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REVIEW

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The many facets of SC function during *C. elegans* meiosis

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Abstract Sexually reproducing organisms rely on meiosis for the formation of haploid gametes. This is achieved through two consecutive rounds of cell division (meiosis I and II) after one round of DNA replication. During the meiotic divisions, chromosomes face several challenges to ultimately ensure proper chromosome segregation. Unique events unfold during meiosis I to overcome these challenges. Homologous chromosomes pair, synapse, and recombine. A remarkable feature throughout this process is the formation of an evolutionarily conserved tripartite proteinaceous structure known as the synaptonemal complex (SC). It is comprised of two lateral elements, assembled along each axis of a pair of homologous chromosomes, and a central region consisting of transverse filaments bridging the gap between lateral elements. While the presence of the SC during meiosis has been appreciated now for 50 years (Moses, Biophys Biochem Cytol 2:215–218, 1956; Fawcett, J Biophys Biochem Cytol 2:403–406, 1956), its role(s) remain a matter of intense investigation. This review concentrates on studies performed in *Caenorhabditis elegans*, a powerful system for investigating meiosis. Studies in this organism are contributing to the unraveling of the various processes leading to the formation of the SC and the various facets of the functions it exerts throughout meiosis.

Introduction

Meiosis is an essential form of cell division through which most sexually reproducing organisms reduce their chromosome number by half, forming haploid gametes which, upon fertilization, will reconstitute a diploid state. This halving in chromosome number is the result of one round of DNA replication followed by two rounds of cell division: meiosis I (a reductional division) and meiosis II (an equational division). While meiosis II proceeds similarly to a mitotic division, unique events unfold during meiosis I. In particular, during prophase I, homologous chromosomes have to identify each other and pair, followed by the formation of a proteinaceous structure known as the synaptonemal complex (SC) between homologs, and completion of meiotic recombination leading to physical attachments between homologs. These events ultimately ensure proper chromosome segregation upon the first meiotic division. Succeeding in this outcome is crucial given that chromosome nondisjunction leads to seriously deleterious consequences such as infertility, miscarriages, and birth defects in humans (reviewed in Hassold and Hunt 2001; Miyamoto et al. 2003; Judis et al. 2004).

Despite the fundamental importance of meiosis, several questions remain to be answered regarding this biological process, among which is understanding the structure and functions of the SC. Although this tripartite structure is ubiquitously present from yeast to humans, as revealed by electron microscopy (EM) and fluorescent immunocytology studies (Roeder 1997; Zickler and Kleckner 1999; Fig. 1), its functions are still not fully understood. Great progress is being achieved in this regard through studies performed in various model systems. Among these are studies involving the soil nematode *Caenorhabditis elegans*.

This review addresses the current knowledge in the field regarding the macromolecular assembly and the roles played by the SC throughout meiosis in *C. elegans*. It starts by highlighting the advantages of performing such studies in *C. elegans* and by outlining key meiotic events unfolding during prophase I. This is followed by an overview of the SC structure and an introduction of its core components in *C. elegans*. Emphasis is then given to the coordination and interplay between homologous pairing, synapsis, and recombination. The goal is to focus on the currently known or proposed functions of the SC during *C.*

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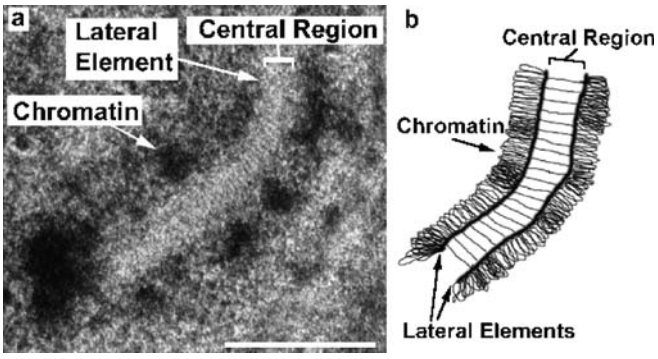

Fig. 1 Morphology of the *C. elegans* SC visualized by TEM. **a** The image is from a 100-nm-thick section of a nucleus from the mid-pachytene region of a wild-type germline. The zipper-like structure, comprised of a central region and a pair of lateral elements, is observed flanked by electron-dense patches of chromatin. *Scale bar* equals 500 nm. **b** Schematic of the SC structure

elegans meiosis, tracing parallels to what is known from other model systems.

C. elegans as a model system for the studies of meiosis

C. elegans is an experimental system especially amenable to coupling the application of powerful cytological approaches to abundant genetic, molecular biology and biochemical tools to address questions regarding meiosis. The germline accounts for more than half of the cells in the adult worm, and its nuclei are distributed throughout the gonad in a defined order, correlating with the sequential stages of meiosis (Schedl 1997; Fig. 2).

High-resolution three-dimensional (3-D) imaging of meiotic chromosomes can be carried out in the context of a well-preserved nuclear architecture, bypassing problems inherent to the analysis of chromosome spreads in other systems (Dernburg et al. 1998). A thorough quantitative and qualitative analysis of pairing interactions between homologs can be performed by fluorescence in situ hybridization (FISH) within the context of these intact nuclei (Dernburg et al. 1998; MacQueen et al. 2002; Colaiacovo et al. 2003). The ability to cytologically monitor progression of meiotic recombination has benefited tremendously from the development of an antibody against a protein involved in strand-exchange during recombination (RAD-51), allowing for both a quantitative and qualitative analysis of meiotic progression in synapsis-defective backgrounds (Colaiacovo et al. 2003; Alpi et al. 2003). *C. elegans* has also proven to be suited for thorough examinations of the SC by electron microscopy within the context of an intact temporal/spatial gradient of meiotic nuclei (Goldstein 1982; Dernburg et al. 1998; MacQueen et al. 2002; Colaiacovo et al. 2003).

Microarray technology applied to the *C. elegans* genome has led to the identification of meiotic gene candidates with germline-enriched expression (Reinke et al. 2000, 2004). Techniques such as RNA-mediated interference (RNAi) and polymarase chain reaction (PCR)-based screens for deletion alleles allow for assessment of the function of germline-active genes (e.g., Jansen et al. 1997; Liu et al. 1999; Wicks et al. 2001; Kamath et al. 2003; Simmer et al. 2003). Studies of the physical interactions between *C. elegans* proteins have been tremendously facilitated by the introduction of approximately 13,000 sequence-verified open reading frames (ORFs) from *C. elegans* into the Gateway cloning system (Reboul et al. 2003), allowing for efficient two-hybrid analyses of particular genes of interest (Walhout and Vidal 2001).

C. elegans is also a powerful genetic system with which to identify and study meiotic genes. This is due to the basis for sex determination in this system, which consists of males (XO) and hermaphrodites (XX), where a self-fertilizing hermaphroditic worm lays mostly hermaphrodit-

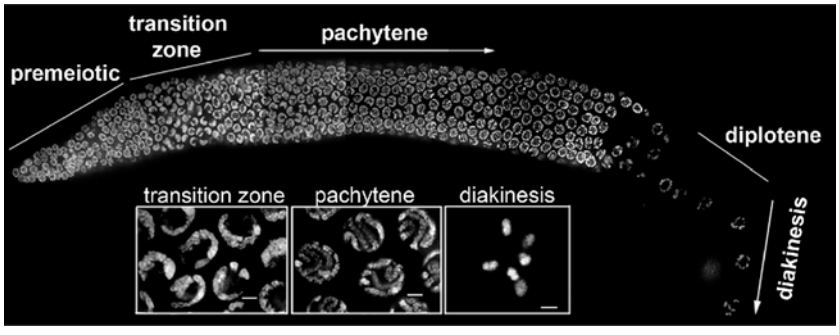

Fig. 2 The progression of meiotic prophase throughout the *C. elegans* germline. Nuclei are displayed throughout the germline in a well-defined temporal/spatial gradient representing the various stages of meiotic prophase. Changes in organization and appearance of DAPI-stained chromosomes allow for prompt identification of these stages. At the most distal region of the germline, nuclei are undergoing mitotic proliferation (premeiotic tip). As nuclei enter into meiosis (transition zone; which corresponds to the leptotene and zygotene stages of meiosis in the *C. elegans* germline), homologous chromosomes begin to pair and they acquire a characteristic polarized organization within the nuclei, imparting a crescent shape morphology to the DAPI signal. As nuclei progress into pachytene, chromosomes redisperse throughout the nuclei and thick parallel tracks of DAPI-stained chromatin become apparent as homologs are fully paired and aligned. The synaptonemal complex is fully formed at this stage and meiotic recombination is completed within this context. Upon exit from pachytene, the SC disassembles and nuclei progress from diplotene into diakinesis. Meanwhile, chromosome condensation and nuclear volume increase. At diakinesis, the six pairs of homologous chromosomes (six bivalents), which remain attached to each other by chiasmata as a result of earlier crossover recombination events, become clearly apparent

ic progeny and produces males at a very low frequency (0.2%) (Hodgkin et al. 1979). Mutations affecting meiotic prophase events, such as homologous chromosome pairing, synapsis, or recombination, result in increased chromosome nondisjunction. In *C. elegans*, this leads to an increase in embryonic lethality due to aneuploidy, accompanied by a *h*igh *i*ncidence of *m*ales (Him phenotype) among the surviving progeny of a mutant hermaphrodite.

This system has also proven more recently to be amenable to biochemical approaches. These range from the tandem immuno-affinity purification of protein complexes (Polanowska et al. 2004; Cheeseman and Desai 2005) to chromatin immunoprecipitations (Whetstine et al. 2005). These are currently being exploited for meiotic studies and will tremendously enhance our understanding of protein interactions and their functions throughout the germline.

Several steps are required during meiosis I to ensure accurate chromosome segregation

The achievement at meiosis I of reducing the chromosome number by segregating homologous chromosomes away from each other sets this cell division apart from either meiosis II or mitosis. Many of the key processes required to achieve this reduction unfold during meiotic prophase. It is upon entrance into prophase of meiosis I that a fully replicated genome encounters its first challenge: chromosomes have to pair with their homologous partners within a 3-D nuclear volume. While in some organisms this is facilitated by pre-meiotic pairing interactions that perdure into meiosis (Burgess 2002; Peoples et al. 2002; Weiner and Kleckner 1994), most organisms have to establish these interactions upon entering meiotic prophase (Dernburg et al. 1998; Li et al. 1999). This is accomplished either through double-strand break (DSB)-dependent or DSB-independent mechanisms and is accompanied by the reorganization of chromosomes within nuclei during early prophase. After pairing, the synaptonemal complex assembles, and it is in the context of a fully formed SC that recombination is completed (Padmore et al. 1991; Villeneuve and Hillers 2001). As these events unfold, the system has to coordinate synapsis, such that it only occurs between homologous partners, and meiotic recombination, such that crossovers will form ensuring physical connections between homologs upon disassembly of the SC. Furthermore, the distribution of these crossover events does not occur at random, as another layer of control is exerted by the mechanism of "crossover interference" (Roeder 1997). These events are then followed by the disassembly of the SC, revealing homologous chromosomes attached by "chiasmata", which are cytologically visible manifestations of earlier crossover events (Jones 1987). Chiasmata are essential for faithful chromosomal segregation, allowing homologs to remain connected to each other until anaphase I and to orient toward opposite poles of the spindle (Nicklas 1974). Additional layers of complexity are added to this process by changes in chromosome condensation throughout meiotic prophase (Zickler and Kleckner 1998, 1999) and the presence of cohesins, which load upon replication and dissociate from chromosomes in a regulated fashion, ensuring sister chromatid cohesion until anaphase of the second meiotic division (Miyazaki and Orr-Weaver 1994; Klein et al. 1999; Eijpe et al. 2003).

The synaptonemal complex

At center stage throughout prophase I is the SC. This "zipper-like" structure is comprised of proteins that align along the axis of each homologous chromosome forming the "axial elements" which later, within the context of a fully formed SC, are called "lateral elements". Additional proteins are involved in the formation of transverse rods or filaments interconnecting the axial elements, forming the "central region" of the SC (von Wettstein et al. 1984).

A remarkable feature of the SC is its ultrastructural conservation across species (reviewed by von Wettstein et al. 1984; Page and Hawley 2004), despite the lack of sequence conservation between the various proteins comprising the transverse filaments identified so far. What such proteins, including C(3)G in flies (Page and Hawley 2001), ZYP1 in plants (Higgins et al. 2005), SCP1 in mammals (Meuwissen et al. 1992), ZIP1 in yeast (Sym et al. 1993), and SYP-1 and SYP-2 in worms (MacQueen et al. 2002; Colaiacovo et al. 2003), all share in common is their secondary structure (reviewed by Hunter 2003; Page and Hawley 2004). These transverse filament proteins consist of an extended coiled-coil region flanked by globular domains. Studies from yeast, mammals, and flies indicate that SC transverse filament proteins, forming homodimers arranged in parallel and in register, anchor to opposing lateral elements through their C-termini and interact head-to-head via their N-termini, bridging the gap between axes (Dobson et al. 1994; Liu et al. 1996; Schmekel et al. 1996; Tung and Roeder 1998; Dong and Roeder 2000; Anderson et al. 2005). These proteins have a tremendous propensity for self-assembly, forming aggregates called polycomplexes that have been observed in various organisms (reviewed by Goldstein 1987). While these are naturally occurring aggregates observed mostly after SC disassembly, they have also been observed when synapsis is perturbed (see review by Zickler and Kleckner 1998) or when transverse filament proteins are either overexpressed in meiotic cells or expressed in mitotic cells (Sym and Roeder 1995; Yuan et al. 1996; Ollinger et al. 2005). When examined by electron microscopy, it has been demonstrated that these polycomplexes resemble the organization and highly ordered structure observed in the SC (Dong and Roeder 2000; Ollinger et al. 2005). This propensity to polymerize is further exemplified by the assembly of transverse filament proteins along nonhomologous chromosomes either in situations where a homologous chromosome is not available, such as in haploid yeast (Loidl et al. 1991), or in meiotic mutants in various species including *C. elegans* (Zickler and Kleckner 1999; Couteau and Zetka 2005; Martinez-Perez and Villeneuve 2005).

These observations raise questions such as: What keeps these components "in check" so that they will not normally self-assemble? How is their assembly regulated such that it normally occurs between homologous chromosomes?

Thus far, the SC has been implicated in a variety of roles during meiosis. These range from participating in the stabilization of homologous pairing interactions, to promoting crossover recombination between homologous chromosomes and regulating chromosome segregation (reviewed in Roeder 1997; Page and Hawley 2003; Page and Hawley 2004). Dissecting apart its roles and understanding the contributions of its various components throughout these processes remain subjects of intense investigation. Superimposed to understanding the functions exerted by this structure and its core components is the importance of understanding what regulates its formation and disassembly. Moreover, how is information, such as the successful identification of a homologous partner and progression of recombination, intertwined and relayed back and forth between the process of SC formation and progression through meiotic prophase? Studies of the SC, thus, serve as a powerful platform from which to address many of the key processes unfolding throughout meiosis.

The SC in *C. elegans*

In *C. elegans*, six SCs per germline nuclei were first observed in the wild-type hermaphrodite and five in the wild-type male by 3-D reconstruction analysis of serial sections from electron microscopy (Goldstein and Slaton 1982; Goldstein 1982). In the XX hermaphrodite, these SCs correspond to the five autosomal bivalents and the XX bivalent, while in the XO male, they correspond to the five autosomal bivalents with the X univalent lacking an SC and remaining heterochromatic throughout pachytene, decondensing at diplotene.

This tripartite structure consists of two lateral elements and a striated central element with a distance between axes of ~100 nm, similar to SCs in flies, plants, yeast, and mammals (Fig. 1). However, only one end of each SC is attached to the nuclear envelope, as determined by EM, and clustering of telomeres at the nuclear periphery is not apparent (Goldstein and Slaton 1982). Thus, there is no evidence of the configuration known as the "bouquet", which facilitates homologous pairing, in which chromosomes are brought into close juxtaposition within a nuclear subdomain as a result of a tighter telomeric association at the nuclear envelope (Gerton and Hawley 2005). In addition, electron-dense structures known as recombination nodules, corresponding to sites of ongoing recombination observed associated with SCs in various other species (Zickler and Kleckner 1999), have not been detected by EM throughout pachytene nuclei in *C. elegans* (Goldstein and Slaton 1982). Altogether, these observations raise several questions. How similar is the SC observed in *C. elegans* to that observed in other species so far? What mechanism(s) drive the search for homology in *C. elegans*?

Below is an outline of the key SC-associated components known to date in *C. elegans*. These are presented due to their relevance to various concepts described later in this review regarding the assembly and roles played by the SC during meiosis. Parallels between them and their counterparts in other systems are pointed out when necessary.

Axis-associated components in *C. elegans*

The meiosis-specific cohesin REC-8

Cohesin proteins load onto chromosomes during DNA replication and play a key role in connecting sister chromatids along their full lengths, possibly by forming rings around the sisters and, consequently contributing to proper chromosome segregation (reviewed by Marston and Amon 2004; Craig and Choo 2005). REC-8 is a 781-amino-acid protein identified in *C. elegans* as the ortholog of the *Schizosaccharomyces pombe* and *Saccharomyces cerevisiae* meiosis-specific cohesin protein Rec8. This was determined by sequence conservation (e.g., it is 17.3% identical and 41.6% similar to the *S. cerevisiae* Rec8p), its RNAi-depletion phenotype, and immunolocalization studies (Pasierbek et al. 2001).

Immunostaining indicated REC-8 is present as small grains throughout mitotic nuclei in the germline before entrance into meiosis. Upon entrance into transition zone, REC-8 localizes to short threads or patches. Later in pachytene, it acquires a continuous linear staining pattern along the longitudinal axes of synapsed chromosomes, fully colocalizing with SMC1 (Chan et al. 2003). REC-8 remains associated with chromosomes through metaphase II, with a progressive decrease in signal intensity between metaphase I and II (Pasierbek et al. 2001).

The role of REC-8 as an axis-associated component is supported by its presence along the axes of unsynapsed chromosomes, such as those observed after SC disassembly, as well as along univalents in diakinesis oocytes in recombination deficient mutants such as *spo-11* (Pasierbek et al. 2001; Nabeshima et al. 2004, 2005). Moreover, REC-8 is required for assembly of the *C. elegans* axial-component HIM-3 and the central region proteins SYP-1 and SYP-2 along chromosomes, but it associates onto chromosomes independently from any of these SC components (Pasierbek et al. 2001; MacQueen et al. 2002; Colaiacovo et al. 2003).

REC-8 is interdependent with SCC-3, another sister chromatid cohesion protein, for its chromosomal association (Chan et al. 2003; Wang et al. 2003). It also depends on the HEAT/Armadillo repeat-containing protein TIM-1, a regulator of chromosome cohesion, for its chromosomal localization (Chan et al. 2003). In *C. elegans*, REC-8's dissociation from chromosomes occurs in a two-step fashion as first demonstrated in yeast (Buonomo et al. 2000; Pasierbek et al. 2001). It is first released from the regions of contact between homologous chromosomes at the onset of the first meiotic division, followed by release from the regions of contact along sister chromatids upon

the second meiotic division. REC-8's cleavage and degradation during meiosis I is dependent on phosphorylation by the aurora-like kinase AIR-2, and on AIR-2 and the polo-like kinase PLK-2 during meiosis II (Rogers et al. 2002).

The axial element component HIM-3

HIM-3 is a 291-amino-acid protein sharing 16% identity and 31% similarity to the N-terminal region of *S. cerevisiae*'s HOP1, a lateral element component of the yeast SC (Hollingsworth and Byers 1989; Hollingsworth et al. 1990; Zetka et al. 1999). It is also homologous to the axial/lateral element of *Arabidopsis* ASY1 (Caryl et al. 2000; Armstrong et al. 2002) and to rice PAIR2 (Nonomura et al. 2004), recently implicated in meiotic pairing and SC formation. A distinctive structural feature of HIM-3 is the presence of a HORMA domain (for *Hop*1p, *R*ev7p, and *MA*D2), a common structural feature of proteins involved in mitotic checkpoints, chromosome synapsis, and DNA repair (Aravind and Koonin 1998).

An analysis of HIM-3 localization indicates that it associates with chromosomes upon entrance into meiosis, initially in a few foci, forming short stretches soon thereafter, and finally acquiring a very continuous staining pattern between paired and aligned homologous chromosomes. It remains associated with chromosomes until the metaphase I to anaphase I transition, a pattern that is distinct from that observed for Hop1 in yeast, which dissociates from chromosomes before SC disassembly (Zetka et al. 1999).

HIM-3 is associated along the axes of unsynapsed chromosomes observed in diplotene and diakinesis nuclei upon disassembly of the SC and in diakinesis oocytes in various recombination mutants, where univalents are apparent due to the lack of chiasmata (Zetka et al. 1999; Nabeshima et al. 2005). This feature and the dependency of HIM-3 on the meiosis-specific cohesin REC-8 for chromosomal association, but not on central region components SYP-1 and SYP-2 (see below), place HIM-3 as an axis-associated component of the SC.

Central region components in *C. elegans*

The structural module of the central region: SYP-1 and SYP-2

Combined approaches involving an RNAi screen and a genetic screen designed for the identification of meiotic genes led to the identification of *syp-1* and *syp-2* (Villeneuve 1994; MacQueen and Villeneuve 2001; Colaiacovo et al. 2002). These genes encode for 489-and 213-amino-acid proteins, respectively. While both possess putative orthologs in a related *Caenorhabditis* species, neither is a homolog of central region components in other species. However, they share similar structural properties with central region components from other species: the presence of coiled-coil domains flanked by globular domains.

SYP-1 and SYP-2 have been proposed to comprise a structural module of the central region of the SC in *C. elegans* (MacQueen et al. 2002; Colaiacovo et al. 2003). They are first observed forming a single large focus per nucleus upon entrance into transition zone, followed by multiple smaller foci and shorter stretches along chromosomes. In pachytene, they localize continuously at the interface between paired and aligned homologous chromosomes (Fig. 3). This chromosomal association is progressively reduced through diplotene and diakinesis and is finally no longer apparent in the last oocyte before fertilization. SYP-1 and SYP-2 are interdependent for their chromosomal localization as SYP-1 no longer associates onto chromosomes in *syp-2* mutants and SYP-2 chromosomal association is no longer observed in *syp-1* mutants. Their timing of association and dissociation from chromosomes parallels that of central region components in other systems (reviewed by Page and Hawley 2004) and differs from that of axis-associated components. Moreover, regions along homologous chromosomes that are not fully synapsed lack SYP-1 or SYP-2 staining while axis-associated components (e.g., HIM-3) are observed localizing continuously along each homologous axis.

SYP-1 and SYP-2's role in SC assembly is further supported by both immunofluorescence and transmission electron microscopy (TEM) studies (MacQueen et al. 2002; Colaiacovo et al. 2003). Axis morphogenesis,

Fig. 3 A nucleus with fully synapsed chromosomes during pachytene in *C. elegans*. DAPI-stained chromosomes are arranged in parallel with SYP-2 localized continuously at the interface between these paired and aligned chromosomes. The image is a projection halfway through a data stack encompassing a whole nucleus prepared using a squash procedure. DAPI-stained chromosomes are in *blue*; α-SYP-2 is in *magenta*. *Scale bar* equals 2 μm. (The image was kindly provided by Dr. Carlos Egydio de Carvalho, Harvard Medical School, Boston.)

determined by HIM-3 and REC-8 immunostaining, does not require either SYP-1 or SYP-2, while these do require normal axis morphogenesis for chromosomal association given that their localization is impaired in either *rec-8* or *him-3* RNAi-depleted or mutant backgrounds (MacQueen et al. 2002; Colaiacovo et al. 2003; Couteau et al. 2004). Moreover, TEM analysis of meiotic prophase nuclei indicates lack of SC formation in both *syp-1* and *syp-2* mutants. These data altogether place SYP-1 and SYP-2 downstream of axis-associated components and indicate that they are required for SC assembly by participating in the formation of the central region of the SC.

Additional SC-associated components

ZHP-3

ZHP-3 is a 387-amino-acid protein identified due to its homology to *S. cerevisiae* ZIP3 with which it shares 18.5% sequence identity (Jantsch et al. 2004; Agarwal and Roeder 2000). Both proteins carry an N-terminal RING finger domain followed by a coiled-coil region and a S-rich C-terminal region. ScZIP3 localization is punctate, forming foci along homologous chromosomes in zygotene through pachytene nuclei. This meiosis-specific protein is a component of recombination nodules observed throughout meiotic chromosomes and postulated to correspond to sites undergoing meiotic recombination. ScZIP3 has been specifically proposed to act at sites of synapsis initiation as a liaison between synapsis and recombination. ZHP-3 (*Z*ip3-*h*omologous *p*rotein) in *C. elegans* has a different pattern of localization. It first acquires a punctate nuclear distribution at transition zone, where it co-localizes with the central region component SYP-1, later assuming a more continuous localization as either confluent dots or lines overlapping with SYP-1's linear staining.

ScZIP3 is required for SC formation. It is necessary for the assembly of the meiosis-specific proteins ZIP2 (synapsis initiation complex component; Chua and Roeder 1998) and ZIP1 (central region component; Sym et al. 1993). Unlike ScZIP3, ZHP-3 is not required for SC formation. Axis-associated components HIM-3 and REC-8, as well as the central region component SYP-1, localize in a *zhp-3* null mutant as they do in a wild-type background. Meanwhile, ZHP-3 chromosomal association depends on SC formation.

What is the role of ZHP-3 in *C. elegans* meiosis? It is not required for homologous pairing as demonstrated by FISH analysis of pairing interactions throughout meiotic prophase (Jantsch et al. 2004). It is interesting that ZHP-3 is required for crossover recombination and consequent chiasma formation. In *zhp-3* mutants, levels of RAD-51 foci are increased by more than twofold and 12 univalents, as opposed to six bivalents, are apparent in oocytes at late diakinesis. The absence of chromosome fragmentation, production of viable progeny (albeit at reduced levels), and timely disappearance of RAD-51 foci in *zhp-3* mutants suggest that repair is unfolding by alternative means leading to either gene conversion, sister-chromatid-mediated repair or nonhomologous end-joining.

The apparent differences between *S. cerevisiae* ZIP3 and *C. elegans* ZHP-3 can be easily reconciled by the fact that, in *S. cerevisiae*, the initiation of meiotic recombination is intimately linked to SC formation while this is not the case in *C. elegans* (see below). What seems to be conserved is the ability to relay information between recombination sites and SC components, where in the case of ZHP-3, DSB-repair is directed to crossover formation in an SC-dependent manner.

HTP-1, HTP-2 and HTP-3

Genomic sequence analysis led to the identification of three *h*im *t*hree *p*aralogs, *htp-1*, *2*, and *3*. All three encode proteins that, similarly to HIM-3, carry a HORMA domain. *htp-1* and *htp-2* encode 352-amino-acid proteins sharing 82% protein sequence identity and 90% nucleotide identity among their coding regions. Both share less than 30% protein sequence identity with HIM-3 and HTP-3. *htp-3* encodes a 739-amino-acid protein that shares 25% protein sequence identity with HIM-3.

Due in part to the high degree of homology shared between HTP-1 and HTP-2, antibodies that will specifically recognize one and not the other have proven difficult to obtain, hence, only localization for HTP-3 has been reported so far (MacQueen et al. 2005; Phillips et al. 2005). HTP-3 localizes along the axes of unsynapsed chromosomes upon entrance into meiosis, before the localization of HIM-3 as well as independently of HIM-3. It then co-localizes with SYP-1 along the interface between paired and aligned homologous chromosomes throughout pachytene. Further examination will determine its precise functions both in the macromolecular assembly of the SC and throughout pairing and recombination.

Neither HTP-1 nor HTP-2 is required for normal axis morphogenesis given that neither in *htp-1* nor *htp-1*; *htp-2 (RNAi)* mutants, localization of REC-8, HIM-3, or HTP-3 is affected. However, both affect SYP-1 and SYP-2 association in early prophase (Couteau and Zetka 2005; Martinez-Perez and Villeneuve 2005). While in wild-type nuclei during early prophase, SYP-1 chromosomal association is only observed after extensive loading of HIM-3 (MacQueen et al. 2002), in *htp-1* mutant germlines, aggregates of SYP-1 are observed before HIM-3 association. This is followed by extensive SYP-1 loading between nonhomologously synapsed chromosomes throughout pachytene (Couteau and Zetka 2005, Martinez-Perez and Villeneuve 2005). Meanwhile, in *htp-1*; *htp-2(RNAi)*, polycomplex-like aggregates are observed in leptotene/zygotene nuclei followed by extensive localization along unsynapsed chromosomes (Couteau and Zetka 2005). These results have implicated HTP-1 and HTP-2 in regulating central region formation in early prophase (Couteau and Zetka 2005; Martinez-Perez and Villeneuve 2005).

Coordinating chromosome organization, pairing, and synapsis throughout early prophase

The coordination between pairing and synapsis and, in particular, how chromosome reorganization is tied into SC assembly are questions currently being addressed through studies of *C. elegans* meiosis. Entrance into meiotic prophase coincides with axis morphogenesis and a redistribution of chromosomes within nuclei, where they cluster towards one side of the nuclei, acquiring a polarized morphology. It is in this configuration that homologous chromosomes identify each other and pair as monitored by FISH (Dernburg et al. 1998). Upon entry into pachytene, the chromosomes redistribute once more, dispersing throughout the nuclear periphery. At this stage, the SC is fully formed and homologs remain stably paired. These two "waves" of chromosome reorganization observed within *C. elegans* germline nuclei in early and mid-prophase are examined below.

Role of axis morphogenesis in achieving a polarized morphology and homologous pairing

In most organisms, the initial recognition and alignment between homologous chromosomes occurs in a discrete time window (leptotene/zygotene) accompanied by the reorganization of chromosomes within nuclei (reviewed by Zickler and Kleckner 1998; Scherthan 2001). During this process, telomeres attach to the inner nuclear membrane, tightly clustering and forming a chromosomal bouquet or, alternatively, chromosomes cluster towards one side of the nucleus. The evidence that telomere clustering indeed facilitates homolog recognition and pairing stems from studies in fission yeast (*taz1* and *rap1*mutants), budding yeast (*ndj1/tam1*mutants) and maize (*pam1* mutants), where the absence of telomere clustering leads to impaired homologous pairing (Chua and Roeder 1997; Conrad et al. 1997; Cooper et al. 1998; Nimmo et al. 1998; Trelles-Sticken et al. 2000; Golubovskaya et al. 2002; Ding et al. 2004). In the case of *C. elegans*, while bouquet formation is not observed (Goldstein and Slaton 1982), the process of chromosomes acquiring a polarized organization during leptotene/zygotene is preserved.

An analysis of *him-3* mutants recently revealed that initial homolog alignment requires the normal formation of chromosome axes (Couteau et al. 2004; Nabeshima et al. 2004). Utilizing FISH, comparisons between a null allele and hypomorphs of *him-3* indicated that, in the absence of HIM-3, there was complete lack of homologous pairing throughout meiotic prophase. An analysis of chromosome morphology in worms homozygous for the null allele indicated that chromosomes failed to acquire the polarized morphology characteristic of transition zone, remaining instead dispersed throughout the nuclear periphery and failing to synapse. In contrast, in the hypomorphs, where HIM-3 expression, albeit reduced, was not completely eliminated and higher levels of SYP-1 chromosomal association were observed, up to 16% of nuclei still acquired some degree of pairing along the autosomes. Moreover, chromosomes succeeded in acquiring a polarized morphology upon entrance into meiosis but failed to properly exit this organization with wild-type kinetics. These studies directly linked HIM-3 and, consequently, axis morphogenesis to the establishment of pairing, to nuclear reorganization during early prophase, and in the coordination of these events with the progression of SC assembly.

While the studies of HIM-3 directly address the role of a structural component of the SC in progression through early prophase, HTP-1 has recently been implicated in the coordination between pairing and synapsis (Martinez-Perez and Villeneuve 2005; Couteau and Zetka 2005). HTP-1 is required for the inhibition of SC polymerization which otherwise can be triggered by failure of chromosome pairing. In *htp-1* mutants, there is extensive SC formation, as determined by chromosomal association of HIM-3, SYP-1, and SYP-2 observed through immunofluorescence. FISH analysis reveals that the observed synapsis is nonhomologous and that occasional pairing partner switches are present. The impact on pairing is observed even in the pairing center regions, which are believed to be involved in the early and localized establishment of pairing (see below). Moreover, there is a reduction in the number of nuclei acquiring the characteristic polarized morphology of early prophase accompanied by the premature formation of SYP-1 aggregates. These observations altogether suggest HTP-1 is required for the early establishment of pairing and for coordinating the progress of homologous pairing with SC polymerization by preventing polymerization between nonhomologous chromosomes. This is further supported by the observations that SC polymerization in *chk-2* mutants (*chk-2* encodes a *C. elegans* ortholog of the Cds1/Chk2 checkpoint protein kinases), which is both late and limited (MacQueen and Villeneuve 2001), now occurs earlier and more extensively in *htp-1*; *chk-2* double mutants (Martinez-Perez and Villeneuve 2005). It is interesting that X chromosome pairing and synapsis are not impaired in *htp-1* mutants. However, in *htp-1*; *htp-2(RNAi)* mutants, while the establishment of pairing on the X chromosome is not affected, the stabilization of pairing and SYP-1/SYP-2 association are no longer observed. This suggests that the stabilization of pairing imparted by synapsis involves either HTP-2 acting alone or both HTP-1 and HTP-2 playing redundant roles (Couteau and Zetka 2005).

How do these two components execute their roles? It has been proposed in these studies that HTP-1 may act by actively preventing the association between SYP-1 and SYP-2, which are interdependent for their role as a structural module of the SC's central region. HTP-1 may alternatively sequester SYP-1 and SYP-2, impeding their non-discriminatory assembly, given their tremendous proclivity for polymerization irrespective of homology. This may translate into the early aggregates of SYP-1 and SYP-2 that are observed upon entrance into meiosis in wild-type nuclei. HTP-1 might also actively promote SYP-1 and SYP-2 degradation, thus, preventing their accumula-

tion. Moreover, HTP-1 and HTP-2 may have antagonistic roles in SC assembly, with HTP-1 regulating HTP-2, which actively promotes SC polymerization (Fig. 4).

Role of SC formation in the stabilization of homologous pairing

Studies of the HIM-3 family of proteins have contributed tremendously to our understanding of the coordination between axis morphogenesis, the early establishment of pairing, and synapsis. The analysis of *syp-1* and *syp-2* mutants meanwhile revealed that the SC plays a role in the stabilization of pairing interactions in *C. elegans* (MacQueen et al. 2002; Colaiacovo et al. 2003). This is based on FISH analysis of homologous pairing throughout meiotic prophase in these mutants. These studies indicated that significant levels of homologous pairing were achieved in *syp-1* and *syp-2* mutants during early prophase, suggesting that there is a synapsis-independent mechanism promoting the establishment of pairing. It is interesting that significantly higher levels of homologous pairing were achieved during early prophase in the pairing center regions of chromosomes (see below) as opposed to other regions throughout chromosomes in *syp-1* and *syp-2* mutants. These studies directly implicated the pairing center regions of chromosomes in this initial synapsis-independent pairing. However, these initial pairing interactions failed to persist, supporting a role for SYP-1 and SYP-2 in the stabilization of these earlier pairing interactions.

In *syp-1* and *syp-2* mutants, chromosomes fail to exit the polarized morphology characteristic of meiotic entry with proper kinetics. The chromosomes instead persist in a polarized configuration through late pachytene, failing to redisperse throughout the nuclear periphery upon entrance into pachytene. Although this may be perceived as a developmental delay or arrest, the chromosomes finally redisperse throughout the nuclear periphery during late pachytene. Moreover, a late prophase DNA damage checkpoint (Gumienny et al. 1999) is still fully active in these mutants, indicating that meiocytes are otherwise competent at this stage to undergo apoptosis. So, why are chromosomes remaining in an extended transition zone morphology? An analysis of both *spo-11*; *syp-1* and *spo-11*; *syp-2* double mutants which lack meiotic DSB formation suggests that the failure in chromosome redispersal observed in both *syp-1* and *syp-2* mutants does not result from a meiotic recombination checkpoint sensing unrepaired intermediates. In both double mutants, the chromosome morphology defect was indistinguishable from that of either *syp-1* or *syp-2* single mutants. This data suggests instead that either the failure to stabilize earlier pairing interactions and/or the inability to polymerize the central region of the SC contribute to this extended transition zone morphology. This argues that this "second wave" of chromosome reorganization in wild-type *C. elegans* may involve as readouts either progression of pairing and/or synapsis for signaling exit into a redispersed organization. This redispersal may alternatively just unfold passively as a result of SC polymerization and, consequently, the formation of a more rigid structure, driving chromosomes out of a polarized morphology.

A recent analysis of *htp-1* mutants has started to shed some light on the regulation of progression from transition zone into pachytene in *C. elegans* (Martinez-Perez and

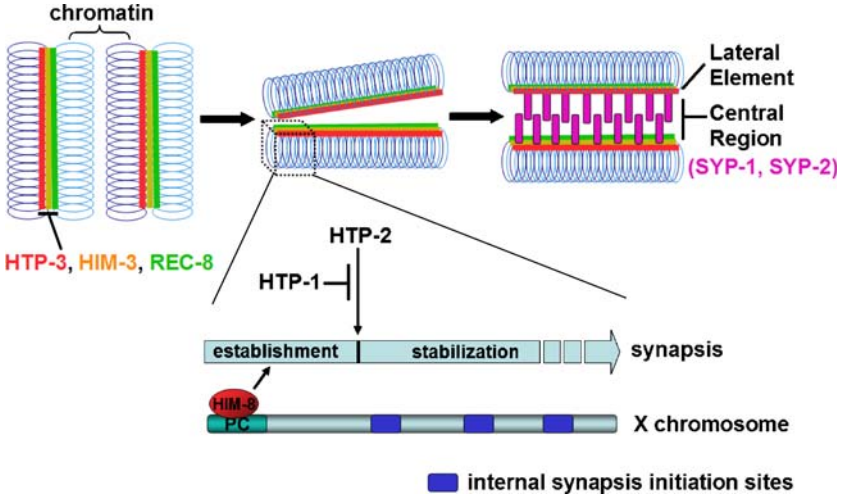

Fig. 4 Model of SC formation in *C. elegans*. A pair of homologous chromosomes are depicted each comprised of a pair of sister chromatids identified through chromatin loops in two different tones of blue. Axes-associated components (HTP-3, HIM-3, and REC-8) are depicted, respectively, in *red*, *orange*, and *green* forming the axial elements along each homologous chromosome. Initial homologous pairing interactions are indicated for the X chromosome. *Inset* focuses on the pairing center (*PC*) region, where HIM-8 associates. Both the PC and HIM-8 are involved in the localized establishment of synapsis at that region. Meanwhile, extensive synapsis is restrained by HTP-1 (shown here hypothetically restraining extensive synapsis promoted by HTP-2). After homologous pairing is established, possibly via kinetic proofreading mediated by the PC, extensive synapsis is promoted involving components such as HTP-2 allowing for stabilization of the earlier pairing interactions. Synapsis may also initiate at additional internal sites along the X chromosome indicated in *dark blue*. The final outcome is the fully formed SC comprised of a pair of lateral elements with the gap between homologous chromosomes bridged by central region components, SYP-1 and SYP-2

Villeneuve 2005). In *htp-1* mutants, although the chromosomes acquire the polarized morphology characteristic of transition zone, they prematurely exit this stage and chromosomes redisperse undergoing extensive nonhomologous synapsis. In *htp-1*; *syp-2* mutants, the chromosomes do not persist in an extended transition zone as observed in *syp-2* single mutants. They instead redisperse prematurely as in *htp-1* mutants, suggesting HTP-1 is involved in inhibiting chromosome redispersal and that SC polymerization may not be a driving force in this redispersal. Moreover, in *htp-1* mutants, autosomal pairing is more severely impaired than in either *syp-1* or *syp-2* mutants. Thus, it is possible that the inability to acquire a "minimum threshold" of initial pairing in *htp-1* mutants leads to the premature release of chromosomes from a polarized morphology. These observations altogether suggest that the efficient progression of pairing is intimately linked to the redispersal of chromosomes during exit from transition zone into pachytene in *C. elegans*.

The role of pairing center ends in pairing and synapsis

The genetic and cytological analysis of various chromosome translocations, inversions, and duplications led to the identification of *cis*-acting regions involved in homologous pairing in organisms such as flies (Hawley 1980; Sherizen et al. 2005), maize (Maguire 1986), and worms (Rosenbluth and Baillie 1981; Herman et al. 1982; Rose et al. 1984; Herman and Kari 1989). In *C. elegans*, these *cis*-acting sites are located at one end of each chromosome and are termed homolog recognition regions or pairing centers (PC). In addition to promoting homologous pairing, these sites have been previously implicated in promoting crossover recombination and the initiation of synapsis in *C. elegans* (McKim et al. 1988; Villeneuve 1994).

FISH analysis of homologous pairing throughout meiotic prophase in *syp-1* and *syp-2* mutants revealed that synapsis-independent pairing can be achieved during early prophase at PCs but not maintained throughout later prophase (MacQueen et al. 2002; Colaiacovo et al. 2003). Recent studies monitoring pairing and synapsis in chromosomes either lacking PCs or carrying only one copy from a pair of PCs (PC heterozygote) revealed that PCs execute two separable functions (MacQueen et al. 2005). First, they act by locally stabilizing pairing interactions at these sites independent of synapsis. Second, they promote synapsis per se. This stems from observations that in a PC-heterozygous situation, the local synapsis-independent stabilization of pairing is lost while synapsis is still achieved. Meanwhile, in a PC-homozygous deletion background, synapsis is severely reduced. Even in the absence of PCs, some degree of synapsis is still surprisingly observed through combined immunofluorescence and FISH approaches (approximately 10% of the observed nuclei carried fully synapsed chromosomes). This indicates the PCs are not absolutely required for SC formation, suggesting that additional discrete sites may act throughout chromosomes to promote synapsis (Fig. 4). The existence of such secondary pairing sites along chromosomes had also been previously proposed by Rosenbluth et al. (1990) and McKim et al. (1993) through the genetic analysis of crossover distributions in various chromosomal rearrangements. These findings have altogether led to the proposal by MacQueen et al. (2005) that PCs may act in a "kinetic proofreading" mechanism through which locally stabilized (but yet reversible) pairing promoted by PCs allows for local evaluation of homology. Once homology is verified, chromosomes progress into a synapsed state. This model fits well with the observation that, in PC heterozygotes, chromosome synapsis increases stochastically throughout pachytene due to the concomitant increase of time during which collisions may unfold between chromosomes.

How does PC function translate into what is known for the coordination between homologous pairing and synapsis in other systems? In *C. elegans*, chromosomes are holocentric during mitosis with spindle attachments observed throughout the entire lengths of chromosomes (Albertson and Thomson 1982). However, during meiosis, spindle attachment seems to be restricted to the ends of chromosomes, indicating a change in kinetic activity (Albertson and Thomson 1993). While there is no evidence that would suggest that the PCs perform the roles of the centromeres regarding microtubule attachments or kinetochore formation, MacQueen et al. (2005) speculate that a centralized or unique region per chromosome involved in initial pairing and synapsis such as the PCs, despite not being a universal feature across species (Zickler and Kleckner 1998) may have been the outcome of centromeres in the *C. elegans* evolutionary branch. It is interesting that recent observations in budding yeast revealed that, in a *spo11* mutant background, ZIP1 localizes to or in the vicinity of centromeres in a ZIP2- and ZIP3-independent fashion (Tsubouchi and Roeder 2005). While this localization differs from that promoted by PCs, which ultimately leads to extensive synapsis in *C. elegans*, it has been demonstrated to be required for "centromere coupling" and proposed to possibly reflect an additional feature of early sorting of homology in budding yeast. In addition, it has suggested that centromeres might serve as early synapsis initiation sites promptly followed by more robust synapsis at noncentromeric sites in *S. cerevisiae*.

How is PC function imparted? Recent studies by Phillips et al. (2005) determined that a C2H2 zinc finger protein (HIM-8) binds specifically to the X chromosome PC and that this locus is associated with the nuclear envelope during prophase. *him-8* was initially identified in a genetic screen for meiotic mutants (Hodgkin et al. 1979) and shown to specifically affect X chromosome segregation (Broverman and Meneely 1994). The recent analysis of *him-8* mutants determined that chromosomes persisted in a transition-zone-like morphology during pachytene although all chromosomes except for the X were synapsed. This implies that a single unsynapsed chromosome pair may be sufficient to trigger this delay (Phillips et al. 2005). Furthermore, neither the PC nor HIM-8 are required for axis morphogenesis. Meanwhile, the presence of both, two copies of the PC and HIM-8, are required for X chromo-

some pairing at the PC and for synapsis. An analysis of a point mutant (*me4*) in which HIM-8 expression and localization as well as PC association with the nuclear envelope are retained indicated that these events, albeit necessary, are not sufficient for achieving proper chromosome pairing and synapsis. It remains to be determined what other functions are exerted by HIM-8 or other as of yet not identified components that mediate this early role. While clear orthologs have not been identified in other species, the identification of a chromosome-specific *trans*-acting factor involved in the early events of pairing and synapsis has nicely merged with the recent studies of PC function. Moreover, they have contributed tremendously to our understanding of what drives homologous pairing and synapsis in systems that do not depend on the initiation of DSBs for these processes.

The interdependencies between initiation of meiotic recombination, pairing, and synapsis

An analysis of pairing, synapsis, and recombination in various organisms has revealed that both DSB-dependent and DSB-independent mechanisms are utilized to achieve homologous pairing (for a review, see Page and Hawley 2003). In *C. elegans*, as in *Drosophila melanogaster*, homologous chromosomes succeed in pairing and synapsis without the initiation of meiotic DSBs (Dernburg et al. 1998; McKim et al. 1998). However, in budding yeast, mice, and *Arabidopsis*, initiation of recombination is required for these events to unfold properly (Roeder 1997; Romanienko and Camerini-Otero 2000; Baudat et al. 2000; Grelon et al. 2001), hence, the observation of axial associations (AA) connecting paired homologous chromosomes in various organisms where pairing is DSB-dependent (for a review, see Page and Hawley 2004) while these structures are not observed in *C. elegans*. Thus, homologous pairing is still observed in *zip1* and *Sycp1*$^{-/-}$ mutants, imparted by the presence of AAs, indicating that the SC is not involved in maintaining such interactions in yeast and mice, while stabilization of homologous pairing is *syp-1*- and *syp-2*-dependent in *C. elegans* (Sym et al. 1993; Nag et al. 1995; MacQueen et al. 2002; Colaiacovo et al. 2003; de Vries et al. 2005). Moreover, proteins such as Mnd1, Hop2, and Dmc1, all involved in AAs, are absent in *C. elegans* (MacQueen et al. 2002). Instead, homologous pairing and initiation of synapsis in *C. elegans* involve axis morphogenesis, the PCs and *trans*-acting factors such as HIM-8. What the analysis of various organisms has allowed us to conclude is that multiple strategies are applied, ultimately leading to the same outcome of successful identification and pairing between homologous chromosomes.

Recent studies indicated that synapsis initiation complexes (SICs) comprised of proteins such as Zip1, Zip2, Zip3, and Msh4 assemble on sites of AAs in yeast (Fung et al. 2004). Further evidence correlating crossover sites with sites of synapsis initiation stemmed from studies involving an allelic series of *spo11* mutants in budding yeast (Henderson and Keeney 2005). These revealed the direct correspondence between varying levels of DSBs on the formation of Zip3 foci, SC formation, and crossovers. The correlation between sites of ongoing recombination and sites of synapsis initiation had also been previously postulated by observations made in maize, *Sordaria*, and *Neurospora* (for reviews, see Bishop and Zickler 2004; Henderson and Keeney 2005). The more recent observation that ZIP1 is associated with centromeres in a *spo11Δ* background has led to the proposal that synapsis may also be initiated at centromeric sites (Tsubouchi and Roeder 2005). In *C. elegans*, PCs are now implicated as synapsis initiation sites (MacQueen et al. 2005). However, extensive synapsis is still observed in a subpopulation of nuclei even in the absence of PCs, indicating that additional sites distributed along chromosomes may also participate in synapsis initiation.

Roles for the SC in crossover formation and sister chromatid exclusion

While the requirement of initiation of meiotic recombination for SC formation is not conserved among species, a more universal theme is the requirement of synapsis for crossing over formation and sister chromatid exclusion. The formation of crossovers is important not only to render genetic diversity but also to form chiasmata which, underpinned by cohesion, allow for proper chromosome segregation at meiosis I. Thus, meiosis has evolved to first ensure that DSBs are formed along chromosomes and, second, to ensure that some of these DSBs are processed into crossover events specifically between homologous chromosomes (for a review, see Villeneuve and Hillers 2001).

Studies in worms, flies, yeast, and mice have demonstrated that, while initiation of meiotic recombination is not impaired in mutants of central region components of the SC (Storlazzi et al. 1996; Colaiacovo et al. 2003; Jang et al. 2003; de Vries et al. 2005), the formation of crossover recombination is impaired (reviewed in Page and Hawley 2004; Zickler and Kleckner 1999). It is interesting to note that the extent of this impairment varies among species. In yeast *zip1* mutants, crossovers are reduced to approximately 30% of wild-type levels (Sym et al. 1993). Meanwhile, crossovers are no longer apparent in *syp-1* or *syp-2* worm mutants and *c(3)G* fly mutants, or are barely detectable in *Sycp1*$^{-/-}$ mouse mutants (Page and Hawley 2001; MacQueen et al. 2002; Colaiacovo et al. 2003; de Vries et al. 2005). This is related to the fact that in some species, such as in *C. elegans*, all crossovers derive from a Msh4/Msh5-dependent pathway (Zalevsky et al. 1999; Kelly et al. 2000) while in others, such as in *S. cerevisiae*, only 50 to 70% of crossovers are Msh4/Msh5-dependent (Ross-Macdonald and Roeder 1994; Hollingsworth et al. 1995). Recent studies have indicated that at least a portion of these Msh4/Msh5-independent crossovers occur through a Mms4- and Mus81-dependent pathway which is SC independent (reviewed in Hollingsworth and Brill 2004).

In *C. elegans*, the development of an antibody against RAD-51 (protein involved in strand invasion/exchange during recombination; Sung 1994) permitted the examination of the progression of DSB repair throughout meiotic prophase (Colaiacovo et al. 2003; Alpi et al. 2003). This analysis revealed RAD-51 foci forming at leptotene/zygotene, peaking in abundance by early to mid-pachytene and disappearing by late pachytene. This indicates that strand exchange intermediates are capable of forming within the context of a fully formed SC. This was further supported by double immunostaining with α-SYP-1 and α-RAD-51, indicating that RAD-51 foci were only present in early prophase nuclei where chromosomes were already undergoing synapsis (Colaiacovo et al. 2003). Moreover, the peak of RAD-51 foci observed during pachytene in *C. elegans* was later than those of either RAD51 and/or DMC1 observed throughout late leptotene and mid-zygotene in yeast, mice, lily, or maize (Bishop 1994; Terasawa et al. 1995; Moens et al. 1997; Franklin et al. 1999). This can be explained by the uncoupling of the initiation of meiotic recombination from both homologous pairing and synapsis observed in *C. elegans* compared to these other organisms (Colaiacovo et al. 2003; Couteau et al. 2004).

An examination of progression of meiotic recombination through RAD-51 immunolocalization in mutants such as *him-3*, *syp-1*, *syp-2*, and *htp-1* indicated that axis morphogenesis, homolog alignment, and synapsis are not required for the initiation of meiotic recombination (Colaiacovo et al. 2003; Couteau et al. 2004; Martinez-Perez and Villeneuve 2005). These studies also revealed that these factors contribute to the barrier against sister-chromatid-mediated repair during meiosis. Previous studies had demonstrated the existence in various organisms of an active meiotic barrier against repair of DSBs between sister chromatids set in place through late pachytene (Schwacha and Kleckner 1997; Blat et al. 2002; Wan et al. 2004; Webber et al. 2004). In the case of *C. elegans*, an analysis of the aforementioned mutants suggests that release from (or an absence of) a polarized morphology allows for release from this constraint (Colaiacovo et al. 2003; Couteau et al. 2004; Martinez-Perez and Villeneuve 2005).

The role of the SC in crossover interference

Meiotic studies in various organisms indicate that at least two strategies have been devised to ensure that chromosomes successfully undergo crossovers leading to functional chiasmata. In some systems such as *S. pombe*, this is achieved by undergoing an excess of crossovers such that at least one unfolds between each pair of homologous chromosomes (Kohli and Bahler 1994; Munz 1994). In most other systems, both the number and distribution of crossovers occur in a more regulated fashion (Jones 1987). One to three crossovers are usually observed per chromosome arm, with the occurrence of a crossover in a particular position reducing the probability that another crossover will occur nearby. This phenomenon known as "crossover interference" has been shown to involve only the Msh4/Msh5- and SC-dependent crossovers in budding yeast (reviewed by Bishop and Zickler 2004). *C. elegans* has, thus, offered a powerful situation in which to investigate the interplay between the SC and crossover interference. This is due to the fact that just a single crossover event is observed per homolog pair and that these crossover events are all Msh4/Msh5- and SC-dependent (Hodgkin et al. 1979; Zalevsky et al. 1999; Kelly et al. 2000; Meneely et al. 2002; MacQueen et al. 2002; Colaiacovo et al. 2003; Hillers and Villeneuve 2003; Nabeshima et al. 2004).

A long-standing question regarding the role of the SC in crossover interference has been whether this regulation is exerted by using the integrity of the axes as a readout or whether it involves the mature SC. Hillers and Villeneuve (2003) contributed in addressing this through the analysis of end-to-end fusions of whole chromosomes involving either two or three of the six chromosomes in *C. elegans*. Their observations indicated that meiotic crossovers in this system are regulated by a chromosome-wide interference mechanism through which the engagement of one DSB event into a crossover involving homologous chromosomes discourages other initiation events from resulting in crossovers. Furthermore, crossover interference is tightly regulated and can be exerted throughout chromosome fusions encompassing up to half of the genome. This indicated that these chromosome fusions were being recognized as a single chromosome unit undergoing a single crossover throughout a region that, for example, as two individualized/non-fused chromosomes would have normally undergone two crossovers (one crossover per chromosome). Moreover, the distribution of these crossovers, which normally occur at the terminal thirds of individual chromosomes, was maintained throughout these chromosome fusions, indicating a preference for off-center exchanges in this system.

To address the functional unit being read by this chromosome-wide interference mechanism, Hillers and Villeneuve (2003) examined crossover distributions in animals heterozygous for fused and unfused chromosomes. For example, crossovers were monitored along a two-chromosome fusion involving chromosomes X and IV paired with unfused homologous partners. This analysis revealed both double and single crossovers, each occurring approximately 50% of the time. These observations indicated that axial discontinuities impaired (but did not completely eliminate) the robust regulation otherwise exerted along these fusions. Moreover, a three-chromosome fusion heterozygote involving paired chromosomes III and IV, lacking a homologous partner for the intervening X chromosome of this fusion in XO males, indicated a mature SC might be necessary for this regulation. In this configuration, interference did not operate along the unpaired X chromosome and each flanking autosome underwent a single crossover event. This indicated that the autosomes were being recognized as individual chromosomes despite being fused to the intervening but unpaired and unsynapsed X. These studies

indicated the presence of a chromosome-wide interference mechanism in *C. elegans* operating either via chromosome axes or SC integrity.

Studies of a *him-3* hypomorph in which the capacity to form crossovers is maintained despite the compromised integrity of chromosome axes further complemented these previous observations (Nabeshima et al. 2004). In *him-3* (*me80*) mutants, axis morphogenesis is impaired, given that levels of HIM-3 protein are reduced and synapsis is consequently incomplete and discontinuous. These defects were accompanied by a significant increase of double crossovers along the analyzed chromosomes (e.g., double crossover levels underwent a 21-fold increase along chromosomes I compared to +/+ or *him-3*(*me80*)/+ controls).

It is interesting that recent studies of synapsis initiation complexes in *S. cerevisiae* indicate that SICs are not distributed randomly and display interference independent of SC assembly (Fung et al. 2004). This is consistent with the analysis of *zmm* mutants (comprised of *mer3*, *msh5*, *zip1*, *zip2*, and *zip3*) suggesting that decisions of whether a DSB will undergo a crossover or noncrossover pathway for repair is also set before SC assembly (Borner et al. 2004). In *D. melanogaster*, crossover interference is still similarly observed in a c(3)G mutant (Page and Hawley 2001). These studies altogether indicate that chromosome axes, instead of a fully formed SC, may either be the readout or transmitting entity of crossover interference.

The regulation of SC disassembly

Despite the importance of SC disassembly and the appearance of chiasmata, how these processes are coordinated and the requirements for them to proceed properly have remained poorly understood until recently. Current analysis of SC disassembly in *C. elegans* is significantly contributing to our understanding of what unfolds during this dynamic reorganization.

First, throughout late pachytene and into diakinesis, the central region components SYP-1 and SYP-2 progressively acquire an asymmetric localization along bivalents. This is in contrast to the situation in early and mid-pachytene when SYP-1 and SYP-2 are present continuously along the region of contact between paired and aligned homologous chromosomes (Fig. 3). In late pachytene, this distribution becomes uneven, with SYP-1 and SYP-2 staining becoming brighter towards one end of each pair of homologous chromosomes. In the pachytene–diplotene transition, the asymmetric distribution of SYP-1 and SYP-2 becomes clearly apparent, while axis-associated components such as REC-8 and HIM-3 persist in their continuous association along the axes of desynapsing chromosomes. Thus, six short stretches of SYP-1 and SYP-2 are observed in each oocyte delineating the remaining region of contact at a single end of each of the six pairs of homologous chromosomes in *C. elegans*. As previously observed by silver-staining of rye and lily chromosomes (Fedotova et al. 1989; Stack 1991), progression throughout diplotene is also accompanied by a progressive increase in axial coiling and a concomitant shortening in the total lengths of chromosomes in *C. elegans*. By diakinesis, the combined increase in both nuclear volume and chromosome compaction allows for the observation of chiasmata. In this configuration, homologous chromosomes are displayed facing away from each other and a cruciform organization becomes apparent. SYP-1 and SYP-2 staining is mostly present along just one of the axes of this cross while axis-associated components continue to stain both axes.

Second, superimposed onto the asymmetric disassembly of the SC is the distribution of crossover recombination events along homologous chromosomes. In the case of *C. elegans*, a single crossover event forms between each pair of homologous chromosomes. Moreover, this event is restricted, as in other species, to the terminal thirds of homologs (Barnes et al. 1995). This asymmetrically distributed event apparently translates into an asymmetric display when chiasmata become evident in diakinesis. Thus, the cruciform organization observed at this stage is comprised of the intersection between a long and a short axis corresponding to the long and short arms of the bivalent. For simplicity, the short arms are referred to as distal to the chiasma. It is interesting that SYP-1 and SYP-2's asymmetric localization consists of an association limited to the short arms of the bivalents.

The potential connection between the asymmetric localization of SYP-1 and SYP-2 with chiasma formation was initially probed by analyzing SYP-1 and SYP-2 localization in various meiotic recombination mutants. In the absence of crossover recombination, 12 univalents are present in oocytes at diakinesis instead of six bivalents. The temporal progression of assembly and disassembly of the SC is not affected by the absence of chiasmata in *C. elegans* (Colaiacovo et al. 2003; Nabeshima et al. 2005). However, the asymmetric disassembly of the SC, observed normally from late pachytene into diakinesis, is disrupted. SYP-1 and SYP-2 instead remain distributed along the full length of chromosomes in late pachytene and of univalents in diakinesis. Further evidence tying the asymmetric localization of SYP-1 and SYP-2 to chiasma formation stems from observations of γ-irradiated *spo-11* mutants (Nabeshima et al. 2005). Upon induction of double-strand breaks in this mutant, both chiasma formation and an asymmetric disassembly of the SC are reconstituted.

An additional layer of regulation is superimposed onto chromosomes during late meiotic prophase. During the first meiotic division, homologous chromosomes have to successfully segregate away from each other, but sister chromatids need to remain connected until their separation at the second meiotic division. Recent studies have demonstrated that this is achieved by a two-step release of sister chromatid cohesion (reviewed by Marston and Amon 2004). Studies in *C. elegans* indicate this involves REC-8 phosphorylation by the Aurora-like kinase AIR-2 (Rogers et al. 2002). It is interesting to note that AIR-2, albeit apparently present in lower levels, co-localizes with SYP-1 and SYP-2 in late pachytene and early diplotene (Nabeshima et al. 2005). It is then no longer observed to be

associated with chromosomes from mid-diplotene through mid-diakinesis. In late diakinesis, it resurges being restricted in its association only to the short arms of the bivalents. The timing of association of AIR-2 with this region coincides with the time of complete dissociation of SYP-1 and SYP-2 from chromosomes. A link between asymmetric disassembly of the SC, chiasma formation, and preparation for AIR-2 association is suggested by several observations. First, AIR-2 localizes along the full length of univalents during late diakinesis in a *spo-11* mutant, in a similar fashion to the localization observed in earlier diakinesis for SYP-1 and SYP-2. Second, its asymmetric localization along the short arms of the bivalents is reinstated upon artificial induction of DSBs by γ-irradiation of *spo-11* worms. Third, the late pachytene–early diplotene localization of AIR-2 is no longer observed in *syp-1* mutants. While a role for the earlier chromosomal association observed for AIR-2 in pachytene and diplotene is yet unclear, these observations altogether suggest a role for the asymmetric localization of SYP-1 and SYP-2 in possibly guiding the association of AIR-2 to this region.

It is interesting that either end of each pair of sister chromatids can become kinetically active during meiosis in *C. elegans*, and which end will acquire this fate in any given meiosis is in part determined by the formation of chiasmata which directs how the bivalents are displayed at the metaphase plate. Thus, short arms remain at the metaphase plate while the long arms orient toward the poles and acquire kinetic activity. Perhaps repression of kinetochore formation distal to the chiasma is accomplished in part through the asymmetric disassembly of the SC accompanied by localization of AIR-2 to this particular region of the bivalents. Alternatively, guiding AIR-2 association through the asymmetric disassembly of the SC may be a necessary strategy for the precise two-step release of cohesion in systems where this process may not be restrained by the presence of protector proteins at localized centromeres (Moore et al. 1998; Kitajima et al. 2004).

Conclusions and pending questions

Significant progress is being made in identifying the structural components of the SC and understanding its roles throughout meiosis. This fresh outlook is due in part to the systematic analysis of this structure in various model systems and the advancements in the technology and reagents available for such studies. The comparisons between the observations in systems as diverged as yeast, worms, flies, plants, and mammals are indicating how different strategies have been devised to ultimately achieve common goals. The key outcome is to successfully segregate homologous chromosomes away from each other upon the first meiotic division. This involves the formation of DSBs and the repair of a subset of these breaks as crossovers. This is superimposed by pairing and synapsis between homologous chromosomes. Some of the differences uncovered thus far revolve around the need of initiating DSBs to pair and synapse. On the other hand, along with such differences, conserved features have emerged, such as the interdependencies between SC formation and Msh4/Msh5-dependent crossover formation.

Studies in *C. elegans* have significantly contributed to our understanding of how the SC interfaces with homologous pairing, progression of meiotic recombination, and chromosome segregation. Yet, several questions remain unanswered. How do SC components assembling in systems such as *C. elegans* ultimately form a structure that is well conserved at the EM level? What is the functional significance of such structural conservation? How is crossover distribution so tightly regulated in *C. elegans* such that only (and always) a single DSB undergoes a crossover event between homologous chromosomes? What is the mechanism involved in sister chromatid exclusion? What are the additional synapsis initiation sites operating along side PCs? What is the involvement of components of the SC with components directly implicated in chromosome segregation? Are there checkpoints set in place specifically monitoring the progression of synapsis? Studies of meiosis from the perspective of the SC's structure and function in *C. elegans* and other systems will certainly continue to shed light on key features of this critically important biological process.

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References

Agarwal S, Roeder GS (2000) Zip3 provides a link between recombination enzymes and synaptonemal complex proteins. Cell 102:245–255

Albertson DG, Thomson JN (1982) The kinetochores of *Caenorhabditis elegans*. Chromosoma 86:409–428

Albertson DG, Thomson JN (1993) Segregation of holocentric chromosomes at meiosis in the nematode, *Caenorhabditis elegans*. Chromosome Res 1:15–26

Alpi A, Pasierbek P, Gartner A, Loidl J (2003) Genetic and cytological characterization of the recombination protein RAD-51 in *Caenorhabditis elegans*. Chromosoma 112:6–16

Anderson LK, Royer SM, Page SL, McKim KS, Lai A, Lilly MA, Hawley RS (2005) Juxtaposition of C(2)M and the transverse filament protein C(3)G within the central region of *Drosophila* synaptonemal complex. Proc Natl Acad Sci USA 102:4482–4487

Aravind L, Koonin EV (1998) The HORMA domain: a common structural denominator in mitotic checkpoints, chromosome synapsis and DNA repair. Trends Biochem Sci 23:284–286

Armstrong SJ, Caryl AP, Jones GH, Franklin FC (2002) Asy1, a protein required for meiotic chromosome synapsis, localizes to axis-associated chromatin in *Arabidopsis* and *Brassica*. J Cell Sci 115:3645–3655

Barnes TM, Kohara Y, Coulson A, Hekimi S (1995) Meiotic recombination, noncoding DNA and genomic organization in *Caenorhabditis elegans*. Genetics 141:159–179

Baudat F, Manova K, Yuen JP, Jasin M, Keeney S (2000) Chromosome synapsis defects and sexually dimorphic meiotic progression in mice lacking Spo11. Mol Cell 6:989–999

Bishop DK (1994) RecA homologs Dmc1 and Rad51 interact to form multiple nuclear complexes prior to meiotic chromosome synapsis. Cell 79:1081–1092

Bishop DK, Zickler D (2004) Early decision; meiotic crossover interference prior to stable strand exchange and synapsis. Cell 117: 9–15

Blat Y, Protacio RU, Hunter N, Kleckner N (2002) Physical and functional interactions among basic chromosome organizational features govern early steps of meiotic chiasma formation. Cell 111:791–802

Borner GV, Kleckner N, Hunter N (2004) Crossover/noncrossover differentiation, synaptonemal complex formation, and regulatory surveillance at the leptotene/zygotene transition of meiosis. Cell 117:29–45

Broverman SA, Meneely PM (1994) Meiotic mutants that cause a polar decrease in recombination on the X chromosome in *Caenorhabditis elegans*. Genetics 136:119–127

Buonomo SB, Clyne RK, Fuchs J, Loidl J, Uhlmann F, Nasmyth K (2000) Disjunction of homologous chromosomes in meiosis I depends on proteolytic cleavage of the meiotic cohesin Rec8 by separin. Cell 103:387–398

Burgess SM (2002) Homologous chromosome associations and nuclear order in meiotic and mitotically dividing cells of budding yeast. Adv Genet 46:49–90

Caryl AP, Armstrong SJ, Jones GH, Franklin FC (2000) A homologue of the yeast HOP1 gene is inactivated in the *Arabidopsis* meiotic mutant asy1. Chromosoma 109:62–71

Chan RC, Chan A, Jeon M, Wu TF, Pasqualone D, Rougvie AE, Meyer BJ (2003) Chromosome cohesion is regulated by a clock gene paralogue TIM-1. Nature 423:1002–1009

Cheeseman IM, Desai A (2005) A combined approach for the localization and tandem affinity purification of protein complexes from metazoans. Sci STKE 266:l1

Chua PR, Roeder GS (1997) Tam1, a telomere-associated meiotic protein, functions in chromosome synapsis and crossover interference. Genes Dev 11:1786–1800

Chua PR, Roeder GS (1998) Zip2, a meiosis-specific protein required for the initiation of chromosome synapsis. Cell 93:349–359

Colaiacovo MP, Stanfield GM, Reddy KC, Reinke V, Kim SK, Villeneuve AM (2002) A targeted RNAi screen for genes involved in chromosome morphogenesis and nuclear organization in the *Caenorhabditis elegans* germline. Genetics 162:113–128

Colaiacovo MP, MacQueen AJ, Martinez-Perez E, McDonald K, Adamo A, La Volpe A, Villeneuve AM (2003) Synaptonemal complex assembly in *C. elegans* is dispensable for loading strand-exchange proteins but critical for proper completion of recombination. Dev Cell 5: 463–474

Conrad MN, Dominguez AM, Dresser ME (1997) Ndj1p, a meiotic telomere protein required for normal chromosome synapsis and segregation in yeast. Science 276:1252–1255

Cooper JP, Watanabe Y, Nurse P (1998) Fission yeast Taz1 protein is required for meiotic telomere clustering and recombination. Nature 392:828–831

Couteau F, Zetka M (2005) HTP-1 coordinates synaptonemal complex assembly with homolog alignment during meiosis in *C. elegans*. Genes Dev 19:2744–2756

Couteau F, Nabeshima K, Villeneuve A, Zetka M (2004) A component of *C. elegans* meiotic chromosome axes at the interface of homolog alignment, synapsis, nuclear reorganization, and recombination. Curr Biol 14:585–592

Craig JM, Choo KH (2005) Kiss and break up—a safe passage to anaphase in mitosis and meiosis. Chromosoma 114:252–262

de Vries FA, de Boer E, van den Bosch M, Baarends WM, Ooms M, Yuan L, Liu JG, van Zeeland AA, Heyting C, Pastink A (2005) Mouse Sycp1 functions in synaptonemal complex assembly, meiotic recombination, and XY body formation. Genes Dev 19:1376–1389

Dernburg AF, McDonald K, Moulder G, Barstead R, Dresser M, Villeneuve AM (1998) Meiotic recombination in *C. elegans* initiates by a conserved mechanism and is dispensable for homologous chromosome synapsis. Cell 94:387–398

Ding DQ, Yamamoto A, Haraguchi T, Hiraoka Y (2004) Dynamics of homologous chromosome pairing during meiotic prophase in fission yeast. Dev Cell 6:329–341

Dobson MJ, Pearlman RE, Karaiskakis A, Spyropoulos B, Moens PB (1994) Synaptonemal complex proteins: occurrence, epitope mapping and chromosome disjunction. J Cell Sci 107:2749–2760

Dong H, Roeder GS (2000) Organization of the yeast Zip1 protein within the central region of the synaptonemal complex. J Cell Biol 148:417–426

Eijpe M, Offenberg H, Jessberger R, Revenkova E, Heyting C (2003) Meiotic cohesin REC8 marks the axial elements of rat synaptonemal complexes before cohesins SMC1b and SMC3. J Cell Biol 160:657–670

Fawcett DW (1956) The fine structure of chromosomes in the meiotic prophase of vertebrate spermatocytes. J Biophys Biochem Cytol 2:403–406

Fedotova YS, Kolomiets OL, Bogdanov YF (1989) Synaptonemal complex transformations in rye microsporocytes at the diplotene stage of meiosis. Genome 32:816–823

Franklin AE, McElver J, Sunjevaric I, Rothstein R, Bowen B, Cande WZ (1999) Three-dimensional microscopy of the Rad51 recombination protein during meiotic prophase. Plant Cell 11:809–824

Fung JC, Rockmill B, Odell M, Roeder GS (2004) Imposition of crossover interference through the nonrandom distribution of synapsis initiation complexes. Cell 116:795–802

Gerton JL, Hawley RS (2005) Homologous chromosome interactions in meiosis: diversity amidst conservation. Nat Rev Genet 6:477–487

Goldstein P (1982) The synaptonemal complexes of *Caenorhabditis elegans*: pachytene karyotype analysis of male and hermaphrodite wild-type and him mutants. Chromosoma 86:577–593

Goldstein P (1987) Multiple synaptonemal complexes (polycomplexes): origin, structure and function. Cell Biol Int Rep 11:759–796

Goldstein P, Slaton DE (1982) The synaptonemal complexes of *Caenorhabditis elegans*: comparison of wild-type and mutant strains and pachytene karyotype analysis of wild-type. Chromosoma 84:585–597

Golubovskaya IN, Harper LC, Pawlowski WP, Schichnes D, Cande WZ (2002) The pam1 gene is required for meiotic bouquet formation and efficient homologous synapsis in maize (*Zea mays* L.). Genetics 162:1979–1993

Grelon M, Vezon D, Gendrot G, Pelletier G (2001) AtSPO11-1 is necessary for efficient meiotic recombination in plants. EMBO J 20:589–600

Gumienny TL, Lambie E, Hartwieg E, Horvitz HR, Hengartner MO (1999) Genetic control of programmed cell death in the *Caenorhabditis elegans* hermaphrodite germline. Development 126:1011–1022

Hassold T, Hunt P (2001) To err (meiotically) is human: the genesis of human aneuploidy. Nat Rev Genet 2:280–291

Hawley RS (1980) Chromosomal sites necessary for normal levels of meiotic recombination in *Drosophila melanogaster*. I. Evidence for and mapping of the sites. Genetics 94:625–646

Henderson KA, Keeney S (2005) Synaptonemal complex formation: where does it start? Bioessays 27:995–998

Herman RK, Kari CK, Hartman PS (1982) Dominant X-chromosome nondisjunction mutants of *Caenorhabditis elegans*. Genetics 102:379–400

Herman RK, Kari CK (1989) Recombination between small X chromosome duplications and the X chromosome in *Caenorhabditis elegans*. Genetics 121:723–737

Higgins JD, Sanchez-Moran E, Armstrong SJ, Jones GH, Franklin FC (2005) The *Arabidopsis* synaptonemal complex protein ZYP1 is required for chromosome synapsis and normal fidelity of crossing over. Genes Dev 19:2488–2500

Hillers KJ, Villeneuve AM (2003) Chromosome-wide control of meiotic crossing over in *C. elegans*. Curr Biol 13:1641–1647

Hodgkin J, Horvitz HR, Brenner S (1979) Nondisjunction mutants of the nematode *Caenorhabditis elegans*. Genetics 91:67–94

Hollingsworth NM, Brill SJ (2004) The Mus81 solution to resolution: generating meiotic crossovers without Holliday junctions. Genes Dev 18:117–125

Hollingsworth NM, Byers B (1989) HOP1: a yeast meiotic pairing gene. Genetics 121:445–462

Hollingsworth NM, Goetsch L, Byers B (1990) The HOP1 gene encodes a meiosis-specific component of yeast chromosomes. Cell 61:73–84

Hollingsworth NM, Ponte L, Halsey C (1995) MSH5, a novel MutS homolog, facilitates meiotic reciprocal recombination between homologs in *Saccharomyces cerevisiae* but not mismatch repair. Genes Dev 9:1728–1739

Hunter N (2003) Synaptonemal complexities and commonalities. Mol Cell 12:533–539

Jang JK, Sherizen DE, Bhagat R, Manheim EA, McKim KS (2003) Relationship of DNA double-strand breaks to synapsis in *Drosophila*. J Cell Sci 116:3069–3077

Jansen G, Hazendonk E, Thijssen KL, Plasterk RH (1997) Reverse genetics by chemical mutagenesis in *Caenorhabditis elegans*. Nat Genet 17:119–121

Jantsch V, Pasierbek P, Mueller MM, Schweizer D, Jantsch M, Loidl J (2004) Targeted gene knockout reveals a role in meiotic recombination for ZHP-3, a Zip3-related protein in *Caenorhabditis elegans*. Mol Cell Biol 24:7998–8006

Jones GH (1987) Chiasmata. In: Moens PB (ed) Meiosis. Academic, Orlando, Florida, pp 213–244

Judis L, Chan ER, Schwartz S, Seftel A, Hassold T (2004) Meiosis I arrest and azoospermia in an infertile male explained by failure of formation of a component of the synaptonemal complex. Fertil Steril 81:205–209

Kamath RS, Fraser AG, Dong Y, Poulin G, Durbin R, Gotta M, Kanapin A, Le Bot N, Moreno S, Sohrmann M, Welchman DP, Zipperlen P, Ahringer J (2003) Systematic functional analysis of the *Caenorhabditis elegans* genome using RNAi. Nature 421:231–237

Kelly KO, Dernburg AF, Stanfield GM, Villeneuve AM (2000) *Caenorhabditis elegans* msh-5 is required for both normal and radiation-induced meiotic crossing over but not for completion of meiosis. Genetics 156:617–630

Kitajima TS, Kawashima SA, Watanabe Y (2004) The conserved kinetochore protein shugoshin protects centromeric cohesion during meiosis. Nature 427:510–517

Klein F, Mahr P, Galova M, Buonomo SB, Michaelis C, Nairz K, Nasmyth K (1999) A central role for cohesins in sister chromatid cohesion, formation of axial elements, and recombination during yeast meiosis. Cell 98:91–103

Kohli J, Bahler J (1994) Homologous recombination in fission yeast: absence of crossover interference and synaptonemal complex. Experientia 50:295–306

Li L, Gerecke EE, Zolan ME (1999) Homolog pairing and meiotic progression in *Coprinus cinereus*. Chromosoma 108:384–392

Liu JG, Yuan L, Brundell E, Bjorkroth B, Daneholt B, Hoog C (1996) Localization of the N-terminus of SCP1 to the central element of the synaptonemal complex and evidence for direct interactions between the N-termini of SCP1 molecules organized head-to-head. Exp Cell Res 226:11–19

Liu LX, Spoerke JM, Mulligan EL, Chen J, Reardon B, Westlund B, Sun L, Abel K, Armstrong B, Hardiman G et al (1999) High-throughput isolation of *Caenorhabditis elegans* deletion mutants. Genome Res 9:859–867

Loidl J, Nairz K, Klein F (1991) Meiotic chromosome synapsis in a haploid yeast. Chromosoma 100:221–228

MacQueen AJ, Villeneuve AM (2001) Nuclear reorganization and homologous chromosome pairing during meiotic prophase require *C. elegans chk-2*. Genes Dev 15:1674–1687

MacQueen AJ, Colaiacovo MP, McDonald K, Villeneuve AM (2002) Synapsis-dependent and -independent mechanisms stabilize homolog pairing during meiotic prophase in *C. elegans*. Genes Dev 16:2428–2442

MacQueen AJ, Phillips CM, Bhalla N, Weiser P, Villeneuve AM, Dernburg AF (2005) Chromosome sites play dual roles to establish homologous synapsis during meiosis in *C. elegans*. Cell 123:1037–1050

Maguire MP (1986) The pattern of pairing that is effective for crossing over in complex B–A chromosome rearrangements in maize. III. Possible evidence for pairing centers. Chromosoma 94:71–85

Marston AL, Amon A (2004) Meiosis: cell-cycle controls shuffle and deal. Nat Rev Mol Cell Biol 5:983–997

Martinez-Perez E, Villeneuve AM (2005) HTP-1-dependent constraints coordinate homolog pairing and synapsis and promote chiasma formation during *C. elegans* meiosis. Genes Dev 19:2727–2743

McKim KS, Howell AM, Rose AM (1988) The effects of translocations on recombination frequency in *Caenorhabditis elegans*. Genetics 120:987–1001

McKim KS, Peters K, Rose AM (1993) Two types of sites required for meiotic chromosome pairing in *Caenorhabditis elegans*. Genetics 134:749–768

McKim KS, Green-Marroquin BL, Sekelsky JJ, Chin G, Steinberg C, Khodosh R, Hawley RS (1998) Meiotic synapsis in the absence of recombination. Science 279:876–878

Meneely PM, Farago AF, Kauffman TM (2002) Crossover distribution and high interference for both the X chromosome and an autosome during oogenesis and spermatogenesis in *Caenorhabditis elegans*. Genetics 162:1169–1177

Meuwissen RL, Offenberg HH, Dietrich AJ, Riesewijk A, van Iersel M, Heyting C (1992) A coiled-coil related protein specific for synapsed regions of meiotic prophase chromosomes. EMBO J 11:5091–5100

Miyamoto T, Hasuike S, Yogev L, Maduro MR, Ishikawa M, Westphal H, Lamb DJ (2003) Azoospermia in patients heterozygous for a mutation in SYCP3. Lancet 362:1714–1719

Miyazaki WY, Orr-Weaver TL (1994) Sister-chromatid cohesion in mitosis and meiosis. Annu Rev Genet 28:167–187

Moens PB, Chen DJ, Shen Z, Kolas N, Tarsounas M, Heng HH, Spyropoulos B (1997) Rad51 immunocytology in rat and mouse spermatocytes and oocytes. Chromosoma 106:207–215

Moore DP, Page AW, Tang TT, Kerrebrock AW, Orr-Weaver TL (1998) The cohesion protein MEI-S332 localizes to condensed meiotic and mitotic centromeres until sister chromatids separate. J Cell Biol 140:1003–1012

Moses MJ (1956) Chromosomal structures in crayfish spermatocytes. J Biophys Biochem Cytol 2:215–218

Munz P (1994) An analysis of interference in the fission yeast *Schizosaccharomyces pombe*. Genetics 137:701–707

Nabeshima K, Villeneuve AM, Hillers KJ (2004) Chromosome-wide regulation of meiotic crossover formation in *Caenorhabditis elegans* requires properly assembled chromosome axes. Genetics 168:1275–1292

Nabeshima K, Villeneuve AM, Colaiacovo MP (2005) Crossing over is coupled to late meiotic prophase bivalent differentiation through asymmetric disassembly of the SC. J Cell Biol 168:683–689

Nag DK, Scherthan H, Rockmill B, Bhargava J, Roeder GS (1995) Heteroduplex DNA formation and homolog pairing in yeast meiotic mutants. Genetics 141:75–86

Nicklas RB (1974) Chromosome segregation mechanisms. Genetics 78:205–213
Nimmo ER, Pidoux AL, Perry PE, Allshire RC (1998) Defective meiosis in telomere-silencing mutants of *Schizosaccharomyces pombe*. Nature 392:825–828
Nonomura KI, Nakano M, Murata K, Miyoshi K, Eiguchi M, Miyao A, Hirochika H, Kurata N (2004) An insertional mutation in the rice PAIR2 gene, the ortholog of *Arabidopsis* ASY1, results in a defect in homologous chromosome pairing during meiosis. Mol Genet Genomics 271:121–129
Ollinger R, Alsheimer M, Benavente R (2005) Mammalian protein SCP1 forms synaptonemal complex-like structures in the absence of meiotic chromosomes. Mol Biol Cell 16:212–217
Padmore R, Cao L, Kleckner N (1991) Temporal comparison of recombination and synaptonemal complex formation during meiosis in *S. cerevisiae*. Cell 66:1239–1256
Page SL, Hawley RS (2001) c(3)G encodes a *Drosophila* synaptonemal complex protein. Genes Dev 15:3130–3143
Page SL, Hawley RS (2003) Chromosome choreography: the meiotic ballet. Science 301:785–789
Page SL, Hawley RS (2004) The genetics and molecular biology of the synaptonemal complex. Annu Rev Cell Dev Biol 20:525–558
Pasierbek P, Jantsch M, Melcher M, Schleiffer A, Schweizer D, Loidl J (2001) A *Caenorhabditis elegans* cohesion protein with functions in meiotic chromosome pairing and disjunction. Genes Dev 15:1349–1360
Peoples TL, Dean E, Gonzalez O, Lambourne L, Burgess SM (2002) Close, stable homolog juxtaposition during meiosis in budding yeast is dependent on meiotic recombination, occurs independently of synapsis, and is distinct from DSB-independent pairing contacts. Genes Dev 16:1682–1695
Phillips CM, Wong C, Bhalla N, Carlton PM, Weiser P, Meneely PM, Dernburg AF (2005) HIM-8 binds to the X chromosome pairing center and mediates chromosome-specific meiotic synapsis. Cell 123:1051–1063
Polanowska J, Martin JS, Fisher R, Scopa T, Rae I, Boulton SJ (2004) Tandem immunoaffinity purification of protein complexes from *Caenorhabditis elegans*. Biotechniques 36:778–780
Reboul J, Vaglio P, Rual JF, Lamesch P, Martinez M, Armstrong CM, Li S, Jacotot L, Bertin N, Janky R et al (2003) *C. elegans* ORFeome version 1.1: experimental verification of the genome annotation and resource for proteome-scale protein expression. Nat Genet 34:35–41
Reinke V, Smith HE, Nance J, Wang J, Van Doren C, Begley R, Jones SJ, Davis EB, Scherer S, Ward S, Kim SK (2000) A global profile of germline gene expression in *C. elegans*. Mol Cell 6:605–616
Reinke V, Gil IS, Ward S, Kazmer K (2004) Genome-wide germline-enriched and sex-biased expression profiles in *Caenorhabditis elegans*. Development 131:311–323
Roeder GS (1997) Meiotic chromosomes: it takes two to tango. Genes Dev 11:2600–2621
Rogers E, Bishop JD, Waddle JA, Schumacher JM, Lin R (2002) The aurora kinase AIR-2 functions in the release of chromosome cohesion in *Caenorhabditis elegans* meiosis. J Cell Biol 157:219–229
Romanienko PJ, Camerini-Otero RD (2000) The mouse Spo11 gene is required for meiotic chromosome synapsis. Mol Cell 6:975–987
Rose AM, Baillie DL, Curran J (1984) Meiotic pairing behavior of two free duplications of linkage group I in *Caenorhabditis elegans*. Mol Gen Genet 195:52–56
Rosenbluth RE, Baillie DL (1981) The genetic analysis of a reciprocal translocation, eT1(III; V), in *Caenorhabditis elegans*. Genetics 99:415–428
Rosenbluth RE, Johnsen RC, Baillie DL (1990) Pairing for recombination in LGV of *Caenorhabditis elegans*: a model based on recombination in deficiency heterozygotes. Genetics 124:615–625
Ross-Macdonald P, Roeder GS (1994) Mutation of a meiosis-specific MutS homolog decreases crossing over but not mismatch correction. Cell 79:1069–1080
Schedl T (1997) Developmental genetics of the germ line. In: Riddle DL, Blumenthal T, Meyer BJ, Priess JR (eds) *C. elegans* II. Cold Spring Harbor, Plainview, New York, pp 241–269
Scherthan H (2001) A bouquet makes ends meet. Nat Rev Mol Cell Biol 2:621–627
Schmekel K, Meuwissen RL, Dietrich AJ, Vink AC, van Marle J, van Veen H, Heyting C (1996) Organization of SCP1 protein molecules within synaptonemal complexes of the rat. Exp Cell Res 226:20–30
Schwacha A, Kleckner N (1997) Interhomolog bias during meiotic recombination: meiotic functions promote a highly differentiated interhomolog-only pathway. Cell 90:1123–1135
Sherizen D, Jang JK, Bhagat R, Kato N, McKim KS (2005) Meiotic recombination in *Drosophila* females depends on chromosome continuity between genetically defined boundaries. Genetics 169:767–781
Simmer F, Moorman C, Van Der Linden AM, Kuijk E, Van Den Berghe PV, Kamath R, Fraser AG, Ahringer J, Plasterk RH (2003) Genome-wide RNAi of using the hypersensitive *rrf-3* strain reveals novel gene functions. PLoS Biol 1(1):E12
Stack SM (1991) Staining plant cells with silver. II. Chromosome cores. Genome 34:900–908
Storlazzi A, Xu L, Schwacha A, Kleckner, N (1996) Synaptonemal complex (SC) component Zip1 plays a role in meiotic recombination independent of SC polymerization along the chromosomes. Proc Natl Acad Sci USA 93:9043–9048
Sung P (1994) Catalysis of ATP-dependent homologous DNA pairing and strand exchange by yeast RAD51 protein. Science 265:1241–1243
Sym M, Roeder GS (1995) Zip1-induced changes in synaptonemal complex structure and polycomplex assembly. J Cell Biol 128:455–466
Sym M, Engebrecht JA, Roeder, GS (1993) ZIP1 is a synaptonemal complex protein required for meiotic chromosome synapsis. Cell 72:365–378
Terasawa M, Shinohara A, Hotta Y, Ogawa H, Ogawa T (1995) Localization of RecA-like recombination proteins on chromosomes of the lily at various meiotic stages. Genes Dev 9:925–934
Trelles-Sticken E, Dresser ME, Scherthan (2000) Meiotic telomere protein Ndj1p is required for meiosis-specific telomere distribution, bouquet formation and efficient homologue pairing. J Cell Biol 151:95–106
Tsubouchi T, Roeder GS (2005) A synaptonemal complex protein promotes homology-independent centromere coupling. Science 308:870–873
Tung KS, Roeder GS (1998) Meiotic chromosome morphology and behavior in zip1 mutants of *Saccharomyces cerevisiae*. Genetics 149:817–832
Villeneuve AM (1994) A *cis*-acting locus that promotes crossing over between X chromosomes in *Caenorhabditis elegans*. Genetics 136:887–902
Villeneuve AM, Hillers KJ (2001) Whence meiosis? Cell 106:647–650
von Wettstein D, Rasmussen SW, Holm PB (1984) The synaptonemal complex and genetic segregation. Annu Rev Genet 18:331–413
Walhout AJ, Vidal M (2001) High-throughput yeast two-hybrid assays for large-scale protein interaction mapping. Methods 24:297–306
Wan L, de los Santos T, Zhang C, Shokat K, Hollingsworth NM (2004) Mek1 kinase activity functions downstream of RED1 in the regulation of meiotic double strand break repair in budding yeast. Mol Biol Cell 15:11–23
Wang F, Yoder J, Antoshechkin I, Han M (2003) *Caenorhabditis elegans* EVL-14/PDS-5 and SCC-3 are essential for sister chromatid cohesion in meiosis and mitosis. Mol Cell Biol 23:7698–7707

Webber HA, Howard L, Bickel SE (2004) The cohesion protein ORD is required for homologue bias during meiotic recombination. J Cell Biol 164:819–829

Weiner BM, Kleckner N (1994) Chromosome pairing via multiple interstitial interactions before and during meiosis in yeast. Cell 77:977–991

Whetstine JR, Ceron J, Ladd B, Dufourcq P, Reinke V, Shi Y (2005) Regulation of tissue-specific and extracellular matrix-related genes by a class I histone deacetylase. Mol Cell 18:483–490

Wicks SR, Yeh RT, Gish WR, Waterston RH, Plasterk RH (2001) Rapid gene mapping in *Caenorhabditis elegans* using a high density polymorphism map. Nat Genet 28:160–164

Yuan L, Brundell E, Hoog C (1996) Expression of the meiosis-specific synaptonemal complex protein 1 in a heterologous system results in the formation of large protein structures. Exp Cell Res 229:272–275

Zalevsky J, MacQueen AJ, Duffy JB, Kemphues KJ, Villeneuve AM (1999) Crossing over during *Caenorhabditis elegans* meiosis requires a conserved MutS-based pathway that is partially dispensable in budding yeast. Genetics 153:1271–1283

Zetka MC, Kawasaki I, Strome S, Muller F (1999) Synapsis and chiasma formation in *Caenorhabditis elegans* require HIM-3, a meiotic chromosome core component that functions in chromosome segregation. Genes Dev 13:2258–2270

Zickler D, Kleckner N (1998) The leptotene–zygotene transition of meiosis. Annu Rev Genet 32:619–697

Zickler D, Kleckner N (1999) Meiotic chromosomes: integrating structure and function. Annu Rev Genet 33:603–754