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Meiotic Recombination and the Crossover Assurance Checkpoint in *Caenorhabditis elegans*

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Abstract

During meiotic prophase, chromosomes pair and synapse with their homologs and undergo programmed DNA double-strand break (DSB) formation to initiate meiotic recombination. These DSBs are processed to generate a limited number of crossover recombination products on each chromosome, which are essential to ensure faithful segregation of homologous chromosomes. The nematode *Caenorhabditis elegans* has served as an excellent model organism to investigate the mechanisms that drive and coordinate these chromosome dynamics during meiosis. Here we focus on our current understanding of the regulation of DSB induction in *C. elegans*. We also review evidence that feedback regulation of crossover formation prolongs the early stages of meiotic prophase, and discuss evidence that this can alter the recombination pattern, most likely by shifting the genome-wide distribution of DSBs.

Introduction

Sexually reproducing organisms rely on correct execution of meiosis to faithfully transmit genetic information from parent to progeny. The reduction in chromosome number during meiosis is achieved by a single round of DNA replication, followed by two consecutive nuclear divisions, in which homologs and then sister chromatids separate from each other. Prior to these divisions, each chromosome must pair with its homologous partner, undergo synapsis (formation of the synaptonemal complex, or SC), and accomplish crossover recombination. This multi-step process leads to the formation of physical linkages known as chiasmata, which enable each chromosome pair to bi-orient and separate during the first meiotic division.

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Crossover formation and all other meiotic recombination events initiate with programmed DNA double-strand breaks (DSBs). The enzyme responsible for DNA cleavage during meiosis was first identified in budding yeast (*Saccharomyces cerevisiae*) as the topoisomerase-related Spo11 protein [1]. A conserved role for Spo11/SPO-11 in metazoan meiosis was first demonstrated in *C. elegans* [2]; this was quickly extended to many other organisms, and is likely a universal feature of meiotic recombination [3]. The activity of Spo11 is tightly regulated, both to ensure that breaks occur in a structural context where they can be repaired efficiently and to optimize the timing, number, and genome-wide distribution of DSBs, the latter of which is a major determinant of the crossover pattern. This regulation shows dramatic variation among eukaryotes. In most mammals, DSB "hotspots" are specified by the genome-wide binding of Prdm9, a sequence-specific DNA-binding protein with histone methyltransferase activity [4]. However, Prdm9-mediated specification of DSB sites is not seen in other lineages. Instead, the meiotic DSB pattern is thought to be influenced by a constellation of chromosome architectural features and chromatin modifications, as well as Spo11 accessory proteins, most of which have poorly understood activities and diverge rapidly during evolution.

The nematode *Caenorhabditis elegans* has emerged as an excellent model system to study meiotic chromosome dynamics. It offers well-developed molecular genetic tools, some idiosyncrasies that facilitate identification and analysis of mutations affecting meiosis [5][1], and advantageous anatomy, in which the complete meiotic progression is observed in a spatio-temporal sequence within the gonad arms (Figure 1A). DSB formation, as well as other aspects of meiotic progression, can be monitored by immunofluorescence and other cytological methods [6]. The genome-wide landscape of meiotic DSBs was recently mapped as part of the modENCODE project [7]. Here we will review our current understanding of the regulation of DSB formation in *C. elegans*. In addition, we summarize evidence from *C. elegans* that crossover formation is regulated by a "crossover assurance" checkpoint that can feed back to impact the abundance and genome-wide distribution of DSBs.

The landscape of DSBs underlies the crossover distribution in *C. elegans*

All eukaryotes display biases in the genome-wide distribution of their meiotic crossovers, but these patterns are quite variable among species. Chromosome-wide crossover patterns have likely coevolved with meiotic chromosome segregation mechanisms to ensure accurate segregation and faithful transmission of genetic information. For example, crossovers are generally suppressed around centromeres, telomeres, and other heterochromatic regions, which likely reflects a tendency for crossovers in centromere-proximal and telomere-adjacent regions to give rise to higher frequencies of meiotic segregation errors.

The haploid genome of *C. elegans* is ~100 Mb, divided among 6 chromosomes of roughly similar size (range: 13.8–21.0 Mb). A large body of recombination data in *C. elegans* revealed that crossover recombination is biased to occur along the distal, or "arm" regions of each chromosome, while the central domains, comprising about half of the physical length

[1]Mutations that disrupt meiosis typically give rise to an easily recognized "High incidence of males" (Him) phenotype. This arises from the *XX/XO* system of sex determination in *C. elegans*, coupled with the fact that even severe meiotic defects do not prevent the production of functional sperm or oocytes, as they do in many organisms.

of each chromosome, show crossover rates severalfold (3–7-fold) lower [8,9]. High-resolution recombination mapping within limited intervals has indicated an absence of true "hotspots," a cold zone for crossovers that spans several hundred kilobases adjacent to each telomere, and an absence of a sharp boundaries between the hotter arms and the cooler centers (Figure 1B) [9,10].

It is currently unknown why the arms have higher recombination rates than the central regions. This is not due to crossover suppression around centromeres: *C. elegans*, like most nematodes, is holocentric, meaning that its kinetochores form at many sites along the length of each chromosome, rather than at a single centromeric locus. Surprisingly, genome-wide mapping has revealed that the recombinogenic arms are more highly enriched than the central regions for features associated with heterochromatin, particularly di- and tri-methylation of lysine 9 on histone H3 (H3K9me2/me3), lamin-associated domains, and many (but not all) classes of transposons and other repetitive elements [11–14]. Thus, *C. elegans* seems to differ from many other eukaryotes in that recombination is positively correlated with these features, at least at low resolution. However, the genome lacks large blocks of tandemly repeated sequences that typically comprise constitutive heterochromatin in eukaryotes, and at finer scale, meiotic recombination is likely associated with regions of transcriptional activity rather than silent regions (see below).

Early steps in meiotic recombination involve endonucleolytic cleavage to remove SPO-11 and resection of the same DNA strands to expose single-stranded 3′ overhangs [15]. This initial processing allows the strand-exchange protein RAD-51 to bind as helical filaments along the ssDNA ends, which is required for strand invasion and thus for all homologous recombination (HR) [16,17]. *Caenorhabditids* lack both the Dmc1 protein that collaborates with Rad51 to execute meiotic recombination in most organisms [16] and the histone variant H2A.X, which is phosphorylated by DNA damage response kinases at DSB sites. Due to the absence of these markers, RAD-51 immunofluorescence is the most widely-used tool for cytological detection of DSBs in *C. elegans*. The appearance of RAD-51 foci depends on SPO-11 activity. Foci are first detected soon after meiotic entry, concomitant with pairing and synapsis. They peak in number at mid-pachytene, and disappear by late pachytene [18].

As part of the *C. elegans* modENCODE project, our group generated a map of meiotic DSBs by RAD-51 chromatin immunoprecipitation from young adult animals and high-throughput sequencing (ChIP-seq) [19,20]. RAD-51 was found to be enriched along chromosome arms relative to the centers. The genome-wide pattern of RAD-51 enrichment largely mirrors the crossover distribution, indicating that the observed arm/center bias in crossover recombination is a consequence of preferential break formation along the arms, similar to findings in many other species. This is consistent with evidence that exposure of *C. elegans* to ionizing radiation during meiosis preferentially increases crossing-over in a cooler central chromosome region [21], presumably reflecting a random distribution of radiation-induced DNA damage. There was one clear disparity between the genetic map and the DSB landscape: RAD-51 was enriched along the full extent of the arms, but crossovers are rarely detected in subtelomeric regions. This implies that DSBs near telomeres are repaired through noncrossover pathways, most likely through intersister repair or gene conversion (interhomolog repair without exchange of flanking markers). RAD-51 ChIP-seq also

confirmed a lack of apparent hotspots at the resolution of the method (~500 bp), although "peaks" of RAD-51, sites typically showing 3–4-fold enrichment over surrounding sequences, could be discerned. Consistent with recombination mapping data, gradual transitions rather than sharp boundaries of RAD-51 enrichment were detected between the arms and the central region of each chromosome (Figure 1B).

Based on cytological analysis of the distribution of RAD-51 foci along pachytene chromosomes, it has been suggested that DSBs do not show arm enrichment, and that the crossover bias is thus imposed at a step subsequent to DSB formation [22]. However, we note that this analysis was performed in *rad-54* mutants, which cannot make crossovers [23] and therefore activate a crossover assurance checkpoint (see below). This results in a highly elevated number of RAD-51 foci, which very likely reflects both an extended duration of DSB formation, and a global shift in the genome-wide distribution of DSBs, as argued below.

Detailed analysis of the RAD-51 ChIP-seq distribution in relation to other chromatin components has not yet been published, but the raw and processed ChIP-seq data from the modENCODE project are publicly available [24]. We found that RAD-51 enrichment correlated positively with histone marks associated with active transcription, including H3K4me3 and particularly H3K36me1, which is strongly associated with long introns within actively transcribed genes on the chromosome arms. While RAD-51 and H3K9 di- and tri-methylation are all enriched on chromosome arms, RAD-51 was inversely correlated with both H3K9me2 and H3K9me3 enrichment when compared on shorter length scales. Moreover, both crossover recombination and RAD-51 enrichment show even more pronounced arm enrichment in *met-2* mutants, which are deficient for H3K9 methylation in the germline (C. Kotwaliwale and AFD, unpublished observations). We interpret these findings to indicate that these histone marks, or other chromatin features associated with them, inhibit the formation of meiotic DSBs, as they do in other species [25–27]. An intriguing possibility is that a recently discovered mark, H3K23me3, which is strongly enriched in the germline of both *Tetrahymena* and *C. elegans*, may play a more direct role in repressing DSB formation by SPO-11 [28]. While the genome-wide distribution of this mark in *C. elegans* has not yet been reported, and nothing is yet known about its regulation, H3K23me3 has been found to be co-enriched on H3 tails that also carry H3K9me2 or H3K9me3 [29].

Beyond SPO-11: other factors required for DSB formation in *C. elegans*

While orthologs of Spo11 are readily recognized by homology and universally required for meiotic DSB formation, accessory proteins that regulate the activity of Spo11 are not well conserved [30]. Among the accessory proteins required for Spo11 to generate DSBs in *S. cerevisiae* (defined here as proteins that are not considered to be directly involved in DNA repair chromosome structure), only Mei4 and Rec114, which form a complex with Mer2 in budding yeast, are known to have orthologs in other phyla, but even these proteins have not been found in some lineages, including *C. elegans* [31]. However, genetic screens have identified other factors that are required to initiate meiotic recombination in *C. elegans*. Two of them, DSB-1 and DSB-2, may play similar roles to the Mei-4-Rec114-Mer2 complex

(Figure 2) [18,32]. These proteins are similar to each other in sequence, and both localize to meiotic chromatin during the time of DSB formation. They do not appear to colocalize extensively at the resolution of diffraction-limited fluorescence microscopy. The chromosomal localization of DSB-2 depends on DSB-1, but DSB-1 does not require DSB-2. DSB-1 is required for all DSBs, while loss of DSB-2 results in a marked reduction in crossover formation that becomes more severe with age. Because DSB-1 and DSB-2 lack recognizable protein domains and have no apparent orthologs outside of nematodes, how they promote break formation remains unclear. They do contain clusters of potential phosphorylation sites for the ATM/ATR family of kinases, suggesting they might regulate the abundance of DSBs through negative feedback, similar to Rec114 [33]. Future efforts to map the distribution of DSB-1/2 along chromosomes or to identify interacting factors may help to illuminate DSB regulation.

Localization of DSB-1 and DSB-2 to chromosomes depends on the activity of CHK-2 [18,32], a meiosis-specific serine/threonine kinase that was also identified through a genetic screen in *C. elegans* [34]. While CHK-2 is homologous to the well-studied mammalian DNA-damage kinase Chk2, its function has clearly diverged: it lacks the clusters of SQ/TQ motifs that mediate regulation of Chk2 by ATM and ATR in response to DNA damage, and is dispensable for DNA damage-mediated cell cycle arrest in the *C. elegans* germline [34,35]. However, it is essential for both DSBs and other key processes of early meiotic prophase, including homolog pairing and synapsis. Direct substrates of CHK-2 that are important for homolog pairing and synapsis have recently been identified (see below), but the role of this kinase in DSB formation is less well defined.

Several other proteins also influence meiotic DSB formation in *C. elegans* (Figure 2). Mutations in the *rec-1* gene, the first known meiotic mutant in *C. elegans,* cause an increase in centrally located crossovers at the expense of arm crossovers [36,37], and thus likely affects the underlying DSB pattern; however, *rec-1* does not affect the overall crossover frequency or fidelity of chromosome segregation. Recombination defects observed in *him-17* null mutants can be rescued by exogenous breaks, indicating that the chromatin-associated THAP domain protein HIM-17 promotes meiotic DSBs [38]. Two additional chromosome-associated proteins, XND-1 and HIM-5, are required for DSB formation specifically on the X chromosome [39,40]. It is unsurprising that the X-chromosome would have distinct requirements for DSB formation during meiosis, since unlike the autosomes, it is transcriptionally silent during meiosis [41]. However, both XND-1 and HIM-5 are enriched on the autosomes relative to the X chromosomes [39,40]. Recent work has identified the *rec-1* gene and shown that it encodes a paralog of HIM-5 [41]. While REC-1 is conserved among related nematodes, HIM-5 may be the product of a recent gene duplication specific to *C. elegans*. HIM-5 and REC-1 appear to play partially overlapping roles in promoting DSB formation, since *rec-1; him-5* double mutants show a severe, genome-wide DSB deficiency, unlike either single mutant [41]. REC-1 may be expressed only at low levels, since a REC-1-GFP transgene could not be detected cytologically [41]. Its molecular role thus remains mysterious.

Unlike DSB-1 and DSB-2, which localize to meiotic chromosomes specifically during the temporal window of DSB formation, HIM-17, HIM-5, and XND-1 are detected in nuclei

throughout more extensive regions of the germline, including in premeiotic nuclei [38–40], and are all enriched on autosomes relative to the X chromosome. HIM-17 and XND-1 were both included among the ChIP-seq targets for the modENCODE project [42]. Genome-wide mapping revealed that both HIM-17 and XND-1 are strongly enriched at the promoters of many germline-expressed genes, suggesting that they may act primarily to regulate transcription. This is consistent with their enrichment on autosomes, since the X chromosome lacks protein-coding genes that are expressed in the germline [41]. Additionally, the idea that HIM-17 and XND-1 are transcription factors can explain their pleiotropic effects on germline functions, which include partial sterility, meiotic entry defects, and temperature sensitivity [39,40,43], in addition to their reported effects on germline histone modifications. The genes whose promoters are bound by HIM-17 and XND-1 overlap extensively and include those encoding the DSB-promoting factors *dsb-2, him-5, and rec-1*, which may explain the effects of *him-17* and *xnd-1* mutations on recombination initiation. Consistent with the idea that HIM-17 and/or XND-1 influence recombination by promoting expression of other factors, HIM-5 is not detected on chromosomes in *him-17* or *xnd-1* mutants [40], and DSB-2 immunofluorescence shows reduced intensity in *him-17* mutants [32].

Initial analysis of *him-17* and *xnd-1* mutants was interpreted to indicate that these proteins promote DSB formation by altering chromatin structure [38,39]. However, it now seems unlikely that the reduction in H3K9 trimethylation observed in germline nuclei of *him-17* mutants [38] is directly related to DSB formation, since disruption of *met-2*, which encodes the major H3K9 methyltransferase in the *C. elegans* germline, does not impair break formation or meiotic recombination [44], although it does impact meiotic surveillance mechanisms [44,45]. By similar logic, the role of XND-1 in promoting breaks is more likely due to its regulation of downstream genes, particularly *him-5*, than to a direct role in chromatin modification, since mutations in *him-5* similarly impair DSB formation on the X chromosome but without the increase in acetylation of H2A lysine 5 observed in *xnd-1* mutants [39,40]. The observation that HIM-5 is enriched on autosomes throughout the premeiotic and early meiotic stages of the germline suggests that it may also play a role in transcription (by extension, the HIM-5 paralog REC-1 may have a similar function), but efforts to map the genome-wide HIM-5 distribution during the modENCODE project were unsuccessful so it is unclear whether the protein is associated with active genes.

Meiotic chromosome structure and DNA repair proteins promote initiation of recombination

A hallmark of meiotic prophase is the reorganization of chromosomes into an “axis-loop” configuration, which is a prerequisite for DSB formation, pairing, and synapsis (Figure 2) [46,47]. This structural remodeling is likely driven by cohesin complexes, which are diffusely distributed throughout interphase chromatin but coalesce to form a linear chromosome axis upon meiotic entry. How this reorganization occurs is unknown, but it may be in part a consequence of the replacement of canonical mitotic cohesin proteins by meiosis-specific variants, a phenomenon observed across eukaryotic phyla. In *C. elegans*, the major mitotic kleisin SCC-1 is replaced by three meiosis-specific kleisins, REC-8,

COH-3, and COH-4 [48,49]. COH-3 and COH-4 are highly similar and functionally redundant, at least by current assays. While REC-8 or COH-3/4 alone can maintain association between sister chromatids at diakinesis, these two types of kleisins play distinct roles during chromosome segregation, and also contribute differentially to the function of the chromosome axis much earlier, during leptotene/zyogtene [48,50]. DSBs are formed in mutants that lack either REC-8 or COH-3/4, but RAD-51 foci are severely reduced in triple *rec-8; coh-4 coh-3* mutants [50], and DSB formation is similarly impaired in other cohesion-defective mutants.

In addition to cohesins, the meiotic chromosome axis in *C. elegans* contains four related meiosis-specific HORMA domain proteins: HIM-3, HTP-1, HTP-2 and HTP-3 [51–54]. Mammals have two such proteins (HORMAD1 and HORMAD2), while budding yeast has one (Hop1) [55,56]. HORMA domains bind to short, linear peptide motifs within other proteins [57]. The four *C. elegans* proteins associate with the chromosome axis through a hierarchical complex [58]. The C-terminal tail of HTP-3 contains binding sites that are specific for the other HORMA proteins, and are required to recruit them to the axis [58]. HTP-3 is required for DSB formation, consistent with the requirement for HORMA domain proteins to generate meiotic DSBs in other organisms [56,59–61], but this activity does not depend on its recruitment of HIM-3 or HTP-1/2 to the chromosome axis [53,58]. HTP-3 has been found to interact with MRE-11 and RAD-50 [53], which may be related to its role in promoting DSBs (see below). As cohesins are required for the association of HTP-3 with meiotic chromosomes [48,53], HTP-3 is likely recruited to the axis by binding of its HORMA domain to a cohesin subunit.

While cohesins and HTP-3 at the chromosome axis promote DSB formation, condensins, which also play important roles in meiotic chromosome architecture, have an inhibitory effect on DSBs. Downregulation of components of either the Condensin I or Condensin II complex leads to axis elongation, and results in an increased number of SPO-11-dependent RAD-51 foci and crossovers [23,62]. This may reflect an “opening” of chromatin structure or perturbation of the regulatory mechanisms that control the timing and number of DSBs. The DNA damage kinases ATM (ATM-1) and/or ATR (ATL-1) likely contribute to this regulation, as they do in other organisms. [33,63,64].

Initiation of meiotic recombination in *C. elegans*, as in other organisms, also depends on a subset of the DNA repair machinery. Spo11/SPO-11 forms covalent adducts with DNA when it generates DSBs, and its removal by endonucleolytic cleavage is essential for downstream steps of meiotic recombination (Figure 2). The mechanisms of Spo11 removal and end resection are conserved among eukaryotes and require the MRN/MRX complex, comprised of Mre11, Rad50, and Nbs1 or Xrs2 [15,65]. Orthologs of Mre11 and Rad50 have been shown to be required for meiotic recombination in *C. elegans* [65,66], however, a third member of this complex has not yet been identified.

Because RAD-51 does not bind to DNA when early DSB processing steps are abrogated, the dependence of DSBs on these factors was investigated through experiments in which worms were irradiated to produce exogenous breaks. At diakinesis in *mre-11* and *rad-50* mutants, twelve individual achiasmate chromosomes, or univalents, are observed, but when these

animals are irradiated during meiosis, the chromosomes become fragmented [67,68]. Further, mutations in *mre-11* or *rad-50* are able to suppress the fragmentation and aggregation of chromosomes seen at diakinesis in *rad-51* and *brc-2* mutants, which are unable to repair breaks through HR [16,69]. These findings indicate that although they act "downstream" of DSBs, MRE-11 and RAD-50 also promote DSB formation in *C. elegans* [67,68], and that RAD-51 and BRC-2 are dispensable for breaks. Similarly, the MRX complex promotes meiotic DSBs in budding yeast, but separation-of-function alleles of *RAD50* and *MRE11* that permit Spo11 cleavage while still impairing their roles in break processing have been identified [70,71]. A similar allele of *mre-11* has also been identified in *C. elegans* [72]. Reduction of the meiosis-specific cohesin REC-8 results in an increase in RAD-51 foci [17,68], and also allows SPO-11 to make breaks in the absence of MRE-11 and RAD-51 [68], indicating that this complex is not absolutely required for DSBs but may counteract inhibitory effects of chromosome structure. COM-1, the *C. elegans* ortholog of Sae2/CtIP, was initially implicated in processing breaks to enable RAD-51 binding, but a subsequent study demonstrated that RAD-51 foci and crossovers are restored in *com-1* mutants when nonhomologous end joining (NHEJ) proteins are also removed, indicating that the primary role of COM-1 may be to inhibit repair of meiotic DSBs by NHEJ, thereby promoting repair through RAD-51–dependent HR mechanisms [73,74].

Interplay between DSB formation, pairing, and synapsis in *C. elegans*

Analysis of *C. elegans spo-11* mutants first revealed that, unlike in budding yeast, plants, and mammals, homolog pairing and synapsis occur independently of DSBs in this organism [2]. All other known proteins involved in meiotic recombination, including RAD-50, MRE-11, COM-1, RTEL-1, BRC-2, RPA-1, BRD-1, RAD-51, RAD-54, MSH-4, MSH-5, COSA-1, etc. are also dispensable for timely and accurate pairing and synapsis [17,67,68,73,75–78]. Nevertheless, DSB formation coincides with homolog pairing and synapsis during early meiotic prophase, and occurs independently of pairing and synapsis. During the leptotene-zygotene stage, known as the "transition zone" region in *C. elegans*, special regions near one end of each chromosome known as meiotic pairing centers [79] tether chromosomes to the nuclear envelope and establish a linkage to microtubules and the dynein motor through the SUN/KASH domain proteins SUN-1 and ZYG-12 [80,81]. This pairing center-mediated chromosome motion is an evolutionary variant of the telomere-led movements seen in most organisms, which often results in a clustering of chromosome ends at the nuclear envelope known as the "meiotic bouquet" [82]. These cytoskeleton-mediated movements promote homolog pairing and synapsis in *C. elegans* (Figure 1A) [81,83,84].

Pairing centers are specified by a family of zinc-finger proteins (HIM-8, ZIM-1, ZIM-2, and ZIM-3) that bind short sequence motifs enriched within these regions [85–87]. They become active at the onset of meiotic prophase by recruiting at least two meiosis-specific kinases, CHK-2 and PLK-2 [88–90]. Both of these kinases are required for pairing center-led chromosome motion, while CHK-2 is also required, independently of its role at pairing centers, for induction of DSBs [88–90]. Stable homolog pairing at the pairing center regions is extended along the chromosomes by synapsis, defined as the assembly of the synaptonemal complex (SC) between chromosome axes [91,92]. In *C. elegans*, synapsis initiates at the pairing centers and spreads along the chromosomes [84,91]. Synapsis results

in stable side-by-side alignment of homologous chromosomes and is essential for all crossover recombination [92].

Feedback control of CHK-2 influences the duration of DSB formation

While pairing and synapsis are not required for DSB formation in *C. elegans*, a body of evidence has revealed that defects in homolog interactions can affect the duration and distribution of DSBs through a feedback mechanism. Early evidence revealed that mutations in *him-8*, which are specifically defective in meiotic segregation of the X chromosome, also alter genetic distances within some autosomal intervals [93]. Molecular identification and characterization of *him-8* revealed that it encodes a protein that binds to the X chromosome pairing center [87], and that its effect on the autosomes is thus indirect. Further analysis confirmed that alteration of the genetic map is a general feature of mutations that disrupt pairing or synapsis of one or more chromosomes [94–96]. Such mutations also result in a phenotype known as the "extended transition zone," because the crescent-shaped configuration of chromosomes normally observed during the leptotene-zygotene stages, accompanied by displacement of the large nucleolus to one side of the nucleus, persists for a much longer region within the germline [87,92,97–99]. Immunofluorescence detection of RAD-51 foci also reveals an extended region of positive nuclei in most mutants with synapsis or crossover defects. This early evidence that meiotic errors might lead to a delay in meiotic progression led us to propose that altered crossover distributions seen in such situations might be a consequence of delays in crossover designation [94]. Similar models have been proposed to explain the "interchromosomal effect" in *Drosophila melanogaster*, in which crossover suppression along one chromosome alters the recombination map on other chromosomes [100,101].

Further evidence that defects in pairing, synapsis, and crossover formation result in meiotic cell cycle delays came with the development of additional molecular markers for meiotic events. The "patchy" aggregates of SUN-1 and ZYG-12 seen at the nuclear envelope in transition zone nuclei persist longer, as does phosphorylation of SUN-1 at several N-terminal serine/threonine residues [80,81,102,103], in diverse mutants with synapsis or crossover defects. These nuclear envelope markers likely reflect the presence of the Polo-like kinase PLK-2 at pairing centers, which is also prolonged under these conditions [89,104]. The disappearance of DSB-1 and DSB-2 from meiotic chromosomes shows a similar temporal dependence on the ability to establish crossover intermediates on all chromosomes [18,32]. All of these molecular markers of early prophase have been shown to depend on the CHK-2 kinase. We recently reported that pairing center proteins HIM-8, ZIM-1, ZIM-2, and ZIM-3 are direct substrates of CHK-2 [88]. By developing a phospho-specific antibody that recognizes these proteins only when they are phosphorylated by CHK-2, we demonstrated that CHK-2 becomes active upon meiotic entry. Its activity normally declines during early pachytene, but is prolonged in most mutants that fail to achieve a crossover on every chromosome pair, including *him-5* mutants, which are defective in crossover formation but not homolog pairing or synapsis. Thus, a prolonged leptotene/zygotene stage, during which DSBs continue to be generated and crossover designation is delayed, can be explained as downstream effects of prolonged CHK-2 activity in response to failures in establishment of crossover intermediates. This feedback

mechanism acts in a cell-autonomous fashion and maintains the status of the meiotic cell cycle at which errors first arise [18,88]. DSB-1 and DSB-2 remain associated with all chromosomes, as do RAD-51 foci, suggesting that genome-wide DSB formation is affected. As cells advance spatially through the germline, the feedback signal appears to be overridden eventually to allow meiotic progression even if crossovers are not present on all chromosomes. Some evidence indicates that this “override” signal depends on the activation of MPK-1 (ERK) [68], which is spatially regulated within the germline [105]. A subset of oocytes containing unsynapsed chromosomes or unrepaired recombination intermediates will undergo apoptosis [106,107], but many escape this error-culling mechanism to mature and undergo fertilization even if they have failed to establish crossovers on every chromosome. This feature of *C. elegans* meiosis provides an experimental advantage, since it enables genetic analysis of these aberrant meioses among the progeny of affected animals.

A variety of evidence suggests that prolonged periods of DSB formation are associated with an altered genome-wide pattern of DSBs and crossovers. In cases where some chromosomes are proficient for crossover formation, as in *him-8* or *zim* mutants, the effect can be observed by measuring the crossover pattern on those chromosomes. The most extreme cases are those of *rad-54* mutants and *helq-1, rfs-1* double mutants, which make no crossovers because they cannot process recombination intermediates to remove RAD-51 [23,75]; in these genotypes the number of RAD-51 foci per nucleus gradually increases severalfold from their normal peak levels in early pachytene through the end of pachytene. While estimates of their numbers have varied among laboratories, and likely depend on the resolution of the microscope system used, several groups have reported detection of over 50 foci per nucleus in *rad-54* mutants [22,108], an order of magnitude higher than the peak numbers typically observed in wild-type oocyte nuclei. However, this does not imply that DSBs occur at 10-fold higher levels, since the foci observed in wild-type animals represent a steady-state number of recombination intermediates, whereas their turnover is blocked in *rad-54* mutants.

Crossover designation appears to act fairly late in *C. elegans*. COSA-1, a molecular marker for crossover sites [77], is only detected as foci along chromosomes in mid/late pachytene, after CHK-2 activity and RAD-51 foci have abated, and is delayed in situations that prolong CHK-2 activity. Thus, even when induced in mid-meiotic prophase, DSBs can compete effectively to become the locus of the single designated crossover [108]. It follows that the genetic map is not a direct readout of early breaks, but can be altered if break formation persists later than usual, since crossover designation is also delayed under such conditions. Taken together, the available evidence indicates that the alteration in genetic map distances observed in mutants with defects in crossover formation on one or more chromosomes is likely to reflect an altered pattern of DSBs, with more breaks occurring in the central regions of chromosomes under such conditions. This can make it difficult to interpret such changes; for example, autosomal recombination was found to be altered in *him-5* mutants [40], with an increase in crossing-over in the central region of the chromosome, but this could be a consequence of checkpoint activation in response to defects in X chromosome crossover designation rather than a direct effect of HIM-5 deficiency. While it is not yet possible to quantify the number of DSBs induced during meiosis in *C. elegans* reliably, work in budding yeast has revealed an increase in the number of DSBs and a shift in their distribution in

response to defects in “homolog engagement” [109]. While this term is deliberately vague, the evidence summarized here indicates that “engagement” corresponds to the act of crossover designation in *C. elegans*, which depends on and occurs subsequent to synapsis, rather than as a prerequisite for synapsis, as in many other organisms.

A shift in the DSB pattern as more breaks arise could be a consequence of “DSB interference” [64], if early breaks on the arms inhibit subsequent breaks in the same regions. This phenomenon has only been studied in detail in budding yeast, where it acts locally to suppress DSBs over a scale of tens of kilobases. An effect on this scale might not be sufficient to reshape the recombination landscape along metazoan chromosomes, but the range of DSB interference may scale with chromosome size. Alternatively, there may be changes in gene expression, chromatin, or chromosome structure that make the central chromosome regions more permissive of break formation at later times in meiotic prophase.

A central role for meiotic HORMA domain proteins in crossover assurance

As described above, mutations that impair crossover formation on one or more chromosomes induce a “crossover assurance checkpoint” that prolongs CHK-2 activity, RAD-51 foci, and other features of the leptotene-zygotene stages of prophase. Using mutations that disrupt specific interactions between the meiotic HORMA proteins in *C. elegans*, we established that the recruitment of HIM-3 and HTP-1/2 to the axis is critical for this checkpoint (Figure 3) [88]. Therefore, HTP-3, which contains four binding sites for HIM-3 and two for HTP-1/2 [58], can be regarded as a scaffold for checkpoint activation.

Budding yeast Hop1, a founding member of the HORMA domain family, also localizes to meiotic chromosome axes, where it interacts with another axis protein, Red1, to recruit a meiosis-specific kinase, Mek1, and mediates a cell-cycle arrest in response to meiotic defects [110–113]. Mammalian HORMAD1 and HORMAD2 localize to unsynapsed axes and recruit ATR to activate a meiotic checkpoint [60,61,114]. Although details of these mechanisms may differ among species, the involvement of checkpoint kinases and meiotic HORMA domain proteins appears to be a general theme of meiotic regulation. In budding yeast and mouse, HORMA domain proteins are depleted from synapsed chromosomes by the conserved AAA+ ATPase Pch2/Trip13 [56,115–117]. As Hop1 and HORMAD1 are required for the normal level of DSB formation [56,59–61], which in turn promotes crossover designation and SC assembly in these organisms [118–120], depletion of HORMA domain proteins by Pch2/Trip13 may provide a mechanism to downregulate DSB formation after crossover designation and synapsis of all chromosomes [109,121]. Interestingly, all four meiotic HORMA domain proteins in *C. elegans* remain associated with synapsed chromosomes until late pachytene [51,53,122], and the *C. elegans* ortholog of Pch2/Trip13 PCH-2 is largely dispensable for axis remodeling. Therefore, it is not clear how the HORMA domain proteins monitor synapsis and crossover formation in *C. elegans*. It will be interesting to test whether the network of meiotic HORMA domain proteins undergoes structural or biochemical changes during meiotic progression to monitor synapsis and crossover formation. Molecular mechanisms by which these proteins mediate the meiotic checkpoint also remain to be identified. It is exciting to note that structures of meiotic HORMA domain proteins are very similar to the kinetochore protein Mad2 [58], which is an

essential signaling transducer of the spindle assembly checkpoint [123]. Future identification of signaling effectors downstream of the meiotic HORMA domain proteins that directly regulate CHK-2 activity will help to reveal how the chromosome axis coordinates meiotic processes.

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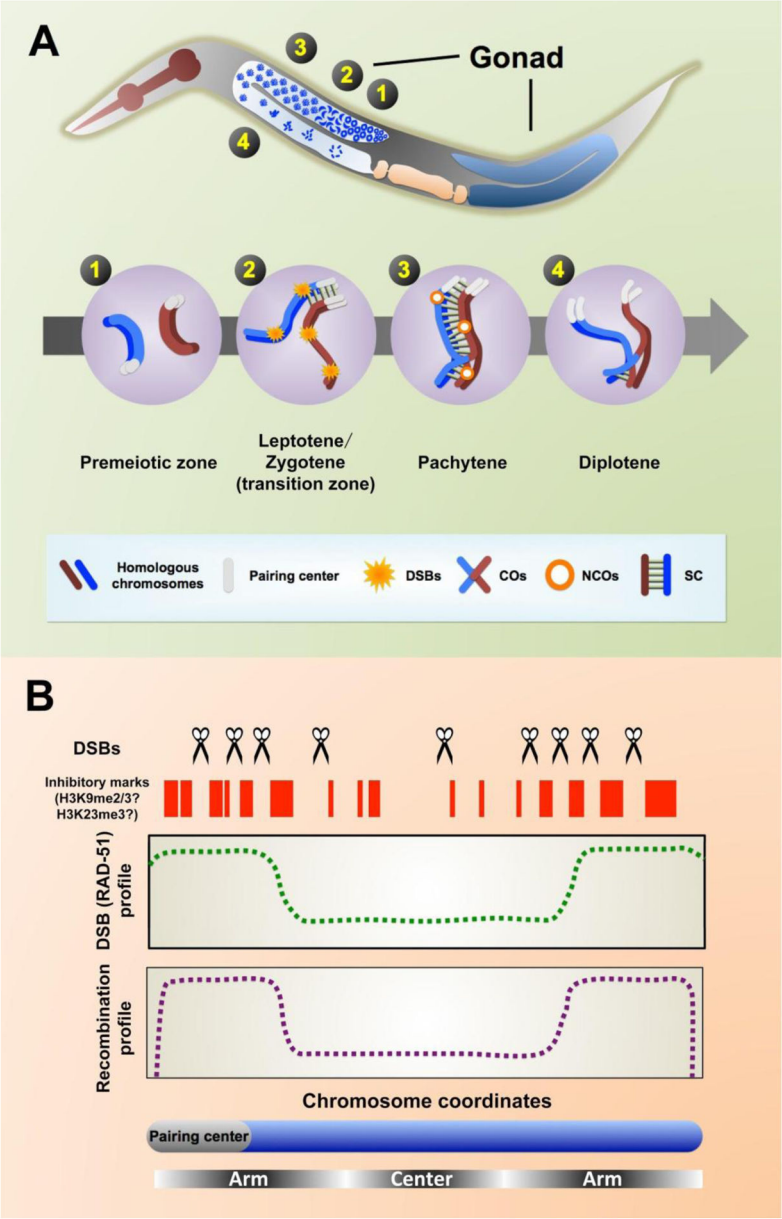

Figure 1. Programmed double-strand break formation during meiosis in *C. elegans*

A) Schematic of *C. elegans* germline anatomy and chromosome configurations during early meiotic prophase. Meiotic recombination initiates via programmed DSB formation during the leptotene/zygotene stages (transition zone), and crossover formation is completed during pachytene. A single recombination intermediate is designated to become a crossover, and excess breaks are repaired through non-crossover pathways.

B) The distribution of meiotic DSBs, as determined by RAD-51 ChIP-seq, mirrors the distribution of crossover frequencies along the *C. elegans* chromosomes, except in subtelomeric regions, which show DSB enrichment but few or no crossovers. DSBs, like crossovers, are enriched on the chromosome arms relative to the centers. Locally they are associated with actively transcribed genes, and are negatively correlated with H3K9 di- and

tri-methylation, although these marks are enriched on the arms. A newly discovered mark, trimethylated H3K23, may also antagonize DSBs.

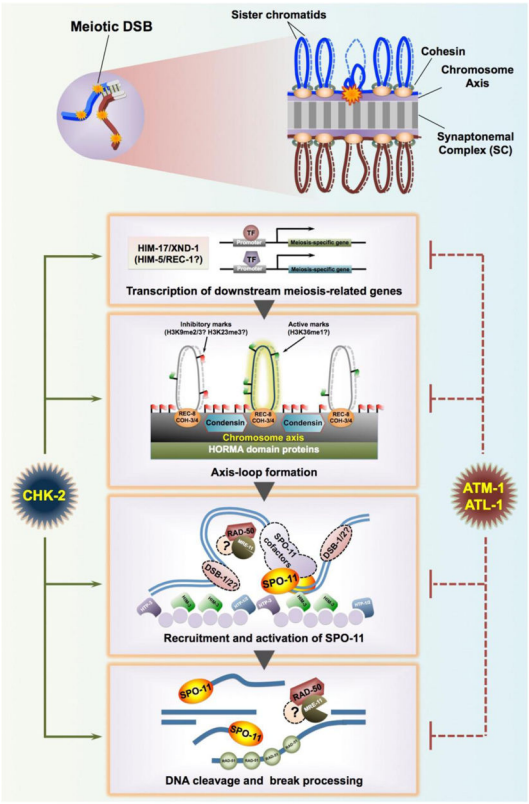

Figure 2. Chromosome structure and recombination initiation machinery in *C. elegans*

Upon meiotic entry, chromosomes are reorganized around a central axis comprised of cohesins and meiosis-specific HORMA proteins. The formation of DSBs depends on this remodeling. The interplay between cohesins and condensins is thought to control axis length. Several *C. elegans* proteins, including HIM-17, XND-1, HIM-5, REC-1, DSB-1, and DSB-2, promote breaks or affect the distribution of breaks. ChIP-seq evidence, as well as mutant analysis, suggests that HIM-17 and XND-1 regulate the expression of downstream genes. HIM-5 is also associated with the autosomes and depleted on the transcriptionally-inactive X chromosome. DSB-1 and DSB-2 bind to meiotic chromosomes during the time of DSB induction and may contribute to recruiting and/or activating SPO-11. SPO-11-DNA adducts are subsequently removed by nucleolytic activity. This requires the MRE11-RAD-50 complex, which also is required for SPO-11-dependent DSB formation. The formation of DSBs also depends on the meiosis-specific CHK-2 kinase and is likely negatively regulated by the DNA damage kinases ATR (ATL-1) and/or ATM (ATM-1).

Figure 3A

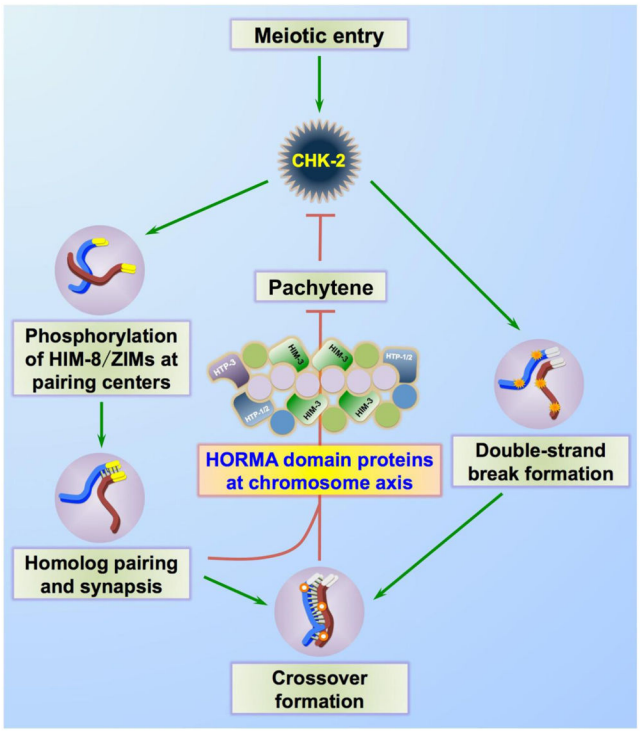

Figure 3B

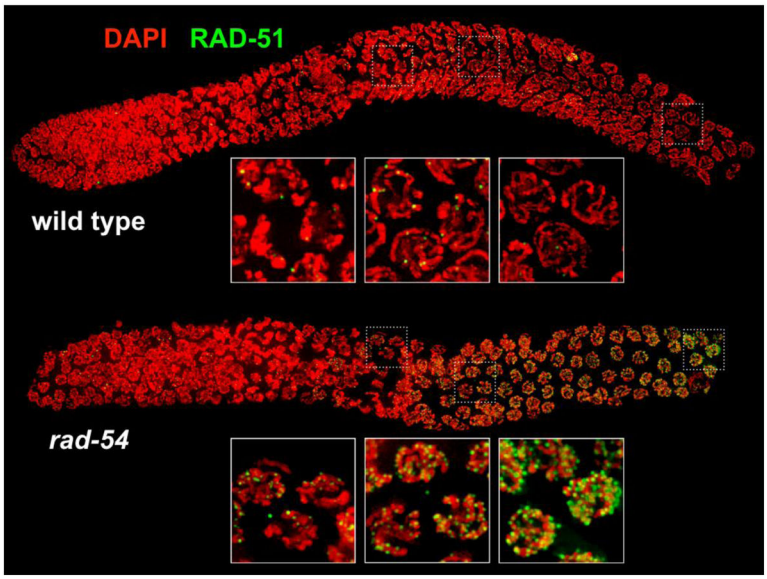

Figure 3. Feedback regulation of meiotic progression through CHK-2 impacts the timing and distribution of DSBs

A) CHK-2 acts as a master regulator of meiotic prophase. Its kinase activity is required for DSBs and also for homolog pairing and synapsis. CHK-2 activity normally declines during early pachytene, once all chromosomes synapse. If crossover intermediates fail to be established on all chromosomes, CHK-2 activity is prolonged. HORMA domain proteins at

the chromosome axis are critical for this crossover assurance mechanism, but it is unknown how they sense or transduce crossover failures to prolong CHK-2 activity. When the checkpoint is activated, DSB-1 and DSB-2 persist along meiotic chromosomes, and (if breaks are made) the number of RAD-51 foci typically rises to higher levels than seen in wild-type animals. Biased crossover formation on chromosome arms is greatly reduced under these conditions, suggesting that the central chromosome regions experience more DSBs.

B) When crossovers cannot be generated on all chromosomes, an extended zone of RAD-51 foci is observed, and more foci are detected per nucleus. Here, two gonad arms immunostained for RAD-51 are shown, one from a wild-type hermaphrodite (top) and one from a *rad-54* mutant (bottom). Insets below each gonad show higher-magnification views of the boxed regions. In wild-type gonads RAD-51 foci peak in number at early pachytene and disappear by late pachytene, while in *rad-54* mutants they progressively increase in number until nuclei reach the most proximal region of the gonad, and disappear as the chromosomes condense at diakinesis. These foci can be difficult to resolve and count reliably at such high densities, but frequently exceed 50 per nucleus. Other mutants with crossover defects have similar but less extreme effects than *rad-54* mutants, which are defective in removal of RAD-51 from recombination intermediates.