Synaptonemal complex central region proteins promote localization of pro-crossover factors to recombination events during *Caenorhabditis elegans* meiosis

Cori K. Cahoon*, Jacquelyn M. Helm*, and Diana E. Libuda*

* University of Oregon, Department of Biology, Institute of Molecular Biology, Eugene, Oregon, 97403
Running title: Synaptonemal complex and meiotic crossover proteins in C. elegans

Keywords: meiosis, recombination, crossovers, synaptonemal complex, C. elegans, chromosome axis, cohesin

Corresponding Author Information:
Diana E. Libuda
University of Oregon
Institute of Molecular Biology
1229 Franklin Blvd
Eugene, OR 97403
541-346-5092 (phone)
541-346-4854 (fax)
dlibuda@uoregon.edu
Abstract

Crossovers (COs) between homologous chromosomes are critical for meiotic chromosome segregation and form in the context of the synaptonemal complex (SC), a meiosis-specific structure that assembles between aligned homologs. During Caenorhabditis elegans meiosis, central region components of the SC (SYP proteins) are essential to repair double-strand DNA breaks (DSBs) as COs. Here, we investigate the relationships between the SYP proteins and conserved pro-CO factors by examining the immunolocalization of these proteins in meiotic mutants where SYP proteins are absent, reduced, or mislocalized. Although COs do not form in syp null mutants, pro-CO factors COSA-1, MSH-5, and ZHP-3 nevertheless co-localize at DSB-dependent sites during late prophase, reflecting an inherent affinity of these factors for DSB repair sites. In contrast, in mutants where SYP proteins are present but form aggregates or display abnormal synapsis, pro-CO factors consistently track with SYP-1 localization. Further, pro-CO factors usually localize to a single site per SYP-1 structure, even in SYP aggregates or in mutants where SC forms between sister chromatids, suggesting that CO regulation occurs within these aberrant SC structures. Moreover, we find that the meiotic cohesin REC-8 is required to ensure SC formation occurs between homologs and not sister chromatids. Taken together, our findings support a model in which SYP proteins promote CO formation by promoting the localization of pro-CO factors to recombination events within an SC compartment, thereby ensuring that pro-CO factors identify a recombination event within an SC structure and that CO maturation occurs only between properly aligned homologous chromosomes.
**Article Summary**

Errors during meiosis are the leading cause of birth defects and miscarriages in humans. Thus, the coordinated control of meiotic events is critical for the faithful inheritance of the genome each generation. The synaptonemal complex (SC) is a meiosis-specific structure that assembles between homologs chromosomes and is critical for the establishment and regulation of crossovers, which ensure the accurate segregation of the homologous chromosomes at meiosis I. Here we show that SC proteins function to regulate crossing over by controlling the localization of proteins involved in promoting the formation of crossovers.
Introduction

During sexual reproduction, generation of haploid gametes from diploid germ cells involves substantial reorganization of chromosomes within the nucleus and formation of specialized meiosis-specific chromosome structures. In preparation for segregating to opposite poles at the meiosis I division, homologous chromosome pairs align along their full lengths and assemble a structure known as the synaptonemal complex (SC) between them. The SC structure is composed of axial elements that assemble along the lengths of conjoined pairs of sister chromatids (known as lateral elements in the context of assembled SC), and a set of proteins that comprise the central region of the SC that link the parallel-aligned homolog axes (CAHOON and HAWLEY 2016; CAHOON and LIBUDA 2019). Multiple SC central region proteins function together to span the distance between the lateral elements and are required for normal meiosis in all organisms that assemble the SC. How the SC central region and its constituent proteins contribute to a successful outcome of meiosis remains a subject of active investigation.

Four different components of the SC central region have been identified in Caenorhabditis elegans, termed SYP-1, SYP-2, SYP-3 and SYP-4 (synaptonemal complex protein). These SYP proteins localize between the lateral elements of the SC and are interdependent for localization and stability (COLAIACOVO et al. 2003; SCHILD-PRUFERT et al. 2011). Analysis of syp mutants has demonstrated that the SYP proteins are required both to stabilize homolog pairing between homologous chromosomes and to promote the formation of crossover (CO) recombination events, which are required for proper chromosome segregation during meiosis I (MACQUEEN et al. 2002; COLAIACOVO et al. 2003; SMOLIKOV et al. 2007b; SMOLIKOV et al. 2009). In addition to this role in promoting CO formation, the SYP proteins also play a role in limiting the number of COs that form during meiosis. A partial depletion of SYP proteins by RNAi causes an increase in the number of chromosomes with more than one CO and an attenuation of CO interference (LIBUDA et al. 2013). Further, recent studies have suggested a liquid crystalline-like behavior of the SC central region proteins and revealed
dynamic properties of the SYPs that change during the course of meiotic prophase progression (ROG and DERNBURG 2015; MLYNARCZYK-EVANS and VILLENEUVE 2017; PATTABIRAMAN et al. 2017; ROG et al. 2017). Moreover, studies have found that CO recombination events are linked to post-translational modifications of the SYP proteins (KIM et al. 2015; NADARAJAN et al. 2016; NADARAJAN et al. 2017; PATTABIRAMAN et al. 2017). Despite these advances in understanding, how the SYP proteins promote the formation of COs between homologs during meiosis remains poorly understood.

In the context of an assembled SC, a set of pro-CO factors (MSH-5, COSA-1, ZHP-1, ZHP-2, ZHP-3, and ZHP-4) are loaded on chromosomes during *C. elegans* meiosis to promote and license the repair of a subset of programmed double strand DNA breaks (DSBs) as COs between homologs (KELLY et al. 2000; JANTSCH et al. 2004; BHALLA et al. 2008; YOKOO et al. 2012; NGUYEN et al. 2018; ZHANG et al. 2018). Following the formation of DSBs by the conserved endonuclease SPO-11, the pro-CO factor MSH-5 (a component of the meiosis-specific MutSγ complex) and COSA-1 (a cyclin-related protein specific to metazoan meiosis) form multiple DSB-dependent foci in early pachytene prior to reducing down in number in late pachytene, marking the 6 CO sites (one CO per chromosome) in *C. elegans* meiosis (KELLY et al. 2000; YOKOO et al. 2012; WOGLAR and VILLENEUVE 2018). In contrast, ZHP-1, ZHP-2, ZHP-3, and ZHP-4 (RING domain containing proteins) coat the SC in early pachytene before reducing and retracting down in a DSB-dependent manner to distinct foci that colocalize with the 6 CO sites marked by MSH-5 and COSA-1 in late pachytene (JANTSCH et al. 2004; BHALLA et al. 2008; YOKOO et al. 2012; NGUYEN et al. 2018; ZHANG et al. 2018). Although analysis of null mutants of the pro-CO factors demonstrates that these proteins are interdependent for their localization and are required for CO formation (YOKOO et al. 2012; ROG et al. 2017), the mechanism of how the pro-CO factors function with one another and the SC to establish a CO is unknown. Recent evidence in *C. elegans* indicates that the SC proteins envelop CO-designated
sites marked by the pro-CO factors (WOGLAR and VILLENUEVE 2018); however, the relationship
between the pro-CO factors and the SC is still largely unclear.

Here we address how the SC central region proteins promote formation of interhomolog
COs by investigating how the SYPs contribute to localization of conserved pro-CO factors that
normally localize at CO sites. Our findings indicate that meiotic chromosome structures
collaborate together with recombination events to control the localization of pro-CO factors,
such that: 1) SYP proteins dictate the context in which pro-CO factors attempt to locate
recombination intermediates; and, 2) correctly assembled chromosome axes restrict SYP
proteins to load only between paired homologs. These features likely promote formation of
interhomolog COs by ensuring that CO maturation occurs only in a productive manner, between
properly aligned and synapsed homologous chromosomes.

Materials and Methods

C. elegans strains, genetics, and culture conditions. All strains are from the Bristol N2
background and were maintained and crossed at 20°C under standard conditions.
Temperatures used for specific experiments are indicated below. For all experiments with
meiotic mutants, homozygous mutant worms were derived from balanced heterozygous parents
by selecting progeny lacking a dominant marker (Unc and/or GFP) associated with the balancer.

The following strains were used in this study:

N2: Bristol wild-type strain.
AV198:  spo-11(ok79) IV; syp-1(me17) V / nT1[unc-?(n754) let-? qls50] (IV;V).
AV276:  syp-2(ok307) V / nT1[unc-?(n754) let-?(m435)] (IV;V).
AV278:  spo-11 IV; syp-2(ok307) V / nT1[unc-?(n754) let-? qls50] (IV;V).
AV307: syp-1(me17) V / nT1[unc-?(n754) let-? qIs50] (IV;V).

AV596: cosa-1(tm3298)/ qC1[qIs26] (III).

AV630: mels8[unc-119(+)] Ppie-1::gfp::cosa-1] II.

AV647: mels8[unc-119(+)] Ppie-1::gfp::cosa-1] II; spo-11(me44) IV / nT1[unc-?(n754) let-? qIs50] (IV;V).

AV671: mels8[unc-119(+)] Ppie-1::gfp::cosa-1] II; him-3(e1256) IV.

AV686: mels8[unc-119(+)] Ppie-1::gfp::cosa-1] II; rec-8(ok978) IV / nT1[qIs51] (IV;V).

AV687: syp-3(ok758) I / hT2[bli-4(e937) let-?(q758) qIs48] (I;III); mels8[unc-119(+)] Ppie-1::gfp::cosa-1] II.

AV688: mels8[unc-119(+)] Ppie-1::gfp::cosa-1] II; syp-2(ok307) V / nT1[unc-?(n754) let-?(m435)] (IV;V).

AV689: mels8[unc-119(+)] Ppie-1::gfp::cosa-1] II; him-3(gk149) IV / nT1[qIs51] (IV;V).

AV695: mels8[unc-119(+)] Ppie-1::gfp::cosa-1] II; mnT12 (X;IV).

AV697: mels8[unc-119(+)] Ppie-1::gfp::cosa-1] II; htp-3(y428) ccls4251 I / hT2[bli-4(e937) let-?(q782) qIs48] (I,III).

AV699: mels8[unc-119(+)] Ppie-1::gfp::cosa-1] II; syp-1(me17) V / nT1[unc-?(n754) let-? qIs50] (IV;V).

AV700: him-3(gk149) IV / nT1[qIs51] (IV;V); syp-2(ok307) V / nT1[qIs51] (IV;V).

CB1256: him-3(e1256) IV.

CV2: syp-3(ok758) I / hT2[bli-4(e937) let-?(q758) qIs48] (I;III).

TY4986: htp-3(y428) ccls4251 I / hT2[bli-4(e937) let-?(q782) qIs48] (I,III).

VC418: him-3(gk149) IV / nT1[qIs51] (IV;V).

VC666: rec-8(ok978) IV / nT1[qIs51] (IV;V).

DLW1: cosa-1(tm3298)/ qC1[qIs26] III; rec-8(ok978) IV/nT1 [qIs51] (IV;V).

DLW12: GFP::COSA-1 II; rec-8 (ok978)/ nT1 [qIs51] (IV;V); syp-2(ok307) V/nT1 (IV;V).
Additional information on strains:

$qls48$ contains $[P\text{myo-2::gfp}; \ P\text{pes-10::gfp}; \ P\text{ges-1::gfp}]$.

$qls50$ contains $[P\text{myo-2::gfp}; \ P\text{pes-10::gfp}; \ P\text{F22B7.9::gfp}]$.

$qls51$ contains $[P\text{myo-2::gfp}; \ P\text{pes-10::gfp}; \ P\text{F22B7.9::gfp}]$.

**syp-1 partial depletion by RNAi.** Partial depletion of $syp-1$ by RNAi was performed as in (LIBUDA et al. 2013). Notably, partial depletion of $syp-2$ and $syp-3$ has been shown to also function similarly to partial depletion of $syp-1$ with regards to affecting CO numbers in *C. elegans* (LIBUDA et al. 2013). Worms were synchronized at the L1 phase by bleaching adults and allowing resultant eggs to hatch on unseeded NGM plates at 20°C for 20-24 hrs. Synchronized L1s were then washed off of the unseeded NGM plates with M9 and placed on NGM+IPTG+Amp plates that were poured within 30 days of use and freshly seeded one day before use with *Escherichia coli* HT115 cells containing either a fragment of the $syp-1/F26D2.2$ gene in the L4440 vector (Ahringer Lab RNAi library) or, the empty vector (referred to as “control RNAi” in figures and text). The RNAi plates with L1s were then placed at 25°C for 40-48 hrs and then their gonads dissected for immunofluorescence.

**Immunofluorescence.** Immunofluorescence was performed as in (LIBUDA et al. 2013). Gonads from adult worms at 18-24 hours post-L4 stage were dissected in 1x egg buffer with 0.1% Tween on VWR Superfrost Plus slides, fixed for 5 min in 1% paraformaldehyde, flash frozen with liquid nitrogen, and then fixed for 1 minute in 100% methanol at -20°C. Slides were washed 3 x 5 min in 1x PBST and blocked for one hour in 0.7% BSA in 1x PBST. Primary antibody dilutions were made in 1x PBST and added to slides. Slides were covered with a parafilm coverslip and incubated in a humid chamber overnight (14-18 hrs). Slides were washed 3 x 10 min in 1x PBST. Secondary antibody dilutions were made at 1:200 in 1x PBST using Invitrogen
goat or donkey AlexaFluor labeled antibodies and added to slides. Slides were covered with a parafilm coverslip and placed in a humid chamber in the dark for 2 hrs. Slides were washed 3 x 10 min in 1x PBST in the dark. All washes and incubations were performed at room temperature, unless otherwise noted. 2 μg/ml DAPI was added to slides and slides were subsequently incubated in the dark with a parafilm coverslip in a humid chamber. Slides were washed once for 5 min in 1x PBST prior to mounting with Vectashield and a 20 x 40 mm coverslip with a 170 ± 5 μm thickness. Slides were sealed with nail polish immediately following mounting and then stored at 4°C prior to imaging. For structured illumination microscopy imaging (SIM), slides were made as described above with the following modification. All SIM slides were mounted in Prolong Gold (ThermoFisher, P36930) and left to harden at room temperature for 2-3 days prior to imaging. All slides were imaged (as described below) within two weeks of preparation. The following primary antibody dilutions were used: rabbit anti-GFP (1:1000) (YOKOO et al. 2012); chicken anti-GFP (1:1000) (Abcam 13970); guinea pig anti-ZHP-3 (1:500) (BHALLA et al. 2008); rabbit anti-MSH-5 (1:10000) (Novus #3875.00.02); guinea pig anti-SYP-1 (1:200) (MACQUEEN et al. 2002); goat anti-SYP-1 (1:1500) (HARPER et al. 2011); guinea pig anti-HIM-8 (1:250) (PHILLIPS et al. 2009), and chicken anti-HTP-3 (1:500) (MACQUEEN et al. 2005).

**Imaging.** Immunofluorescence slides were imaged at 512 x 512 pixel dimensions on an Applied Precision DeltaVision microscope with a 63x lens with 1.5x optivar. Images were acquired as Z-stacks at 0.2 μm intervals and deconvolved with Applied Precision softWoRx deconvolution software. For quantification of GFP::COSA-1 foci, nuclei that were in the last 4-5 rows of late pachytene and were completely contained within the image stack were analyzed. Foci were quantified manually from deconvolved three-dimensional stacks. For quantification of chiasmata and visualization of chiasmata, individual chromosomes from a single diakinesis nucleus were
cropped and rotated in three dimensions using Volocity three-dimensional rendering software. Images shown are projections through three-dimensional data stacks encompassing whole nuclei, generated with a maximum-intensity algorithm with the softWoRx software. For quantification of HIM-8, early to mid pachytene regions of the gonad were visualized in IMARIS and the distance between each HIM-8 focus was determined using the “Measurements” tool in IMARIS. HIM-8 foci were considered paired if the distance between the foci was ≤0.7 µm and unpaired if the distance was >0.7 µm. To determine SC association with the HIM-8 foci, the nuclei were visualized and rotated in 3D using IMARIS to track the SC traces in and out of each HIM-8 focus. A single SC trace was determined by following a single SC track in and out of the HIM-8 focus. For two SC traces, the HIM-8 focus displayed two SC tracks coming in and two SC tracks coming out of the HIM-8 focus.

For SIM, slides were imaged at 2430 x 2430 pixel dimensions on a Zeiss ELYRA S.1 / LSM 880 microscope with a Plan Apochromat 63x (1.4 NA) oil lens. Images were acquired as a Z-stack at 0.110 µm interval with 3 rotations and were processed using the Zeiss ZEN software for both SIM reconstruction and channel alignment (alignment calibration based off 100-nm TetraSpeck beads from ThermoFisher). Maximum-intensity projections were generated using FIJI (NIH). Images were adjusted for brightness and contrast. For quantification of SC trace length, individual nuclei were 3D cropped from the mid-pachytene region using IMARIS. The “Filament tracer” tool in IMARIS was used to trace the SC in each cropped nucleus.

**Statistics.** Most of the p-values reported are two-tailed and calculated from Mann-Whitney tests, which are robust non-parametric statistical tests appropriate for the relevant data sets. A Wilcoxon test was used for the *rec-8* mutant SC trace comparisons. Each test used is indicated next to the reported p-value in the Results section.
Data Availability. All strains are available upon request. Figure S1A shows localization of COSA-1, ZHP-3, and MSH-5 in syp-1 and syp-3 mutants. Figure S1B has unadjusted images of GFP::COSA-1 localization in wild-type, syp-2, and syp-3 mutants. Figure S2A shows the localization of COSA-1 and MSH-5 in wild-type, him-3, htp-3, and rec-8 mutants. Figure S2B shows localization of COSA-1 and ZHP-3 in wild-type and rec-8 mutant. Figure S3 shows localization of COSA-1 and SYP-1 in syp-1 partial depletions combined with wild-type, him-3, and rec-8 mutants. Figure S4 shows representative images and the quantification of DAPI staining bodies at diakinesis from wild-type, rec-8, cosa-1, rec-8; cosa-1, and rec-8; syp-2 mutants. Figure S5 shows the localization of RAD-51 in wild-type, syp-2 (ok307), and syp-2; rec-8 mutants at both late pachytene (A) and diakinesis (B). Table S1 has the number of GFP::COSA-1 foci per nucleus in late pachytene from wild-type, syp-1, syp-2, syp-3, rec-8, rec-8; syp-2, and him-3 mutants. Supplemental materials available at Figshare.

Results

Pro-crossover factors co-localize to DSB-dependent events in late pachytene of mutants lacking synaptonemal complex central region proteins

Many of the pro-CO factors display distinct localization patterns that often co-localize with the SYP proteins during pachytene (Figure 1). ZHP-3 (an E3 ligase) localizes along the lengths of the chromosomes in nuclei at the mid-pachytene stage of meiotic prophase, and MSH-5 forms foci in excess of the number of COs at this stage (JANTSCH et al. 2004; BHALLA et al. 2008; ZHANG et al. 2018). Upon transition to the late pachytene stage, COSA-1 is detected as 6 bright foci at nascent CO sites that colocalize with MSH-5 and correspond to the 6 CO sites (1 per chromosome) in C. elegans [(YOKOO et al. 2012); Table S1]. Additionally, at late pachytene, the
ZHP-3 tracks gradually retract in a DSB-dependent manner to form distinct foci that colocalize with COSA-1 and MSH-5 (Yokoo et al. 2012).

In syp null mutants, meiotic recombination is initiated by the formation of DSBs, but repair of these breaks does not yield interhomolog COs and the germ lines exhibit a prolonged clustering stage (MacQueen et al. 2002; ColaiacoVo et al. 2003; Smolikov et al. 2007b; Smolikov et al. 2009). Despite the lack of interhomolog COs, syp null mutants display a few foci of COSA-1 and MSH-5, specifically during late pachytene when the prolonged clustering stage ends (Figure 1A; Figure S1). As was also shown in previous studies (Jantsch et al. 2004; Zhang et al. 2018), localization of ZHP-3 as tracks along the chromosomes in early-mid pachytene requires the SYP proteins, and upon eventual release from the prolonged clustering in syp mutants, ZHP-3 localizes as foci during late pachytene. Further, we found that most of these ZHP-3 foci in the syp mutants colocalize with MSH-5 or COSA-1 (Figure 1A; Figure S1). Notably, the ZHP-3, MSH-5, and COSA-1 foci observed in the syp mutants are of weaker intensity than those observed in wild-type or other mutant situations where SYP proteins are present (Figure S1B). In contrast to the highly reproducible number of COSA-1 foci (6 per nucleus) detected in wild-type, the number of COSA-1 foci observed in syp mutants are reduced and significantly different from wild-type (Figure 1B; Table S1; P<0.0001, Mann Whitney). Thus, in the absence of the SYPs, pro-CO factors are able to associate into foci, even though these foci are incapable of forming COs.

To determine if these pro-CO factor foci in syp mutants are forming at DSB sites, we assessed pro-CO factor localization in syp mutants that lacked SPO-11, the conserved endonuclease that forms programmed DSBs during meiosis (Dernburg et al. 1998). Similar to the spo-11 single mutant [which lacks endogenous DSBs; (Dernburg et al. 1998)], late pachytene nuclei in spo-11; syp-1 and spo-11; syp-2 double mutants typically have only an occasional MSH-5 focus (0-1 focus per nucleus; Figure 2). This result demonstrates that in the absence of synapsis, the localization of pro-CO factors is dependent on programmed DSBs.
Further, this result reflects the proclivity of these factors to associate both with each other and with abnormal recombination intermediates present on the chromosomes in this context (Pattabiraman et al. 2017).

**Pro-crossover factors specifically associate with SYP stretches along chromosomes in mutants with limited synapsis**

To further understand the relationship between the SC and the loading of pro-CO factors, we examined localization of pro-CO factors and SYP-1 in mutants with abnormal SC formation. Specifically, we assessed worms homozygous for partial loss-of-function mutations affecting the chromosome axis protein HIM-3 (Figure 3; Figure S2). In *him-3(e1256)* mutants, SYP-1 loads only on a subset of chromosome pairs (usually four chromosomes) (Zetka et al. 1999). In these *him-3(e1256)* mutants, we found COSA-1 and MSH-5 foci only associated with the synapsed chromosomes displaying extensive SYP-1 stretches during late pachytene (Figure 3A and S2). Further, for *him-3(e1256)* mutants the average number of COSA-1 foci detected in late pachytene nuclei corresponded to the eventual number of chiasmata present in diakinesis stage oocytes (Figure 3B; Table S1; \(P<0.0001\), Mann Whitney), indicating that the COSA-1 foci detected in this mutant represent *bona fide* CO events. In comparison, the *him-3(me80)* mutants exhibit a more severe synapsis defect where only short discontinuous stretches of synapsis occur on a subset of autosomes (Figure 3A; Couteau et al. 2004; Nabeshima et al. 2005)). Additionally, *him-3(me80)* mutants also display increases in double COs determined by both genetic assays and diakinesis bivalents containing two chiasmata (Couteau et al. 2004; Nabeshima et al. 2004)). Similar to *him-3(e1256)*, COSA-1 foci in *him-3(me80)* were also invariably associated with the limited SYP-1 stretches (Figure 3A). Similar to prior studies in wild-type and in strains partially depleted for *syp-1* (Yokoo et al. 2012; Libuda et al. 2013), we found all COSA-1 foci were associated with a SYP-1 stretch in either *him-3* partial loss of
function mutant (90/90 nuclei contain COSA-1 associated with SC in him-3(e1256); 90/90 nuclei contain COSA-1 associated with SC in him-3(me80)).

To determine if CO regulation is still occurring within the SC structures of him-3(e1256), we decided to test whether we could experimentally perturb CO interference in this mutant. In previous work, we showed that partial depletion of any of the SYP proteins (SYP-1, SYP-2, and SYP-3) by 60-70% results in an increased number of COSA-1 foci and attenuates CO interference (LIBUDA et al. 2013). Combining the partial depletion of SYP-1 with the him-3(e1256) mutant resulted in an increased occurrence of SYP-1 stretches with greater than 2 COSA-1 foci (Figure S3). In 14% of control RNAi treated him-3(e1256) nuclei, we observe two COSA-1 foci along a SYP-1 stretch. Upon syp-1 partial RNAi treatment, the number of SYP-1 stretches containing more than two COSA-1 foci significantly increased to 41%. This result suggests that CO interference is likely still acting along the SYP-1 stretches that form on the chromosomes in him-3(e1256) partial loss of function mutants.

**Pro-crossover factors associate with SYP protein aggregates formed in null mutants lacking meiotic chromosome axis components**

To determine if the localization of pro-CO factors is directed by SYP proteins, we examined pro-CO factor localization in mutants where SYPs form aggregates in the nucleoplasm (Figure 4; Figure S2). Mutants null for the lateral elements HTP-3 and HIM-3 both are unable to form COs and are unable to load SYP proteins onto chromosomes, instead forming a SYP protein aggregate within the nucleoplasm (COUTEAU et al. 2004; GOODYER et al. 2008). Specifically, him-3(gk149) null mutants typically contain a single elongated SYP-1 aggregate in late pachytene nuclei (Figure 4A). Likewise, htp-3(y428) null mutants usually contain one or sometimes two aggregates per nucleus in late pachytene (Figure 4A). Both him-3 and htp-3 mutants displayed COSA-1 and MSH-5 foci associated with the SYP aggregate (Figure 4; Figure S2). Notably, this similar result for pro-CO factor localization in him-3 and htp-3 null
mutants occurs despite the fact DSBs are formed in him-3(gk149) but not in htp-3(y428) (COUTEAU et al. 2004; GOODYER et al. 2008). Further, in most cases, we find that only a single COSA-1 focus was associated with a given SYP-1 aggregate (Figure 4A). Similarly, Rog et al. 2017 also showed that the pro-CO factors ZHP-3 and COSA-1 also localize to SC aggregates as a single focus in htp-3(tm3655) null mutants. Collectively, these data suggest that COSA-1 has a strong tendency to associate with SYP-1 and that the ability to limit COSA-1 foci to a single site on a given SYP-1 structure is retained even when the SYP proteins are concentrated in a nucleoplasmic aggregate.

To test whether the pro-CO factors are held in a SYP-dependent manner within the aggregates formed in the him-3(gk149) null mutant, we assessed MSH-5 and ZHP-3 localization in an him-3(gk149); syp-2(ok307) double mutant. In contrast to the him-3(gk149) null mutant, we found that the him-3(gk149); syp-2(ok307) double mutant (Figure 4) looked similar to the syp-2(ok307) single mutant (Figure 1), where MSH-5 and ZHP-3 localize to multiple DSB-dependent chromosomal sites in late pachytene nuclei. This finding suggests that aggregation of SYP proteins to a single site preferentially stabilizes the association of pro-CO factors and directs them to colocalize together with SYP-1 in a single compartment. When this constraint is released, the pro-CO factors are free to associate together at DSB-dependent chromosomal sites.

**rec-8 mutants inappropriately assemble SC between sister chromatid pairs**

The proclivity of pro-CO factors to be targeted to synapsed regions raised the possibility that inappropriate synapsis between nonhomologous chromosomes and/or sister chromatids might also direct the localization of pro-CO factors to these incorrectly synapsed regions. Multiple studies have suggested that worms mutant for a meiosis-specific cohesin protein REC-8 may undergo nonhomologous and/or sister chromatid synapsis due to extensive homolog pairing defects (PASIERBEK et al. 2001; SEVERSON et al. 2009). Further, electron microscopy in rec-8
mutant mouse spermatocytes indicates SC formation may occur between sister chromatids in this context (Xu et al. 2005).

To characterize chromosome synapsis along the 12 sets of sister chromatids in a C. elegans rec-8(ok978) null mutant, we used super resolution microscopy. We found that both wild-type and rec-8 null mutants displayed tripartite SC with SYP-1 localizing between the two HTP-3 tracks suggesting that the overall structure of the SC is unaltered in rec-8 null mutants (Figure 5A). Notably, after tracing the SC segments in mid pachytene nuclei, we observed that the number of SC segments in rec-8(ok978) null mutants is significantly higher than wild-type. While wild-type always displayed six SC traces per nucleus representing the six homolog pairs, rec-8 null mutants showed on average 10 SC traces per nucleus (Figure 5B) suggesting that inappropriate synapsis is occurring among the 12 pairs of sister chromatids (P<0.0001, Wilcoxon test). Further, the length of the SC traces in rec-8 mutants were on average shorter than the average length of the wild-type traces (3.13 µm and 5.53 µm respectively; P<0.0001, Mann Whitney test; Figure 5C). Some of these shorter traces in the rec-8 mutants had unsynapsed HTP-3 segments extending from the synapsed region (Figure 5A and 5D), likely representing regions of partial synapsis along the chromosome. However, it is also possible that some of these short-synapsed regions are the result the chromosome self-synapsing.

Some of the SC traces in the rec-8 null mutants displayed lengths longer than the wild-type traces with traces ranging from 8 µm to nearly 12 µm long. We determined that these long SC traces were created by multi-chromosome and/or chromatid synapsis events (Figure 5D). Among these events, we observed 3 different classes of multi-chromosome synapsis: (1) a single branching Y-shaped structure, (2) a bubble-shaped structure, and (3) very large multi-branching structures (as if four chromatids are synapsing along different regions) (Figure 5D). Taken together, the formation of these large aberrant synapsis events and the average 10 SC traces observed in rec-8 mutants suggests that these mutants are assembling SC between the sister chromatids and possibly with nonhomologous chromosomes.
To further determine if rec-8 null mutants are assembling SC between sister chromatids, we also assayed homolog pairing on the X chromosome using the pairing center protein HIM-8, which binds to the pairing center region on one end of the X chromosome and is required for X chromosome pairing (MacQueen et al. 2005; Phillips et al. 2009). In wild-type, 100% of HIM-8 foci are paired (≤0.7 µm apart) and only have a single SC track extending from the paired HIM-8 focus, indicating the SC assembled between the homologs (Figure 6). While previous work using FISH has shown that rec-8 RNAi nuclei display extensive homolog pairing defects along the autosomes (Pasierbek et al. 2001), we found that rec-8 null mutants displayed only a slight X-chromosome pairing defect with ~80% of the HIM-8 foci being paired. Strikingly, nearly 60% of these paired HIM-8 foci in rec-8 null mutants displayed two SC tracks extending from the HIM-8 focus (Figure 6). Additionally, of the 20% unpaired HIM-8 foci, 92% of these foci had SC associated with each unpaired HIM-8 focus. The occurrence of two sets of SC tracks extending from paired HIM-8 foci and SC associating with both unpaired HIM-8 foci strongly suggests that rec-8 null mutants are assembling SC between sister chromatids.

Localization of pro-crossover factors tracks with SYP stretches when synapsis occurs incorrectly between sister chromatid pairs

To test whether localization of pro-CO factors could be mistargeted to events along incorrectly synapsed regions, we investigated pro-CO factor localization in the rec-8(ok978) null mutant. We found that the inappropriately synapsed sister chromatids in rec-8(ok978) mutants still enable localization of pro-CO factors to sites along the synapsed sisters. Specifically, rec-8 null mutants displayed COSA-1 foci along the SYP-1 stretches in late pachytene nuclei (Figure 7A) and between condensed pairs of sister chromatids in diplotene and diakinesis phase nuclei (Figure 7C). Interestingly, 99% of rec-8 mutants localized COSA-1 to no more than 12 sites in each late pachytene nucleus (Figure 7B; Table S1). Further, ZHP-3 and MSH-5 similarly
associate with COSA-1 foci strongly suggesting that recombination may be occurring between sister chromatids in this mutant (Figure S2).

The average number of COSA-1 foci formed in rec-8(ok978) null mutants was consistent with the average number of SC traces per nucleus (10.4 ± 1.2 COSA-1 foci per nucleus and 10 SC traces per nucleus; Figures 5A and 7B; Table S1). This observation in rec-8(ok978) null mutants may reflect an imposed limitation of COSA-1 foci by CO interference occurring along synapsed sister chromatids and multi-chromatid synapsis events. In support of this suggestion, partial depletion of SYP-1 in the rec-8(ok978) null mutant background resulted in the frequent occurrence of SYP-1 stretches harboring two COSA-1 foci, even while reducing the fraction of chromosomes associated with SYP-1 stretches (Figure S3). Together, our data reinforce the suggestion that the SC central region may regulate CO numbers and distribution along the length of a chromosome (or chromatid) in which the SC has assembled.

**Regions of desynapsis in rec-8 null mutants fail to repair DSBs**

Previous studies have shown that rec-8 null mutants will frequently equationally separate the sister chromatids at the first meiotic division and that the sister chromatids are held together in a DSB-dependent manner prior to the first meiotic division (SEVERSON et al. 2009; SEVERSON and MEYER 2014). As COSA-1 localization is DSB-dependent (YOKOO et al. 2012), our finding of COSA-1 foci between condensed sister chromatid pairs during diakinesis in rec-8 null mutants suggests sister chromatids are held together in a COSA-1 dependent manner at a DSB site (Figure 7C). Previous studies found that in rec-8 mutants, the absence of COSA-1 causes the separation of the sister chromatids at diakinesis (CRAWLEY et al. 2016) and the absence of the SC results in severe chromosome fragmentation (COLAIACOVO et al. 2003) (Figure S4). Further, we found that the number of COSA-1 foci are significantly reduced in the rec-8; syp-2 double mutant (Figure 7B; Table S1). Thus, the SC central region between sister chromatids in rec-8 mutants is important for the efficient loading of COSA-1. Additionally, the loading of COSA-1 in
rec-8 mutants is likely marking a DSB-dependent event occurring between sister chromatids that may be used to equationally separate the sister chromatids at meiosis I.

The previously reported striking chromosome fragmentation defect in the rec-8; syp-2 double mutant [(COLAIACOVO et al. 2003); Figure S4] suggests the intersister associations created by the SC central region proteins in rec-8 mutants are required to complete DSB repair. Therefore, in the absence of the SC, rec-8; syp-2 double mutants likely lose both intersister and interhomolog associations and accumulate unrepaired DSBs, which leads to the extensive chromosome fragments, DNA bridges, and DNA aggregates observed in the majority of the diakinesis nuclei of rec-8; syp-2 mutants [(COLAIACOVO et al. 2003); Figure S4]. In accordance with this chromosome fragmentation phenotype during diakinesis in rec-8; syp-2 double mutants, the recombinase RAD-51 [a marker of DSBs, (COLAIACOVO et al. 2003)] is accumulated extensively along chromosomes through diakinesis in this context (Figure S5), unlike in syp-2 mutants which only accumulate RAD-51 through late pachytene (COLAIACOVO et al. 2003). Thus, if synapsis during pachytene is required to enable DNA repair in rec-8 single mutants, then the unsynapsed regions observed in rec-8 mutants should accumulate DNA damage. In support of this hypothesis, RAD-51 does indeed accumulate on late pachytene chromosome stretches where SYP-1 is absent in rec-8 single mutants (Figure 8). Similar to rec-8 single mutants, rec-8; cosa-1 double mutants also accumulate RAD-51 on unsynapsed chromosome regions at late pachytene (Figure 8). Thus, the maintenance of sister chromatid interactions by the SC in rec-8 mutants is indeed critical for DSB repair during meiosis. Overall, these data and previous published results suggest that meiotic DSB repair requires the SC central region proteins to promote partner associations critical for accessing DNA repair templates.

Discussion

Relationship between the synaptonemal complex and pro-crossover factors
Many studies have implicated a connection between the SC proteins and crossing over in *C. elegans* (COLAIACOVO et al. 2003; NABESHIMA et al. 2004; NABESHIMA et al. 2005; SMOLIKOV et al. 2007a; MARTINEZ-PEREZ et al. 2008; LIBUDA et al. 2013; PATTABIRAMAN et al. 2017; WOGLAR and VILLENEUVE 2018; ZHANG et al. 2018). To regulate where COs can form, our data indicate that the SC central region proteins in *C. elegans* have the capacity to promote the localization of the pro-CO factors COSA-1, MSH-5, and ZHP-3 to recombination events. Previous data in *C. elegans* demonstrate that the pro-CO factors, which are interdependent for localization, normally load in the context of an assembled SC (YOKOO et al. 2012; WOGLAR and VILLENEUVE 2018). Even in the context of an SC aggregate, the pro-CO factors are still interdependent for localization (ROG et al. 2017). Moreover, recent studies in *C. elegans* have shown that the SC central region proteins are preferentially stabilized on chromosomes containing CO or CO-like events (MACHOVINA et al. 2016; NADARAJAN et al. 2016; PATTABIRAMAN et al. 2017). Further, our previous work demonstrated that meiotic chromosome structures both limit and respond to CO formation (LIBUDA et al. 2013). Collectively, these previous results paired with our current findings suggest a close reciprocal relationship between the SYP proteins and COs. Notably, a recent study in *S. cerevisiae* identified a site on Zip1 (SC central region protein that is an analog of SYP-1) that is required for normal Zip3/ZHP-3 localization in meiosis (VOELKEL-MEIMAN et al. 2019). Interestingly, this site on Zip1 is directly adjacent to a site required for synapsis. Thus, the *C. elegans* SC central region proteins may directly interact with the pro-CO factors to restrict them to DSB events that occur within the context of the SC.

The localization of pro-CO factors to DSB-dependent sites in *syp* mutants (in which COs fail to form) suggests that pro-CO factors have an inherent capacity to associate with a DSB repair intermediate prior to CO formation. Previous studies have suggested that the pro-CO factors associate with a DSB repair intermediate after RAD-51 unloading, but before double Holliday junction resolution (SCHVARZSTEIN et al. 2014). Since the CO fate of a DSB is thought to occur very early in DSB repair (reviewed in LAKE and HAWLEY 2016), if not at the formation of
the DSB, it is possible that in syp mutants the pro-CO factors could be associating with an early CO-competent repair intermediate that ultimately fails in establishing a CO and is resolved through a different repair pathway. Alternatively, the pro-CO factors could be associated with a repair intermediate that they normally do not localize to in a wild-type situation. However, it is possible that there are other models that could explain this relationship between the SC and pro-CO factors. Thus, future studies assessing how DSBs are repaired in syp mutants may provide insight into the types of repair intermediates that the pro-CO factors have an inherent affinity for in the absence of SYP proteins.

**Stabilized pro-crossover factor localization may require both SYPs and a recombination intermediate**

Based on our data and previous data from other groups, we suggest that pro-CO factors have a set of conditions that need to be met for strong localization to a recombination intermediate (COLAIACOVO et al. 2003; NABESHIMA et al. 2004; NABESHIMA et al. 2005; SMOLIKOV et al. 2007a; MARTINEZ-PEREZ et al. 2008; LIBUDA et al. 2013; CRAWLEY et al. 2016; PATTABIRAMAN et al. 2017; WOGLAR and VILLENEUVE 2018; ZHANG et al. 2018). First, pro-CO factors are drawn to an SC compartment. Second, once inside the SC compartment, the pro-CO factors locate a recombination intermediate at which point the pro-CO factors may be stabilized by the SC and/or trigger a reorganization of the SC compartment locally around that repair intermediate, the latter of which has been shown to occur in *C. elegans* (WOGLAR and VILLENEUVE 2018). Alternatively, this reorganization of SC proteins might promote a structural change in the recombination intermediate that has a higher affinity for pro-CO factors influencing their stabilization at the repair intermediate. Intriguingly, pro-CO factors fail to form a focus along chromosomes with SC in *spo-11* mutant nuclei that do not form any DNA lesions (YOKOO et al. 2012; PATTABIRAMAN et al. 2017); however, pro-CO factors can form a focus within an SC aggregate that lacks recombination events in *htp-3* mutants (ROG et al. 2017). This difference in
pro-CO factor localization between spo-11 and htp-3 suggests that chromosome-associated SC may be inherently different from a nucleoplasmic SC aggregate. Thus, the formation of an SC compartment in conjunction with recombination intermediates may be dictating the ability of pro-CO factors to stably co-localize with one another.

**REC-8 and chromosome synapsis**

During *C. elegans* meiosis, the meiotic-specific cohesin protein REC-8 functions in both sister chromatid cohesion and homolog pairing (PASIERBEK et al. 2001; SEVERSON et al. 2009; SEVERSON and MEYER 2014). For both of these functions, REC-8 works together with two other meiotic-specific cohesins (COH-3 and COH-4), but genetic mutant analysis of all three of the cohesin proteins suggests that REC-8 may have additional roles separate from the sister chromatid cohesion and pairing role with COH-3/COH-4 (SEVERSON et al. 2009; SEVERSON and MEYER 2014; CRAWLEY et al. 2016). We have identified a possible role for REC-8 in establishing synapsis between homologs, thereby enabling the recruitment of pro-CO factors to recombination events between homologs.

In multiple organisms it has been shown that the SC is two vertically stacked layers with each layer connecting one sister chromatid of each homolog (CAHOON et al. 2017; KOHLER et al. 2017). However, it is unclear what is ensuring that the SC is assembled between the homologs and not between sister chromatids since each sister contains a chromosome axis that has the lateral element proteins. Our data in the rec-8 null mutant indicate that at the pairing center in wild-type situations, the initiation of SC assembly is regulated to ensure the SC assembles between the homologs and not the sisters. Further, we find that occasionally the SC assembles between multiple chromatids in rec-8 null mutants. Given that the SC assembles between pairs of sister chromatids in this mutant, it is compelling to suggest that these multi-chromatid synapsis events may represent the four sister chromatids for the same chromosome engaging with one another along different corresponding chromosomal regions. Alternatively, these
events could represent non-homologous synapsis. While it is clear REC-8 is required to assemble SC between the homologs, it is unknown if REC-8 performs this SC assembly function by: 1) promoting SC assembly between the homologs; or, 2) by preventing SC assembly between the sisters. Future studies are needed to elucidate the relationship between SC assembly and REC-8.

**Meiotic DSB repair and sister chromatid associations in rec-8 mutants**

Several studies indicate that the vast majority, if not all, of DSB repair during meiotic prophase I is by recombination-based mechanisms which require access to a repair template (reviewed in HUNTER 2015). Previous studies have found that unlike other meiotic chromosome structure mutants [such as *syp* mutants and *him*-3 null mutants in which all DSBs are repaired by late pachytene and mid-pachytene, respectively (COLAIACOVO et al. 2003; COUTEAU et al. 2004)], *rec-8* null mutants are unable to efficiently repair DSBs by the end of late pachytene, thereby resulting in both the persistence (or continued formation) of DSBs through diplotene and the eventual fragmentation of chromosomes in diakinesis (ALPI et al. 2003; HAYASHI et al. 2007). While homologs are unpaired in *syp* and *him* null mutants, sisters are still held together by cohesin and therefore, DSBs are able to repair, albeit not as COs (COLAIACOVO et al. 2003; COUTEAU et al. 2004). In the absence of both the SC central region and cohesion, as occurs in *rec-8; syp*-2 double mutants, DSBs are unable to repair, thereby resulting in prevalent chromosome fragmentation. Further, in *rec-8* single mutants, we also found the persistence (or continued formation) of DSBs along single chromatids that were not associated with their sister via SC formation. Taken together, these results reinforce the notion that: 1) interactions between chromatids or homologs are required for accessing DNA templates during recombination-based repair; and, 2) recombination is required for repair of most DSBs during meiotic prophase I.
Crossover events between homologs are known to be required in most organisms to maintain connections between homologs during diakinesis (reviewed in HUNTER 2015). In C. elegans, an extreme form of CO interference exists such that only one CO is formed between homologs, therefore the pro-CO factors are localized to a single CO event per pair of homologs during late pachytene. In rec-8 null mutants, we find that the pro-CO factors are largely recruited to single events along the SC formed between sister chromatids in late pachytene. Further, sister chromatid pairs are held together in a COSA-1 dependent manner at diakinesis in rec-8 null mutants. Given these results, it is compelling to hypothesize that the pro-CO factors are marking and enabling CO formation between sisters, thereby joining sister chromatids together as pairs during diakinesis in rec-8 null mutants. Future experiments investigating the specific DSB repair outcomes that can occur in rec-8 null mutants may elucidate the nature of these events marked by the pro-CO factors.

**Meiotic chromosome structures and limiting crossovers**

The SC central region proteins are required for both promoting and inhibiting crossing over during meiosis (MACQUEEN et al. 2002; COLAIACOVO et al. 2003; HAYASHI et al. 2010; LIBUDA et al. 2013; PATTABIRAMAN et al. 2017). Even in the context of compromised or aberrant SC between either sister chromatids or homologs, we find that an assembled SC between DNA molecules is still capable of regulating the amount of crossing over. For example, the numbers we observed for COSA-1 foci formation in rec-8(ok978) null mutants is consistent with interference occurring along synapsed sister chromatid pairs. As there are 12 pairs of sister chromatids in the rec-8(ok978) null mutants and we only very rarely observe greater than 12 COSA-1 foci (0.8% of all nuclei), this result may reflect an imposed limitation of COSA-1 foci by the number of pairs of sister chromatids. Further, we see an increase in COSA-1 foci along stretches of SYP-1 upon perturbing CO interference, in wild-type, him-3(e1256) partial loss of function, and rec-8(ok978) null strains. Given our previous study demonstrating a role for the SC
in promoting CO interference (LIBUDA et al. 2013), in these cases of sister chromatid synapsis within the *rec-8(ok978)* null mutant, it is possible that these partially synapsed sister chromatid pairs are being recognized as a signal “module” or chromosome in which interference can act. Hence, interference, which is occurring along one set of sisters, may be transmitted along the other set of sister chromatids to which the pair is partially synapsed. Overall, these results further support the hypothesis that a fully assembled SC may serve as the scaffolding along which a signal may be propagated in *C. elegans*.

**Acknowledgements**

We thank A. Dernburg, A. Villeneuve, and M. Zetka for antibodies and the CGC (funded by National Institutes of Health (NIH) P40 OD010440), B. Meyer, and A. Villeneuve for strains. We thank A. Villeneuve, K. Hillers, and members of the Libuda Lab, especially N. Kurhanewicz and E. Toraason, for comments on the manuscript. This work was supported by the National Institutes of Health R00HD076165 and R35GM128890 to DEL and a Jane Coffin Childs Postdoctoral Fellowship to CKC. DEL is also a Searle Scholar and recipient of a March of Dimes Basil O’Connor Starter Scholar award.

**Figure Legends**

**Figure 1. DSB-dependent colocalization of pro-CO factors to late pachytene foci in syp mutants.** (A) Immunofluorescence images of mid-late pachytene region of germ lines from wild-type and *syp-2(ok307)* mutant worms, with meiotic prophase progressing from left to right. In wild-type nuclei at the mid-pachytene stage (left sides of wild-type panels), ZHP-3 is localized along the lengths of the chromosomes, MSH-5 is detected as foci in excess of the eventual
number of COs, and COSA-1 foci are not detected. Upon transition to late pachytene, COSA-1 foci are detected at nascent CO sites, colocalized with MSH-5, and ZHP-3 tracks gradually reduce and retract toward the COSA-1 foci. In the syp-2 mutant panels, nuclei at the left sides of the images exhibit characteristic DAPI signals reflecting prolonged persistence of chromosome clustering and chromosome movement (dashed white line). However, upon eventual release from chromosome clustering and transition to a late pachytene-like dispersed chromosome organization, ZHP-3 is detected as foci, most of which colocalize with MSH-5 (top) or GFP::COSA-1 (bottom). Both Jantsch et al. 2004 and Zhang et al. 2018 have also published ZHP-3 localization in syp null mutants. As the ZHP-3, MSH-5 and COSA-1 foci in the syp mutants are of weaker intensity than in wild-type, signal intensities in the syp-2 images were boosted relative to controls to enable visualization of the foci (see Figure S1 for unadjusted images). Dashed box indicates the nucleus that is enlarged in the adjacent image and scale bar on the enlarged images represents 2 µm. All other scale bars represent 5 µm. (B) Quantitation of GFP::COSA-1 foci in late pachytene nuclei for syp null mutants (Table S1). Number of asterisks represent degree of statistical significance from a Mann Whitney test (** = P < 0.0001). Error bars represent standard deviation. Number of nuclei scored for GFP::COSA-1: wild-type, n=505; syp-1(me17), n=223; syp-2(ok307), n=101; syp-3(ok758), n=99.

**Figure 2. Colocalization of pro-CO factors in syp mutants is DSB-dependent.**

Immunolocalization of MSH-5 and ZHP-3 in representative late pachytene nuclei from wild-type, spo-11, syp-1(me17), spo-11; syp-1(me17), and spo-11; syp-2(ok307). Similar to the spo-11 single mutant, late pachytene nuclei in spo-11; syp-1 and spo-11; syp-2 double mutants typically have only an occasional MSH-5 focus (0-1), indicating that the presence of multiple foci in the syp single mutants is DSB dependent. Representative images of the syp-2 single mutant are in Figure 1. Scale bar represents 5 µm.
Figure 3. GFP::COSA-1 specifically associates with synapsed chromosome segments in mutants with limited synapsis. (A) Immunofluorescence images of representative nuclei in the late pachytene regions of germ lines from worms of the indicated genotypes, in which the SC central region protein SYP-1 (red) localizes: along the lengths of paired homologs (wild-type); along the lengths of a subset of chromosomes (him-3(e1256) mutant); or in several short stretches (him-3(me80) mutant). All GFP::COSA-1 (green) are associated with the chromosomes or chromosome segments where SYP-1 localized. (B) Immunofluorescence image of a representative diakinesis nucleus from the him-3(e1256) mutant, shown with DAPI (blue) and chromosome axis component HTP-3 (yellow) to visualize the chiasmata. Chiasmata were visualized and counted using 3D-rotations; solid arrowheads indicate bivalents connected by chiasmata, while carets indicate achiasmate chromosomes (univalent) (dashed caret indicates a univalent hidden in this projection). (C) Bar graph depicting quantitation of GFP::COSA-1 foci in late pachytene nuclei (bars without a pattern) and chiasmata (bars with diagonal lines) in diakinesis nuclei for wild-type (blue bars) and the him-3(e1256) (purple bars) partial loss-of-function chromosome axis mutant (Table S1); error bars indicate standard deviation ($P<0.0001$, Mann Whitney). Number of late pachytene nuclei scored for COSA-1 foci: wild-type, n=505; him-3(e1256), n=161. Number of nuclei scored for chiasmata: wild-type, n=28; him-3(e1256), n=40. All scale bars represent 5 μm.

Figure 4. Pro-CO factors associate with SYP-1 aggregates in mutants lacking meiotic chromosome axis components. (A) Immunolocalization of SYP-1 (red) and GFP::COSA-1 (green) in nuclei from the late pachytene regions of null mutants lacking chromosome axis components HIM-3 or HTP-3. SC assembly is severely impaired in both the him-3(gk149) and htp-3(y428) null mutants, and SYP proteins instead assemble into abnormal aggregates known as polycomplexes. GFP::COSA-1 localization is consistently associated with these abnormal
SYP-1 structures in both mutants. The representative image of late pachytene in wild-type is repeated from Figure 3. Rog et al. 2017 also showed that ZHP-3 and COSA-1 also localize to SC aggregates in htp-3(tm3655) null mutants. (B) Immunolocalization of MSH-5 (green) and ZHP-3 (red) in nuclei from late pachytene from him-3(gk149) and him-3 (gk149); syp-2 (ok307) double mutant. Whereas MSH-5 and ZHP-3 are usually detected together at a single site per nucleus in the him-3(gk149) mutant, multiple foci are detected in nuclei in the him-3 (gk149); syp-2 (ok307) double mutant (as in the syp-2 single mutant, see Figure 1). All scale bars represent 5 μm.

Figure 5. Synapsis occurs between sister chromatid pairs in rec-8 mutants. (A) Structured illumination microscopy images of SYP-1 (red) and chromosome axis component HTP-3 (green) in representative mid pachytene nuclei, showing that SYP-1 localizes between pairs of HTP-3 tracks in both wild-type and rec-8(ok978); this indicates that synapsis occurs between sister chromatid pairs in the rec-8 mutant. White dashed box indicates the enlarged region of SC depicted in the smaller images on the right. The solid arrowhead identifies a region where both lateral elements of the SC are visible indicated by the two tracks of HTP-3. The carets indicate a region of unsynapsed HTP-3, which is enlarged in panels d’, e’, and f’ with a cartoon diagram of the unsynapsed region below panel f’ (red = SYP-1; green = HTP-3). Scale bars for whole nucleus images represent 2 µm and scale bars for smaller enlarged SC segments represents 250 nm. (B) Box plot depicting the number of SC tracks per nucleus showing that rec-8 (ok978) (purple) mutants display on average 10 SC tracks per nucleus, while wild-type (yellow) only has 6 SC tracks per nucleus. (C) Violin plots showing the distribution of the SC track length in µm from wild-type (yellow) and rec-8 (ok978) (purple). Number of mid pachytene nuclei traced for the SC: wild-type n=15 (3 total gonads); rec-8(ok978) n=15 (3 total gonads). (D) 3D surfaces, generated in IMARIS, showing the SC traces (white) in each nucleus from rec-
Each SC trace contains both HTP-3 (green) and SYP-1 (red). Multiple SC synapsis structures were observed in rec-8 (ok978) mutants: single SC track, bubble SC track, Y-shaped branching SC track, and multi-branching SC track. A representative example of each SC synapsis structure is outlined in yellow and depicted as a diagram below each 3D surface image with the orange dashed line representing the SC trace.

Figure 6. Paired X-chromosomes in rec-8 mutants exhibit two stretches of SC. (A)

Immunolocalization of HIM-8 (green) and SYP-1 (red) in early pachytene nuclei from rec-8 (ok978) and wild-type. As some SCs from the top and bottom halves of the nuclei are superimposed in the full projections encompassing whole nuclei, partial projections showing half nuclei are shown. Colored dashed boxes indicate the enlarged region of the HIM-8 focus and SC depicted in the smaller images below with the color indicating paired (yellow) or unpaired (blue) HIM-8 foci. Scale bars represent 5 µm. (B) Stacked bar plot showing the fraction of nuclei displaying paired (yellow) or unpaired (blue) HIM-8 foci. HIM-8 foci were considered paired if the distance between the foci was ≤0.7 µm (see methods). All HIM-8 foci in wild-type are paired and in rec-8 (ok978) mutants, 80% of the HIM-8 foci are paired. In wild-type, all of the paired HIM-8 foci are associated with one SC track (solid bar) indicating SC between homologous chromosomes. However, within rec-8 mutant nuclei, we observed differences in the number of SC tracks associating with either the paired or unpaired HIM-8 focus/foci. Among the paired HIM-8 foci (yellow), the majority of the rec-8 mutant nuclei displayed HIM-8 foci associated with two SC tracks (dotted bar) suggesting SC assembly between sister chromatids. Paired HIM-8 foci were also observed not associated with SC tracks (vertical striped bar) or associated with one SC track (solid bar). Among the unpaired (blue) HIM-8 foci in rec-8 mutants, the majority of the nuclei displayed HIM-8 foci where each is associated with an SC track (horizontal striped bar) also suggesting that the SC is assembling between sister chromatids in this context. Unpaired HIM-8 foci were also observed where only one focus was associated with an SC track.
(diagonal striped bar). Number of early pachytene nuclei scored for HIM-8: wild-type, n=90; rec-8 (ok798), n=116.

**Figure 7. Pro-CO factors associate with SYP-1 stretches and between sister chromatid pairs in rec-8 mutants**

(A) Immunolocalization of SYP-1 and GFP::COSA-1 in fields of nuclei from the late pachytene regions of wild-type, rec-8(ok978), and rec-8; syp-2 germ lines. Average number of COSA-1 foci per nucleus and standard deviation is labeled on the image for each genotype. Scale bar represent 5 μm. (C) Stacked bar graph showing percentages of nuclei with indicated numbers of GFP::COSA-1 foci in late pachytene for wild-type, rec-8(ok978), and rec-8; syp-2 (Table S1). Number of late pachytene nuclei scored for COSA-1 foci: wild-type, n=505; rec-8(ok978), n=245; rec-8; syp-2, n= 204. (C) Immunolocalization of GFP::COSA-1 in DAPI stained diplotene and diakinesis bivalents from rec-8(ok978) germ lines.

**Figure 8. Regions of desynapsis in rec-8 null mutants fail to repair DSBs.**

Immunolocalization of RAD-51 and SYP-1 in the late pachytene regions of wild-type, rec-8(ok978), and cosa-1; rec-8(ok978) germ lines. Arrowheads indicate chromatids that failed to load SYP-1 and have accumulated RAD-51 foci. Scale bars represent 5 μm.

**References**

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

Bhalla, N., D. J. Wynne, V. Jantsch and A. F. Dernburg, 2008 ZHP-3 acts at crossovers to couple meiotic recombination with synaptonemal complex disassembly and bivalent formation in *C. elegans*. PLoS Genet 4: e1000235.
Cahoon, C. K., and R. S. Hawley, 2016 Regulating the construction and demolition of the synaptonemal complex. Nat Struct Mol Biol 23: 369-377.

Cahoon, C. K., and D. E. Libuda, 2019 Leagues of their own: sexually dimorphic features of meiotic prophase I. Chromosoma.

Cahoon, C. K., Z. Yu, Y. Wang, F. Guo, J. R. Unruh et al., 2017 Superresolution expansion microscopy reveals the three-dimensional organization of the Drosophila synaptonemal complex. Proc Natl Acad Sci U S A 114: E6857-E6866.

Couteau, F., K. Nabeshima, A. Villeneuve and M. Zetka, 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.

Dernburg, A. F., K. McDonald, G. Moulder, R. Barstead, M. Dresser et al., 1998 Meiotic recombination in *C. elegans* initiates by a conserved mechanism and is dispensable for homologous chromosome synapsis. Cell 94: 387-398.

Goodyer, W., S. Kaitna, F. Couteau, J. D. Ward, S. J. Boulton et al., 2008 HTP-3 links DSB formation with homolog pairing and crossing over during *C. elegans* meiosis. Dev Cell 14: 263-274.

Harper, N. C., R. Rillo, S. Jover-Gil, Z. J. Assaf, N. Bhalla et al., 2011 Pairing centers recruit a Polo-like kinase to orchestrate meiotic chromosome dynamics in *C. elegans*. Dev Cell 21: 934-947.

Hayashi, M., G. M. Chin and A. M. Villeneuve, 2007 *C. elegans* germ cells switch between distinct modes of double-strand break repair during meiotic prophase progression. PLoS Genet 3: e191.
Hayashi, M., S. Mlynarczyk-Evans and A. M. Villeneuve, 2010 The synaptonemal complex shapes the crossover landscape through cooperative assembly, crossover promotion and crossover inhibition during Caenorhabditis elegans meiosis. Genetics 186: 45-58.

Hunter, N., 2015 Meiotic Recombination: The Essence of Heredity. Cold Spring Harb Perspect Biol.

Kelly, K. O., A. F. Dernburg, G. M. Stanfield and A. M. Villeneuve, 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.

Kim, Y., N. Kostow and A. F. Dernburg, 2015 The chromosome axis mediates feedback control of CHK-2 to ensure crossover formation in C. elegans. Dev Cell 35: 247-261.

Kohler, S., M. Wojcik, K. Xu and A. F. Dernburg, 2017 Superresolution microscopy reveals the three-dimensional organization of meiotic chromosome axes in intact Caenorhabditis elegans tissue. Proc Natl Acad Sci U S A 114: E4734-E4743.

Lake, C. M., and R. S. Hawley, 2016 Becoming a crossover-competent DSB. Semin Cell Dev Biol 54: 117-125.

Machovina, T. S., R. Mainpal, A. Daryabeigi, O. McGovern, D. Paoneskou et al., 2016 A surveillance system ensures crossover formation in C. elegans. Curr Biol 26: 2873-2884.

MacQueen, A. J., M. P. Colaiacovo, K. McDonald and A. M. Villeneuve, 2002 Synapsis-dependent and -independent mechanisms stabilize homolog pairing during meiotic prophase in C. elegans. Genes Dev 16: 2428-2442.
MacQueen, A. J., C. M. Phillips, N. Bhalla, P. Weiser, A. M. Villeneuve et al., 2005
Chromosome sites play dual roles to establish homologous synapsis during meiosis in *C. elegans*. Cell 123: 1037-1050.

Martinez-Perez, E., M. Schvarzstein, C. Barroso, J. Lightfoot, A. F. Dernburg et al., 2008 Crossovers trigger a remodeling of meiotic chromosome axis composition that is linked to two-step loss of sister chromatid cohesion. Genes Dev 22: 2886-2901.

Mlynarczyk-Evans, S., and A. M. Villeneuve, 2017 Time-course analysis of early meiotic prophase events informs mechanisms of homolog pairing and synapsis in *Caenorhabditis elegans*. Genetics 207: 103-114.

Nabeshima, K., A. M. Villeneuve and M. P. Colaiacovo, 2005 Crossing over is coupled to late meiotic prophase bivalent differentiation through asymmetric disassembly of the SC. J Cell Biol 168: 683-689.

Nabeshima, K., A. M. Villeneuve and K. J. Hillers, 2004 Chromosome-wide regulation of meiotic crossover formation in *Caenorhabditis elegans* requires properly assembled chromosome axes. Genetics 168: 1275-1292.

Nadarajan, S., T. J. Lambert, E. Altendorfer, J. Gao, M. D. Blower et al., 2017 Polo-like kinase-dependent phosphorylation of the synaptonemal complex protein SYP-4 regulates double-strand break formation through a negative feedback loop. Elife 6.

Nadarajan, S., F. Mohideen, Y. B. Tzur, N. Ferrandiz, O. Crawley *et al.*, 2016 The MAP kinase pathway coordinates crossover designation with disassembly of synaptonemal complex proteins during meiosis. Elife 5: e12039.
Nguyen, H., S. Labella, N. Silva, V. Jantsch and M. Zetka, 2018 C. elegans ZHP-4 is required at multiple distinct steps in the formation of crossovers and their transition to segregation competent chiasmata. PLoS Genet 14: e1007776.

Pasierbek, P., M. Jantsch, M. Melcher, A. Schleiffer, D. Schweizer et al., 2001 A Caenorhabditis elegans cohesion protein with functions in meiotic chromosome pairing and disjunction. Genes Dev 15: 1349-1360.

Pattabiraman, D., B. Roelens, A. Woglar and A. M. Villeneuve, 2017 Meiotic recombination modulates the structure and dynamics of the synaptonemal complex during C. elegans meiosis. PLoS Genet 13: e1006670.

Phillips, C. M., X. Meng, L. Zhang, J. H. Chretien, F. D. Urnov et al., 2009 Identification of chromosome sequence motifs that mediate meiotic pairing and synapsis in C. elegans. Nat Cell Biol 11: 934-942.

Rog, O., and A. F. Dernburg, 2015 Direct visualization reveals kinetics of meiotic chromosome synapsis. Cell Rep 10: 1639-1645

Rog, O., S. Kohler and A. F. Dernburg, 2017 The synaptonemal complex has liquid crystalline properties and spatially regulates meiotic recombination factors. Elife 6: e21455.

Schild-Prufert, K., T. T. Saito, S. Smolikov, Y. Gu, M. Hincapie et al., 2011 Organization of the synaptonemal complex during meiosis in Caenorhabditis elegans. Genetics 189: 411-421.

Schvarzstein, M., D. Pattabiraman, D. E. Libuda, A. Ramadugu, A. Tam et al., 2014 DNA helicase HIM-6/BLM both promotes MutSgamma-dependent crossovers
and antagonizes MutSgamma-independent interhomolog associations during

*Caenorhabditis elegans* meiosis. Genetics 198: 193-207.

Severson, A. F., L. Ling, V. van Zuylen and B. J. Meyer, 2009 The axial element protein HTP-3 promotes cohesin loading and meiotic axis assembly in *C. elegans* to implement the meiotic program of chromosome segregation. Genes Dev 23: 1763-1778.

Severson, A. F., and B. J. Meyer, 2014 Divergent kleisin subunits of cohesin specify mechanisms to tether and release meiotic chromosomes. Elife 3: e03467.

Smolikov, S., A. Eizinger, A. Hurlburt, E. Rogers, A. M. Villeneuve et al., 2007a Synapsis-defective mutants reveal a correlation between chromosome conformation and the mode of double-strand break repair during *Caenorhabditis elegans* meiosis. Genetics 176: 2027-2033.

Smolikov, S., A. Eizinger, K. Schild-Prufert, A. Hurlburt, K. McDonald et al., 2007b SYP-3 restricts synaptonemal complex assembly to bridge paired chromosome axes during meiosis in *Caenorhabditis elegans*. Genetics 176: 2015-2025.

Smolikov, S., K. Schild-Prufert and M. P. Colaiacovo, 2009 A yeast two-hybrid screen for SYP-3 interactors identifies SYP-4, a component required for synaptonemal complex assembly and chiasma formation in *Caenorhabditis elegans* meiosis. PLoS Genet 5: e1000669.

Voelkel-Meiman, K., S. Y. Cheng, M. Parziale, S. J. Morehouse, A. Feil et al., 2019 Crossover recombination and synopsis are linked by adjacent regions within the N terminus of the Zip1 synaptonemal complex protein. PLoS Genet 15: e1008201.
Woglar, A., and A. M. Villeneuve, 2018 Dynamic architecture of DNA repair complexes and the synaptonemal complex at sites of meiotic recombination. Cell 173: 1678-1691.e1616.

Xu, H., M. D. Beasley, W. D. Warren, G. T. van der Horst and M. J. McKay, 2005 Absence of mouse REC8 cohesin promotes synapsis of sister chromatids in meiosis. Dev Cell 8: 949-961.

Yokoo, R., K. A. Zawadzki, K. Nabeshima, M. Drake, S. Arur et al., 2012 COSA-1 reveals robust homeostasis and separable licensing and reinforcement steps governing meiotic crossovers. Cell 149: 75-87.

Zetka, M. C., I. Kawasaki, S. Strome and F. Muller, 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.
Figure 1

A

wild-type

syp-2 (ok307)

MSH-5

ZHP-3

DAPI

GFP::COSA-1

ZHP-3

GFP::COSA-1

ZHP-3

GFP::COSA-1

DAPI

B

Average # GFP::COSA-1 foci per nucleus in late pachytene

wild-type

syp-1 (mex17)

syp-2 (ok307)

syp-3 (ok758)
Figure 2

Late Pachytene
Figure 3

A

| Wild-type | him-3 (e1256) | him-3 (me80) |
|-----------|---------------|--------------|
| GFP::COSA-1 | SYP-1 DAPI | GFP::COSA-1 | SYP-1 |

B

Diakinesis

C

Average # per nucleus

| Wild type | him-3 (e1256) |
|-----------|---------------|
| GFP::COSA-1 (late pachytene) | chiasmata (diakinesis) |
Figure 4

A

GFP::COSA-1  SYP-1  DAPI

wild-type

GFP::COSA-1  SYP-1

him-3 (gk149)

htp-3 (y428)

B

Late Pachytene

MSH-5

MSH-5  ZHP-3  DAPI

him-3 (gk149)

him-3 (gk149);
syp-2 (ok307)

Late Pachytene
Figure 5

A

Wild-type and rec-8 (ok978) SC trace length (µm)

B

SC tracks per nucleus

C

SC track length (µm)

D

Single, bubble, and branching SC synapsis structures
Figure 6

A

|        | wild-type | rec-8 (ok978) |
|--------|-----------|---------------|
| paired | 1 SC/1 HIM-8 | 2 SC/1 HIM-8 |
|        | paired | paired | unpaired |
| 1 SC/1 HIM-8 | 1 SC/1 HIM-8 | 2 SC/2 HIM-8 |

Early Pachytene

B

| fraction of nuclei |
|-------------------|
| 1.0               |
| 0.9               |
| 0.8               |
| 0.7               |
| 0.6               |
| 0.5               |
| 0.4               |
| 0.3               |
| 0.2               |
| 0.1               |

wild type | rec-8 (ok978) |
|-----------|---------------|
| paired    | paired        |
| 2 SC/1 HIM-8 focus | 1 SC/1 HIM-8 focus |

unpaired 1 SC/2 HIM-8 foci 2 SC/2 HIM-8 foci
**Figure 7**

(A) Fluorescence images showing wild-type, rec-8(ok978), and rec-8; syp-2 germline nuclei stained with GFP::COSA-1 and DAPI. The images are labeled with the number of GFP::COSA-1 foci per nucleus and the genotype of the nuclei.

(B) Bar graph showing the percentage of nuclei with the indicated number of foci in late pachytene. The percentages are for wild-type, rec-8(ok978), and rec-8; syp-2.

(C) Fluorescence images of diplotene and diakinesis stages showing the patterns of GFP::COSA-1 and DAPI staining in rec-8(ok978) germline nuclei.
Figure 8

Late Pachytene