding of crossover regulation may also arrive from the enhanced spatial information provided by super-resolution microscopy experiments that allow us to approach the size scale of recombination intermediates (Brown et al., 2015; Cahoon et al., 2017; Köhler et al., 2017; Slotman et al., 2020; Woglar and Villeneuve, 2018).

Supplementing genetic and cytological experiments with *in vitro* reconstitution (for examples see Cannavo et al., 2020; Kulkarni et al., 2020) is an attractive way to enhance our understanding of the factors that mediate crossover repair and regulation. Although it has been possible to assemble parts of the SC-CR (Dunce et al., 2018) and axis (Kim et al., 2014) *in vitro*, more work remains to be done. Successful reconstitution of complex nuclear assemblies such as centrosomes (Woodruff et al., 2017), the establishment of standard approaches to study condensates (Alberti et al., 2019) and the revolutionary power of cryo-electron microscopy to study large molecular assemblies (Beck and Baumeister, 2016; Kühlbrandt, 2014) suggest that *in vitro* reconstitution of the meiotic chromosome environment, or of other key steps in dHJ formation, designation and resolution, may not be far away. Such feats would allow a rigorous discrimination between models of crossover interference in a controlled environment where biochemical, biophysical and temporal parameters could be measured and perturbed, allowing unprecedented insight into the physical mechanisms of crossover repair.

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Competing interests

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