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# Meiotic recombination in *Caenorhabditis elegans*

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## Abstract

The faithful segregation of homologous chromosomes during meiosis is dependent on the formation of physical connections (chiasma) that form following reciprocal exchange of DNA molecules during meiotic recombination. Here we review the current knowledge in the *Caenorhabditis elegans* meiotic recombination field. We discuss recent developments that have improved our understanding of the crucial steps that must precede the initiation and propagation of meiotic recombination. We summarize the pathways that impact on meiotic prophase entry and the current understanding of how chromosomes reorganize and interact to promote homologous chromosome pairing and subsequent synapsis. We pay particular attention to the mechanisms that contribute to meiotic DNA double-strand break (DSB) formation and strand exchange processes, and how the *C. elegans* system compares with other model organisms. Finally, we highlight current and future areas of research that are likely to further our understanding of the meiotic recombination process.

## *Caenorhabditis elegans*: a model system to study meiotic prophase progression

Meiosis is the specialized cell division by which diploid organisms generate haploid gametes key to sexual reproduction (Roeder 1997, Page & Hawley 2003). The reduction in chromosome number in the gametes is achieved by one round of DNA replication followed by two divisions (known as meiosis I and meiosis II); in the first division, homologous chromosomes are segregated, followed by the segregation of sister chromatids in the next round of division corresponding to meiosis II. To achieve correct chromosome segregation during meiosis I, homologous chromosomes must first recognize each other and align along their lengths (Zickler & Kleckner 1998). Once alignment and pairing is achieved synapsis occurs between homologous chromosomes with the assembly of a proteinaceous structure known as the synaptonemal complex (SC), which ensures that homologs remain in close proximity until the obligate crossover has been generated by double-strand break (DSB)-induced recombination (Roeder 1997, Zickler & Kleckner 1999). It is within the context of the SC that crossover recombination occurs, which ultimately gives rise to crossovers that visibly manifest as chiasma. In addition to shuffling genes, crossover recombination is crucial because the chiasma is a physical link between homologs that enable them to correctly orient toward opposite spindle poles, an event that is critical for faithful segregation at the first meiotic division (Petronczki *et al.* 2003). Failure to correctly align chromosomes on the meiotic spindle frequently

results in aneuploidy, which has catastrophic consequences for embryonic development (Hassold & Hunt 2001).

*C. elegans* is a well-established model organism for the study of meiosis. The nematode is amenable to genetic manipulation through forward and reverse genetic approaches, which permits dissection of complex pathways. This system also possesses well-developed cytological tools, such as fluorescence *in-situ* hybridization (FISH) (Dernburg *et al.* 1998) and immunolocalization (Zetka *et al.* 1999), useful for meiotic mutant characterization. Meiosis in *C. elegans* not only displays canonical features with other eukaryotes but the linear progression through meiotic events within the gonad makes the adult hermaphrodite a particularly attractive model system to study meiosis (Albertson *et al.* 1997 *C. elegans* II). In the adult *C. elegans* hermaphrodite, germline nuclei in the distal tip of the gonad arm divide mitotically and thereafter enter meiotic prophase I as they progress away from the distal end of the gonad. The prophase of meiotic division I is traditionally divided into five sequential stages – leptotene, zygotene, pachytene, diplotene and diakinesis – defined by a number of characteristic morphological changes associated with homolog alignment, pairing, synapsis and desynapsis. Direct visual observation of the hallmarks of each phase has been a useful starting point for the analysis of meiotic mutants in *C. elegans* (Figure 1). Following pre-meiotic S-phase, initial pairing events between homologous chromosomes takes place in the transition zone (Dernburg *et al.* 1998) when the polarized redistribution of chromosomes give rise to the characteristic 'crescent'-shaped DNA. In early pachytene, homolog synapsis resulting from formation of the SC can be recognized in nuclei as parallel DAPI-stained chromosomes that track either side of the SC (Goldstein & Slaton 1982, Dernburg *et al.* 1998, MacQueen *et al.* 2002, Colaiacovo *et al.* 2003). Synapsis is visibly complete by mid-pachytene. Once the obligate crossover has formed desynapsis is initiated and chromosome condensation begins during diplotene (Nabeshima *et al.* 2005). It is at this stage that the emerging chiasmata can be first seen holding homologous chromosomes together. Finally, oocyte nuclei arrested at diakinesis can be recognized in the proximal region of the germline with six distinct DAPI-stained bodies or bivalents, that correspond to highly condensed homologous chromosome pairs attached by the chiasma. After fertilization, in the subsequent metaphase I, all the bivalents line up on the spindle, and at anaphase I the two duplicated homologs separate from each other and move to the opposite poles of the spindle, and the cell divides. To produce haploid gametes, a second cell division is required in which sister chromatids are segregated (McCarter *et al.* 1999).

A number of different genetic screens have been particularly useful in defining genes that impact on meiotic progression in *C. elegans*. The 'high incidence of males' or Him phenotype (Hodgking *et al.* 1979) is a powerful diagnostic of chromosome segregation defects. *C. elegans* hermaphrodites possess two X chromosomes (XX), while males are X0. Hermaphrodites produce both sperm and oocytes, and can reproduce either by self-fertilization or by mating with males, which only produce sperm. Males normally arise among the self-progeny of hermaphrodites at low frequency (0.1–0.2%) through stochastic non-disjunction of the X chromosome. Mutations in many meiotic genes lead to errors in X chromosome segregation that manifest as a significant increase in the frequency of males. Frequently, the Him phenotype of meiotic mutants is coupled with extensive embryonic lethality, as a consequence of autosome non-disjunction. An improved screening method for meiotic mutants based on the Him phenotype has been to look for an increase in males produced by hermaphrodites carrying a *Pxol-1::gfp* reporter transgene (Kelly *et al.* 2000). The 'green eggs and Him' screen is based on the fact that *Poxl-1::gfp* transgene expresses only in male embryos. The presence of increased numbers of green-fluorescent embryos therefore correlates with the Him phenotype. Importantly, since many meiotic mutants are inviable, mutagenized hermaphrodites containing elevated numbers of GFP-positive embryos can be immediately crossed to wild-type males to facilitate rescue of lethal meiotic genes. This dual strategy has been very useful in identifying meiotic components required for pairing and recombination processes (see below).

## From mitosis to meiosis

As mitotic cells move away from the distal end of the gonad they undergo pre-meiotic DNA replication as they enter the meiotic program. After chromosomes

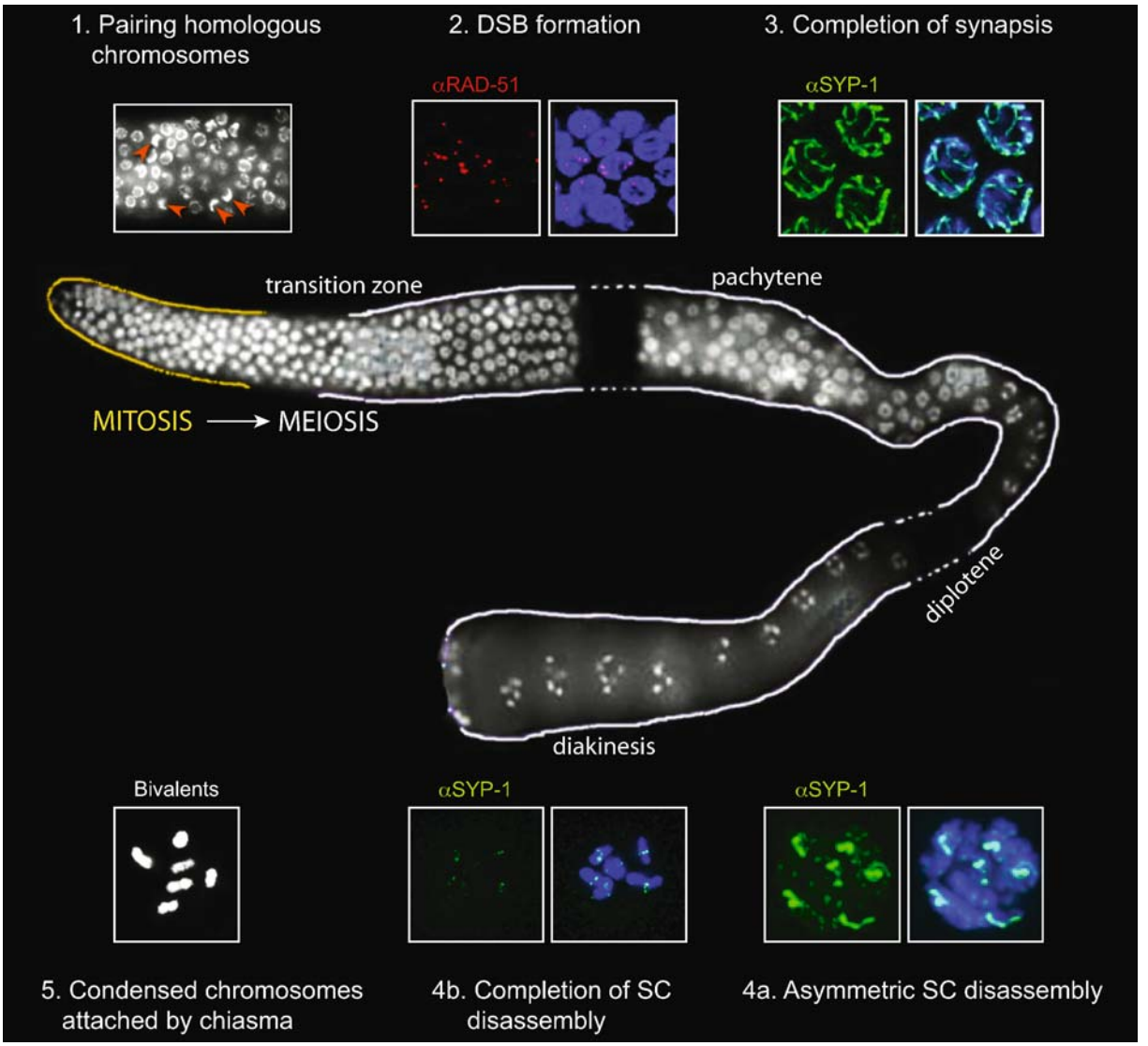


*Figure 1.* Schematic representation of the hallmarks of meiotic prophase progression in the *C. elegans* germline. Shown in a representative image of a DAPI-stained germline extracted from wild-type (N2) worms (modified from J. Maciejowski & E.J. Hubbard), and immunostaining with RAD-51 and SYP-1 antibodies (MacQueen *et al.* 2002, Alpi *et al.* 2003).

have been duplicated during DNA replication, the sister chromatids remain tightly linked along their length by the loading of cohesin complexes (Cohen-Fix 2001, Lee & Orr-Weaver 2001). The cohesion of sister chromatids has been well defined within the context of the mitotic cell cycle, where sister chromatids are separated during a single division cycle through the proteolytic cleavage of the cohesin complexes by separase (Ciosk *et al.* 1998, Uhlmann *et al.* 1999). In contrast, the maintenance of cohesion between sister chromatids is essential for the correct segregation of homologous chromosomes at meiosis I; cohesion is first released along the arms at the onset of anaphase I but sister chromatids remain tethered by cohesin complexes retained in the vicinity of centromeres. These cohesin complexes remain until the second meiotic division. Meiotic cohesin complexes differ from their mitotic equivalents in that certain mitotic components (Scc1/Mdc1/Rad21, Scc3/SA1/SA2, Smc1 and Smc3) are replaced by

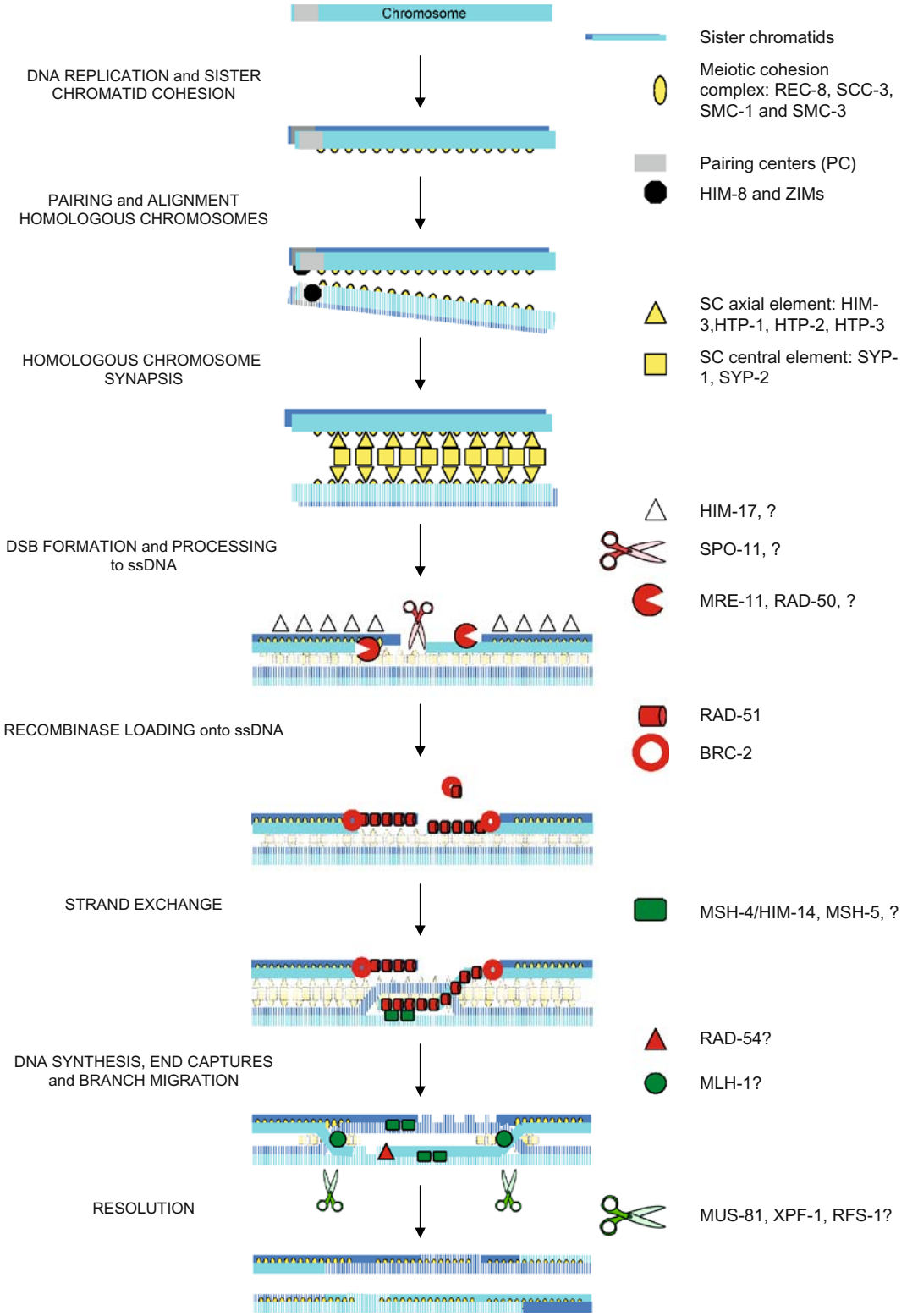


*Figure 2.* Schematic of the sequential steps and the proteins involved during meiotic prophase progression in *C. elegans*.

meiosis-specific variants. In yeast only Scc1 is replaced by meiosis-specific Rec8 (Klein *et al.* 1999, Watanabe & Nurse 1999), whereas in mammalian meiosis two additional replacements occur: Smc1 by Smc1β, and STAG3 for SA1/SA2 (Prieto *et al.* 2001, Revenkova *et al.* 2001).

While single candidates where found for SCC-3 (Wang *et al.* 2003) and the two subunits belonging to the structural maintenance of chromosomes (SMC) family (Pasierbek *et al.* 2003, Chan *et al.* 2003), SMC-1 (or HIM-1) and SMC-3, REC-8 is one of the four Rad21/Scc1/Rec8 homologs present in *C. elegans* genome (Pasierbek *et al.* 2001). REC-8 localizes at chromosomal axes throughout meiosis I, and depletion causes embryonic lethality, a strong Him phenotype and cytological defects associated with defective synapsis and premature separation of sister chromatids prior to the first meiotic division. Evidence suggests that REC-8 is the meiotic cohesin protein homolog to Rad21/Rec8. Reciprocal co-immunoprecipitation of SCC-3, SMC-1, SMC-3 and REC-8 indicates that these four proteins form a complex. Furthermore, SCC-3, SMC-1 and SMC-3 recapitulated the meiotic localization pattern described for REC-8 and their depletion or deletion produced a phenotype with cytological defects analogous to that observed in the absence of *rec-8* (Chan *et al.* 2003). Taken together these data confirm that REC-8, SCC-3, SMC-1 and SMC-3 consitute the *C. elegans* meiotic cohesin complex required for the correct segregation of homologous chromosomes (Figure 2). Although sister chromatids are not held together in worms deficient for meiotic cohesin components, it has been shown that the initial recognition and pairing of homologs in leptotene/zygotene is only decreased. This suggests that the initial homolog interactions leading to pairing are not solely dependent on cohension and SC formation.

## Finding your partner: homolog alignment and pairing

During pairing, homologous chromosomes recognize each other, align and become physically connected along their entire length. How homologous chromosomes recognize each other is still uncertain. For many organisms, models involving complementary DNA base-pair interactions between intact duplex at numerous and widely dispersed sites along chromosomes have been proposed (Weiner & Kleckner 1994, Gerton & Hawley 2005). A number of elegant studies have established that *C. elegans* chromosomes possess specialized pairing regions that are required for stabilizing homolog interactions and for promoting initiation of synapsis. Evidence for such sites came from experiments examining the meiotic behavior of strains carrying various chromosome rearrangements/deletions that led to the identification of *cis*-acting regions on each chromosome which are collectively termed pairing centers (PC) (McKim *et al.* 1993, Villeneuve 1994 , MacQueen *et al.* 2005).

Two recent studies have characterized four related zinc-finger proteins (HIM-8, ZIM-1, ZIM-2 and ZIM-3), which directly interact with the PC and are essential for promoting pairing and homologous chromosome synapsis during meiosis (Phillips *et al.* 2005, Phillips & Dernburg 2006). Analysis revealed that HIM-8 and ZIM-2 interact with the PC on chromosome X and V respectively, ZIM-1 associates with the PC on chromosomes II and III, and ZIM-3 interacts with the PC on chromosomes I and IV (Figure 2). It is currently unclear why the X and V chromosome have unique PC binding proteins or why the two remaining pairs of autosomes share a single protein. This indicates that the specificity of homolog recognition cannot be solely defined by either the role of the PC or by the identity of the corresponding ZIM/HIM-8 family member. It is also interesting to note that the PC-associated protein ZIM/HIM-8 associates with the nuclear envelope and that, in the case of the three ZIM, their subnuclear localization seems to be lost in *chk-2* mutants (Phillips & Dernburg 2006) that are compromised for homolog alignment and pairing (MacQueen *et al.* 2001).

CHK-2 is a member of the checkpoint kinase Cds1/Rad53 family (MacQueen & Villeneuve 2001, Oishi *et al.* 2001). Analysis of the *chk-2* mutant revealed that CHK-2 is required for the initial establishment of pairing between homologous chromosomes as well as for chiasma formation. Indeed, *chk-2* mutants fail to exhibit the characteristic crescent-shaped nuclear morphology associated with homolog pairing. The pairing defect in *chk-2* mutants can now be partially explained by the mislocalization of the ZIM proteins. However, HIM-8 localization to the X chromosome PC is unaffected in the *chk-2* mutants, implying that the regulation of pairing

differs between autosomes and the X chromosome (Phillips & Dernburg 2006). Despite this, the precise role for CHK-2 in controlling homolog pairing remains to be elucidated. An intriguing possibility is that phosphorylation of ZIM proteins by CHK-2 might be important for their correct subcellular localization. The possibility that phosphorylation events might be more generally involved in controlling the various stages of meiotic prophase progression has not been explored in any great detail. However, studies are currently under way to identify all potential phosphorylation sites in known *C. elegans* meiotic proteins using peptide array technologies. Peptide arrays can be used in conjunction with specific recombinant kinases such as CHK-2 or more generally with *C. elegans* meiotic extracts. Coupling this *in-vitro* approach with *in-vivo* assays, such as mutant complementation, will be important for confirming the relevance of specific phosphorylation events in controlling meiotic prophase progression. This kind of analysis has the potential to further our understanding of the regulation of meiosis in *C. elegans*.

## Synapsis through SC formation

In organisms such as yeast and mammals, initiation of meiotic recombination is a prerequisite to SC formation. In contrast, SC formation occurs independent of initiation of meiotic recombination in *C. elegans* and *Drosophila melanogaster* (reviewed by Gerton & Hawley 2005, Roeder 1997). Indeed, *C. elegans* mutants in genes required for initiation or completion of meiotic recombination assemble the SC as normal (Dernburg *et al.* 1998, Alpi *et al.* 2003). The lack of chiasmata between homologs only becomes apparent cytologically in these mutants as the SC disassembles (see below). The lack of a requirement of DSB to initiate synapsis likely reflects the use of alternative ways of homolog recognition such as the use of pairing centers in the worm and somatic pairing in the fly (MacQueen *et al.* 2005, Vazquez *et al.* 2002). In all organisms, however, the recombination process is completed while homologous chromosomes are held in close juxtaposition by the SC, which likely aids in homology searching prior to strand exchange and heteroduplex formation.

The SC is a highly ordered tripartite structure composed of two parallel lateral elements, corresponding to the proteinaceous scaffolds along the individual chromosomes, and a central region, which contains transverse elements that lie perpendicular to the lateral elements (reviewed by Heyting 1996, Page & Hawley 2004). The axial elements play important roles in chromosome condensation, pairing, synapsis, and inhibiting meiotic recombination between sister chromatids; while the central element forms the basis of synapsis and is likely involved in crossover interference (see below). In yeast, DNA is organized around the lateral element, which contains the Rec8 cohesin and axial elements such Red1 and Hop1 (Hollingsworth *et al.* 1990, Hollingsworth & Johnson 1993). No obvious Red1 homologs have been described in mammals or *C. elegans*, although functional counterparts of Hop1 do exist. Hop1 belongs to the meiosis-specific HORMA domain protein family (mammals: HORMAD1; *C. elegans*: HIM-3, HTP-1, HTP-2 and HTP-3). So far all the *C. elegans* HORMA proteins have been implicated in structural maintenance and dynamics of homolog pairing and synapsis (Zetka *et al.* 1999, Couteau *et al.* 2004, Couteau & Zetka 2005, Martinez-Perez & Villeneuve 2005, MacQueen *et al.* 2005). HIM-3 is a major component of the lateral element, and localizes to chromosomes prior to synapsis where it co-localizes with REC-8 along the chromosome length (Figure 2). Analysis of *him-3* mutants indicates that HIM-3 facilitates synapsis by promoting the polymerization of the SC central core elements: SYP-1 and SYP-2 (Zetka *et al.* 1999, Colaiacovo *et al.* 2003, Couteau *et al.* 2004). It has also been proposed that HIM-3 might perform a role in enforcing recombination between homologs whilst acting as a barrier to repair through the sister, analogous to Hop1, its ortholog in budding yeast (Niu *et al.* 2005).

In *C. elegans* meiotic recombination can be monitored by analyzing the dynamics of assembly and disassembly of the strand exchange protein RAD-51 onto meiotic DSB (Alpi *et al.* 2003, Martin *et al.* 2005). In the absence of synapsis meiotic DSBs are not efficiently repaired and RAD-51 foci persist, presumably because the homolog is not in close juxtaposition, and repair through the sister is temporarily blocked. Indeed, in the *him-3* null mutant homologous chromosomes fail to synapse, yet the kinetics of assembly and disassembly of RAD-51 foci at meiotic DSB resembles that of the wild type (Zetka *et al.* 1999, Colaiacovo *et al.* 2003, Couteau

*et al*. 2004). This suggests that, although DSBs cannot be repaired through the homolog (due to the defect in synapsis), they are repaired with normal kinetics through the sister chromatid. It is therefore likely that HIM-3 normally functions to temporarily prevent DSB repair between sister chromatids to ensure formation of the obligate crossover between homolog pairs, analogous to its yeast counterpart Hop1 (Niu *et al*. 2005).

A similar defect in using the sister chromatid as a template for meiotic DSB repair is observable in *htp-1* mutants, although HTP-1 differs from HIM-3 in that it also prevents the stabilization of inappropriate synapsis between nonhomologous autosomes (Couteau & Zetka 2005, Martinez-Perez & Villeneuve 2005). Exactly how HTP-1 functions to prevent inappropriate synapsis remains unclear. Currently, conclusive evidence for the function of HTP-2 in meiosis has been hampered by the lack of a null mutant for *htp-2,* although RNAi supports a role in promoting SC assembly (Couteau & Zetka 2005). Studies in yeast indicate that Mnd1–Hop2 complex is a meiosis-specific heterodimer required to ensure and promote strand invasion with the correct template DNA (Tsubouchi & Roeder 2002, Pezza *et al*. 2006). It therefore appears that the functions of HTP-1 and HTP-2 resemble that of Mnd1-Hop2 in *S. cerevisiae*, although there is no obvious sequence similarity between the *C. elegans* and yeast proteins.

SYP-1 and SYP-2 are components of the central region of the SC in *C. elegans* (MacQueen *et al*. 2002, Colaiacovo *et al*. 2003). Although there is no obvious sequence similarity among SC core components between different species, these proteins contain a central coiled-coil motif, that is also present in the yeast homolog Zip1 and in mammalian SCP1, which are both required for normal meiotic recombination, respectively (Meuwissen *et al*. 1992, Sym *et al*. 1993). In the absence of SYP-1 or SYP-2 meiotic DSBs are created with normal timing, as revealed by the timely accumulation of RAD-51 foci, but synapsis fails to occur. However, RAD-51 foci persist throughout pachytene and only begin to disappear in diplotene nuclei, presumably following repair of meiotic DSB between sister chromatids, once the barrier to such events has been removed (Colaiacovo *et al*. 2003). The presence of 12 intact univalents at diakinesis in *syp* mutants suggests that meiotic DSB repair has occurred. This situation contrasts with the highly irregular decondensed chromosomes evident at diakinesis in mutants defective for meiotic DSB repair (e.g. *rad-51* or *brc-2* mutants (Rinaldo *et al*. 2002, Martin *et al*. 2005)).

It is known that the yeast SC central region components Zip2 and Zip3 are both required to promote Zip1 assembly between homologous chromosomes (Chua & Roeder 1998, Agarwal & Roeder 2000). It is believed that Zip3 marks the DSB sites that will become a crossover and subsequently recruits Zip2 to finally promote SC formation via Zip1 assembly (Agarwal & Roeder 2000). Although yeast and *C. elegans* differ in their dependence on DSB formation for synapsis a sequence homolog for Zip3 is present in *C. elegans* (ZPH-3 (Jantsch *et al*. 2004)). Contrary to its 'homolog' in yeast, chromosome localization of ZHP-3 is dependent on SYP-1 but independent of DSB formation. Deletion of *zhp-3* does not affect SC or DSB formation, but the presence of univalents at diakinesis indicates that this protein is essential for meiotic recombination at some level (Jantsch *et al*. 2004). The precise function of ZHP-3 in *C. elegans* meiotic recombination remains unclear.

## Control of meiotic DSB formation

Crossover or chiasma formation requires temporal coordination of the initiation of meiotic recombination with progression through prophase, but molecular events ensuring this coordination remain elusive. Meiotic recombination is initiated in all eukaryotes by the formation of a DSB through the action of a specialized topoisomerase enzyme Spo11 (Keeney *et al*. 1997). In *C. elegans*, *spo-11* mutants exhibit extensive embryonic lethality and a severe Him phenotype, indicative of errors in meiotic chromosome segregation (Dernburg *et al*. 1998). Cytological analysis of *spo-11* mutants reveals 12 univalents at diakinesis, instead of the six bivalents in wild-type animals, suggestive of a defect in crossover formation. Consistent with the proposed role for SPO-11 in DSB generation, *spo-11* mutants are partially rescued by artificially inducing DSBs using ionizing radiation, as was shown for the yeast *spo11* mutant (Figure 2). Furthermore, RAD-51 foci fail to form in *spo-11* mutants, indicative of an absence of DSBs. Although the mechanism for initiation of meiotic recombination is conserved throughout eukaryotes, the analysis of *spo-11* mutants revealed that pairing and synapsis

occur normally (Dernburg *et al*. 1998). The fact that SC formation occurs independent of *spo-11* in *C. elegans* permits the analysis of mutants that function in the recombination process *per se* without the added complication that defects observed may result from failure to initiate synapsis.

In addition to Spo11 nine other genes are known to be absolutely required for meiotic DSB formation in yeast, i.e. Rad50, Mre11, Xrs2, Mer2, Mei4, Rec102, Rec104, Rec114 and Ski8. Rad50, Mre11 and Xrs2 encode a conserved protein complex critical for Spo11 function, DSB processing and sensing of DSBs in mitotic cells (see below) (Gerton & Hawley 2005). Several observations suggest that Spo11 acts together with Ski8, Rec102 and Rec104 in a multi-protein complex essential for DSB formation (Arora *et al*. 2004, Kee *et al*. 2004); Ski8 (a WD repeats protein) acts as scaffold protein for Spo11 localization to chromatin and to Rec102/Rec104 complex, whose role is still unclear. No Rec102 or Rec104 sequence homologs are found in *C. elegans* or mammals. Two recent studies suggest that Mer2, Mei4 and Rec114 also form a complex (Henderson *et al*. 2006, Li *et al*. 2006). Moreover, Mer2 is a substrate for the yeast cyclin-dependent kinase Cdc28, suggestive of regulatory connection between cell cycle and meiotic DSB formation (Henderson *et al*. 2006). Phosphorylation of Mer2 appears to modulate its interactions with the Rec114/Mei4 complex but more studies are necessary to clarify the exact function of this complex. Again no sequence homologs have been found in *C. elegans* or mammalians for these three proteins. The absence of homologs in *C. elegans* could be explained by an alternative mechanism of DSB initiation and regulation, due to the different scenarios in which DSB formation is initiated: along paired chromosomes in yeast and mostly within the context of the SC in worms. But this would not explain why the search for homologs in mammalian genomes has been unsuccessful. A more likely scenario is that functional homologs exist in all eukaryotes but are not obvious based on sequence homology alone. It is likely that further analysis of the yeast DSB-initiation complexes may suggest opportunities for identification of functional equivalents in higher eukaryotes.

It is been speculated that the yeast meiotic DSB initiation complexes may somehow affect competence for DSB formation through inducing alterations in chromosome structure, potentially through epigenetic modifications. In this respect a recently characterized protein, HIM-17, is the first reported factor in *C. elegans* that functions to establish competence for DSB formation through inducing modifications in chromatin (Reddy & Villeneuve 2004). *him-17* mutants exhibit 12 univalents at diakinesis, are defect for RAD-51 focus formation and, like the *spo-11* mutant, both phenotypes can be partially rescued by introducing artificial DSB using ionizing radiation. These data place HIM-17 function at the level of meiotic DSB formation. HIM-17 was shown to localize to chromatin throughout the germline, suggestive of a role on chromatin. Indeed, immunostaining of *him-17* mutants with antibodies to lysine 9 methylated histone H3 (H3MeK9), a marker of heterochromatin and transcriptionally inactive DNA, showed an abnormal pattern (Reddy & Villeneuve 2004). These results suggested that HIM-17 might induce changes to chromatin structure that are important for SPO-11 function, potential at the level of chromatin accessibility. To understand how the chromatin modifications induced by HIM-17 regulate meiotic DBS formation is clearly an important area of investigation that may shed further light on the regulation of SPO-11 action.

Following the generation of a DSB by Spo11 the DNA ends at the break site must be first processed into 3′ single-stranded DNA tails by the combined action of helicases and exonucleases. The MRN complex (consisting of MRE11, RAD50, and NBS1/Xrs2) is required for DSB formation, processing, and checkpoint signaling during meiotic cell division in yeast (van den Bosch *et al*. 2003). *C. elegans* possesses homologs for Mre11 and Rad50 (Chin & Villeneuve 2001, Colaiacovo *et al*. 2002) and a putative candidate that shares weak similarity to part of the human NBS1 protein. Consistent with a critical meiotic function *mre-11* mutants exhibit 12 univalents at diakinesis indicative of a defect in meiotic recombination. Unlike *spo-11* mutants, IR-treatment of *mre-11* mutants does not restore crossover formation but instead leads to chromosomal abnormalities at diakinesis. The latter is suggestive of a role in DSB processing but it is currently unclear if meiotic DSB are actually formed in *mre-11* mutants, but are not efficiently processed, or if MRE-11 is required for the generation of DSBs by SPO-11. RNAi knockdown of *rad-50* has been shown to result in univalents at diakinesis analogous to *spo-11* and *mre-11* mutants but confirmation of the

precise meiotic function of *rad-50* will require studies in a deletion mutant.

## Molecular events of meiotic recombination

In all organisms the RecA/Rad51 family of recombinases catalyze the strand-invasion and strand-exchange reactions between homologous DNA molecules (West 2003). Homologs and paralogs have been found in all eukaryotes; among these, Dmc1, which encodes the meiotic ortholog of budding yeast Rad51 (Bishop *et al.* 1992). In *C. elegans* there is only one recombinase protein RAD-51, which acts in both mitotic and meiotic recombination (Rinaldo *et al.* 2002). The processed ssDNA tails at DSBs are the substrate onto which monomers of RAD-51 polymerize to form a nucleoprotein filament, which can be visualized cytologically as discrete nuclear foci that form in a SPO-11-dependent manner at meiotic DSB. The RAD-51-DNA filament executes the central functions in homologous recombination: search for the homologous template and invasion into an intact homologous sequence with the formation of heteroduplex DNA (Figure 2). Although bacterial RecA is a potent recombinase, its eukarytotic counterparts (Rad51), including *C. elegans* RAD-51 (Petalcorin *et al.* 2006) are weak recombinase enzymes that require additional co-factors/mediators to potentiate their activity in recombination. Yeast Rad52 is the prototypical mediator that binds directly to Rad51, facilitates its loading onto DSBs, and stimulates Rad51-mediated strand exchange and D-loop formation. Surprisingly, *C. elegans* does not possess a Rad52 homolog, a situation that is mirrored in *D. melanogaster* and *Arabidopsis thaliana*. Although Rad52 is critical to recombination in yeast, the mammalian homolog is largely dispensable for recombination. Rather it appears that alternative mediator proteins have taken over the role of Rad52 in facilitating Rad51 recombination reactions. Indeed central to recombination in higher eukaryotes is BRCA2, one of the two major breast and ovarian cancer tumor-suppressor proteins.

The human BRCA2 protein contains eight BRC repeats that confer direct binding to Rad51 and three oligonucleotide–oligosaccharide binding (OB-fold) domains that mediate binding to ssDNA/dsDNA transitions (Tutt *et al.* 2001, Venkitaraman 2000, 2001). Based on these observations, and the fact that cells lacking BRCA2 fail to load RAD51 onto DNA breaks, it has been speculated that BRCA2 recruits RAD51 to sites of DNA damage where it may facilitate binding of RAD51 onto processed breaks and could modulate other RAD51 activities (Patel *et al.* 1998, Jasin 2002, Moynahan 2002, Moynahan *et al.* 2001, Wilson & Elledge 2002). How this occurs at the mechanistic level is not well understood. Since human BRCA2 is a 3418aa protein, it has not been possible to express and purify the full-length protein for biochemical analysis.

One of the hallmarks of BRCA2 is the BRC motif. In a search for proteins with similarities to the BRC, a putative homolog of BRCA2 was identified in *C. elegans* (CeBRC-2) (Martin *et al.* 2005). Remarkably, this protein possesses a single BRC and one OB-fold condensed into a 394aa polypeptide. CeBRC-2 was shown to interact directly with RAD-51 *in vitro* and *in vivo* through its single BRC domain (Martin *et al.* 2005). The single OB-fold situated in the C-terminal part of CeBRC-2 confers preferential binding to ssDNA. CeBRC-2 also contains two consensus nuclear localization signals (NLS) that flank the OB-fold domain. It is noticeable that RAD-51 lacks an obvious NLS, analogous to its human counterpart. Indeed, CAPAN1 cells that harbor a truncating mutation in human BRCA2 exhibit cytoplasmic localization of Rad51, indicating that one of the normal functions of BRCA2 is to facilitate transport of Rad51 into the nucleus. A very similar situation exists in *C. elegans*; *Cebrc-2* mutants are defect for efficient nuclear localization of RAD-51. Although CeBRC-2 is approximately one-tenth the size of its human counterpart, deletion mutants in *Cebrc-2* exhibit many other defects associated with *BRCA2* deficiency in human cells, indicating that the critical functions of BRCA2 are conserved in *C. elegans*. Specifically, CeBRC-2 prevents extensive chromosomal fragmentation during early embryogenesis and is required for the repair of both meiotic and IR-induced DSB, defects that are partially attributable to a failure to target Rad51 into the nucleus. In addition to a role in nuclear localization of RAD-51, dominant negative experiments using the BRC domain of CeBRC-2 alone revealed a role in facilitating loading of RAD-51 onto processed meiotic DSB. Unlike *rad-51* mutants, animals deficient for CeBRC-2 accumulate replication protein A (RPA) foci that are dependent on meiotic DSB formation by SPO-11 (Martin *et al.*

2005). These data suggested that, in the absence of CeBRC-2, DSBs are hyper-resected to generate extensive regions of ssDNA. The fact that CeBRC-2 is recruited to meiotic DSBs in the absence of RAD-51, and that *rad-51* mutants do not accumulate RPA foci at meiotic DSB, suggests that CeBRC-2 is able to load onto DSB independent of RAD-51, displace RPA from ssDNA and likely minimizes extensive DSB processing by exonucleases. However, its normal function in wild-type cells is to facilitate nuclear localization and loading of RAD-51 onto meiotic DSB (Figure 2).

Unlike *spo-11* and *mre-11* mutants, animals deficient for *rad-51* or *Cebrc-2* exhibit extensive chromosomal abnormalities and chromatin decondensation at diakinesis (Martin *et al*. 2005). This difference from the situation observed in *spo-11* and *mre-11* mutants likely reflects the role for RAD-51 and CeBRC-2 in all aspects of meiotic DSB repair; crossover and non-crossover formation and repair of DSB using the sister chromatid as a template. The fact that *mre-11* mutants resemble *spo-11* mutants, but not *rad-51* or *Cebrc-2*, also supports the idea that MRE-11 is required for meiotic DSB formation, although this requires further confirmation. Surprisingly, analysis of *Cebrc-2* mutants also suggested functions independent of *rad-51* in an error-prone repair pathway distinct from NHEJ (Martin *et al*. 2005). It was speculated that, since *C. elegans* lack a Rad52 homolog, CeBRC-2 may have taken over the role of Rad52 in regulating RAD-51 during HR and may also function in error-prone repair via the single strand-annealing pathway (SSA).

Biochemical analysis supports a role for CeBRC-2 in facilitating RAD-51 functions post formation of the RAD-51 nucleoprotein filament. It was demonstrated that recombinant CeBRC-2 stimulated RAD-51-mediated D-loop formation and reduced the rate of ATP hydrolysis catalyzed by RAD-51 (Petalcorin *et al*. 2006). These findings, together with related studies using *Ustilago maydis* BRCA2 and RAD51 proteins and fragments of human BRCA2, suggested that CeBRC-2 and its counterparts in other eukaryotes function as mediators of RAD-51-dependent recombination reactions (Kojic *et al*. 2002, Yang *et al*. 2002, 2005). The fact that CeBRC-2 also inhibits ATP hydrolysis by RAD-51 would suggest that CeBRC-2 can prevent RAD-51 nucleoprotein filament depolymerization; ATP hydrolysis leads to depolymerization of the RAD-51-DNA filament (Galletto *et al*. 2006, Joo *et al*. 2006).

Human BRCA2 also associates with a small, highly acidic protein DSS1 that is strongly conserved throughout eukaryotes (Marston *et al*. 1999, Yang *et al*. 2002). DSS1 appears to play an important role in the HR pathway as disruption of Dss1 in *U. maydis* confers meiotic recombination defects that are epistatic with the Rad51 pathway. Furthermore, siRNA of DSS1 in human cells leads to the accumulation of abnormal DNA structures and a defect in Rad51 focus formation at DNA damage sites, analogous to the defects observed in BRCA2-deficient cells (Kojic *et al*. 2003, Gudmundsdottir *et al*. 2004). The *C. elegans* DSS1 ortholog (Y119D3B.15) possesses 75% sequence identity to its human and *U. maydis* counterparts. However, the helical domain-OB1 region that binds DSS1 in human BRCA2 is not obviously conserved in CeBRC-2. Although DSS-1 significantly improves the solubility of CeBRC-2 when the two proteins are co-expressed in *E. coli* (Petalcorin *et al*. 2006), DSS-1 and CeBRC-2 do not co-purify over gel filtration and do not interact in either yeast two-hybrid or in pull-down experiments. Moreover, DSS-1 is dispensable for CeBRC-2 to stimulate RAD-51-mediated D-loop formation (Petalcorin *et al*. 2006). A deletion mutant in *dss-1* has recently become available that will hopefully provide insight into any potential functions in meiotic recombination.

A number of other proteins implicated in meiotic recombination in yeast and/or mammalian cells are also present in *C. elegans*. These include homologs of BRCA1, BARD1, a Rad51 paralog, and Rad54 (BRC-1, BRD-1, RFS-1 and RAD-54, respectively). It appears that BRC-1 and its heterodimeric partner BRD-1 do not play critical roles in meiotic recombination as deletion mutants exhibit six intact bivalents at diakinesis indicative of successful crossover recombination (Boulton *et al*. 2004). However, both mutants exhibit a weak Him phenotype that may suggest a subtle role in recombination. The precise function of BRC-1/BRD-1 in meiosis is currently unclear but this is an area that we are actively working on. Although deletion mutants in *rfs-1* and *rad-54* are available meiotic recombination functions have yet to be reported (Figure 2). Understanding how these accessory proteins interplay with Rad51 is critical to dissect the mechanisms of meiotic

recombination. What remains completely unclear is how RAD-51 dependent homology searching and subsequent strand invasion events are performed within the context of the SC. It is unlikely that the SC simply acts as a scaffold, but may actively facilitate all steps required for initiation, propagation and completion of meiotic recombination.

The processing of strand exchange intermediates produces recombinant products that have either exchanged the flanking DNA arms (crossovers) or have not undergone exchange (non-crossovers). Crossover outcome in *C. elegans* is dependent on the meiosis-specific members of the MutS family, HIM-14/MSH-4 and MSH-5, conserved components of the core meiotic recombination machinery that promote crossover outcomes of initiated recombination events (Zalevsky *et al*. 1999, Kelly *et al*. 2000, Colaiacovo *et al*. 2003). Cytological analysis of *msh-4*/*him-14* and *msh-5* mutants revealed that homologs are paired and aligned and meiotic DSBs are generated as indicated by the formation of RAD-51 foci (Colaiacovo *et al*. 2003). However, the presence of persistent RAD-51 foci and 12 univalents in these mutants suggests that HIM-14/MSH-4 and MSH-5 perform a critical role after the formation of the RAD-51 nucleoprotein filament but upstream of Holliday junction resolution. Although *msh-4*/*him-14* and *msh-5* encode members of the MutS family no difference in spontaneous mutation frequency or defects in IR-induced DSB repair has been detected (Kelly *et al*. 2000). This suggests that HIM-14/MSH-4 and MSH-5, like their orthologs in budding yeast, have no detectable role in mismatch repair but rather may function specifically to promote crossing over during meiosis. In contrast to yeast, where mutants that lack Msh4 and/or Msh5 can still generate 30–50% of the normal crossovers (Ross-Macdonald & Roeder 1994, Hollingsworth *et al*. 1995), *C. elegans* relies exclusively on this pathway to generate crossovers, since the lack of either gene eliminates all crossovers. Although genetic studies have shown that MSH4-MSH5 strongly influences crossover formation their mechanism of action remains unclear. Markers that indicate the position of crossovers are not currently available in *C. elegans*. However, MLH1, a commonly used marker of crossovers in certain organisms (Baker *et al*. 1996), is present in *C. elegans* but remains uncharacterized (Figure 2).

The precise mechanisms that regulate crossing-over remain poorly understood in all organisms. Since crossover formation is critical for accurate chromosome segregation at the first meiotic division specific mechanisms must exist in all organisms to ensure that at least one crossover is generated per homolog pair. Crossover interference, a phenomenon in which the generation of a crossover at a particular site somehow interferes with the coincident occurrence of another crossover, results in crossovers that are widely spaced along the chromosome length (van Veen & Hawley 2003, Bishop & Zickler 2004). In the majority of higher eukaryotes one to three crossovers are formed per homolog pair. However, studies in *C. elegans* have revealed that one crossover is formed per bivalent, suggesting that a single crossover is capable of suppressing the formation of a second crossing over event along the entire chromosome length; this is an extreme case of crossover interference (Hillers & Villeneuve 2003). Support for a role for the chromosome axis in crossover interference has come from analysis of a hypomorphic allele in the axial component *him-3*, which retains the capacity to synapse and undergo crossover formation despite altered chromosome axis composition (Nabeshima *et al*. 2004). The analysis of this mutant provided evidence that the integrity of chromosome axis plays an important role in limiting the number of crossovers per homologous chromosome pair as an increase in double crossovers was observed. It should be noted that this mutant exhibits increased RAD-51 foci, raising the possibility that the increase in double crossover events may result from up-regulation in DSB formation. Understanding the mechanism of crossover interference is one of the outstanding challenges in the meiosis field. *C. elegans* is clearly an intriguing system for studying this phenomenon given the restriction of a single crossover event per homolog pair.

Evidence suggests that the chromosomal location of the crossover is also tightly controlled in *C. elegans*. The distribution of sites of meiotic exchanges on autosomes is restricted to regions that lie outside of gene clusters located in the middle of the chromosome; crossing over tends to occur in the 30% of the chromosome length from each autosome end (Brenner 1974, Hillers & Villeneuve 2003). Curiously the sites of meiotic exchanges on the X chromosome differ from autosomes as they are more

evenly distributed along the chromosome. Genetic studies have identified an uncloned locus, *rec-1*, that alters the distribution of crossovers on autosomes resulting in an increase in the frequency of crossovers within the central gene cluster region (Zetka & Rose 1990, 1995). It is therefore likely that the cloning of the mutation responsible for *rec-1* will provide important insights into the control of crossover formation.

Completion of meiotic recombination requires the resolution of heteroduplex structures that may resemble Holliday junctions. The prototypical resolvase in *E. coli* is RuvABC that enzymatically cleaves Holliday junctions in a symmetrical manner (reviewed in West 1997). Despite the absence of sequence homologs of RuvABC in higher eukaryotes mammalian cells do possess a similar resolvase activity associated with the Rad51C paralog, but the exact components of this enzymatic complex remain elusive (Liu *et al*. 2004). Evidence from fission yeast also suggests that structure-specific endonucleases such as Mus81 play important roles in completing meiotic recombination (Boddy *et al*. 2001, Chen *et al*. 2001, Kaliraman *et al*. 2001). Whilst homologs of Rad51 paralogs, Mus81 and XPF are present in *C. elegans* the endonuclease responsible for completing meiotic recombination is not yet known (Figure 2).

## Summary

During the past decade *C. elegans* has developed as a powerful system for studying meiotic prophase progression. It is clear that the factors that have made *C. elegans* an attractive system for such studies (forward and reverse genetics, spatial organization of the germline, uncoupled meiotic DSB formation and synapsis, etc.) will continue to aid our goal of understanding the genes and pathways that impact on meiosis. The recent identification of chromosome pairing centres and their associated Zn-finger binding

*Table 1*. List of the known *C. elegans* proteins required for the indicated steps in meiosis I in comparison with yeast and mammalian counterparts. Asterisks indicate the putative homologs identified by sequence homology, which remain to be characterized.

| Function | *S. cerevisiae* | Mammals | *C. elegans* |
|---|---|---|---|
| PCs | ? | ? | HIM-8 & ZIMs |
| cohesion | Rec8p | REC8 | REC-8 |
| | Scc3 | STAG3 | SCC-3 |
| | Smc1 | SMC1ß | SMC-1 |
| | Smc3 | SMC3 | SMC-3 |
| SC | Red1 - Hop1 | HORMAD1* | ? HIM-3 |
| | Mnd1-Hop2 | MND1- ? | HTP-1 - HTP-2 |
| | ? | ? | HTP-3 |
| | Zip1p | SCP1 | SYP-1 SYP-2 |
| | Zip3 Zip2 | SCP2 SCP3 | ZHP-3 ? |
| DSB formation | Spo11p | SPO11 | SPO-11 |
| | Ski8 - Rec102 - Rec104 | ? ? ? | ? ? ? |
| | Mer2 - Mei4 - Rec114 | ? ? ? | ? ? ? |
| | Mre11 Rad50 Xrs2 | MRE11 RAD50 NSB1 | MRE-11 RAD-50 XNP-1* |
| Meiotic recombination | Rad51 | RAD51 | RAD-51 |
| | Dmc1 | DMC1 | RAD-51 |
| | Rad55, Rad57 | RAD51B-C-D, XCCC2-3 | RFS-1* |
| | Mei5-Sae3 | ? ? | ? ? |
| | Rad52 ? | (RAD52?) BRCA2 | ? BRC-2 |
| | Rad54 | RAD54 | RAD-54* |
| | Msh4-Msh5 | MSH4 MSH5 | MSH-4/HIM-14 MSH-5 |
| | Mlh1 | MLH1 | MLH-1* |
| | | EME1 | EME-1* |
| | Mus81? | MUS81 | MUS-81* |

factors has placed *C. elegans* at the forefront of meiotic research into homolog recognition, pairing and synapsis. Analogous to the mitotic cell cycle, meiotic progression is likely to be controlled by a plethora of post-translational modifications of which we know very little. The demonstrations that the CHK-2 kinase is critical for homolog alignment and pairing and HIM-17 is important for meiotic DSB formation likely represent the first of many factors that impact on meiotic progression through post-translational modification. Future studies in these areas are likely to advance our understanding of key steps in meiosis in all organisms (Table 1).

In the years ahead *C. elegans* is likely to contribute significantly to our understanding of conserved mechanisms of meiotic prophase progression, such as initiation of meiotic DSB formation and synapsis, whilst mechanistic differences with other models will bring a better understanding of how complex issues can be solved by alternative means.

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