The synaptonemal complex shapes the crossover landscape through cooperative assembly, crossover promotion and crossover inhibition during *C. elegans* meiosis

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The synaptonemal complex (SC) is a highly-ordered proteinaceous structure that assembles at the interface between aligned homologous chromosomes during meiotic prophase. The SC has been demonstrated to function both in stabilization of homolog pairing and in promoting the formation of interhomolog crossovers (COs). How the SC provides these functions and whether it also plays a role in inhibiting CO formation has been a matter of debate. Here we provide new insight into assembly and function of the SC by investigating the consequences of reducing (but not eliminating) SYP-1, a major structural component of the SC central region, during meiosis in *C. elegans*. First, we find an increased incidence of double CO (DCO) meiotic products following partial depletion of SYP-1 by RNAi, indicating a role for SYP-1 in mechanisms that normally limit crossovers to one per homolog pair per meiosis. Second, *syp-1 RNAi* worms exhibit both a strong preference for COs to occur on the left half of the X chromosome and a significant bias for SYP-1 protein to be associated with the left half of the chromosome, implying that the SC functions locally in promoting COs. Distribution of SYP-1 on chromosomes in *syp-1 RNAi* germ cells provides strong corroboration for cooperative assembly of the SC central region and indicates that SYP-1 preferentially associates with X chromosomes when it is present in limiting quantities. Further, the observed biases in the distribution of both COs and SYP-1 protein support models in which synapsis initiates predominantly in the vicinity of Pairing Centers (PCs). However, discontinuities in SC structure and clear gaps between localized foci of PC-binding protein HIM-8 and X chromosome-associated SYP-1 stretches allow refinement of models for the role of PCs in promoting synapsis. Our data suggest that the CO landscape is shaped by a combination of three attributes of the SC central region: a CO-promoting activity that functions locally at CO sites, a cooperative assembly process that enables CO formation in regions distant from prominent sites of synapsis initiation, and CO-inhibitory role(s) that limit CO number.
INTRODUCTION
Reduction in ploidy during sexual reproduction depends on the ability to form pair-wise associations between homologous chromosomes. The homolog pairing process typically culminates in an arrangement in which the homologs are aligned in parallel along their lengths, with a highly ordered proteinaceous structure known as the synaptonemal complex (SC) located at the interface between them. Further, in most organisms, pairwise associations between homologs are solidified through the formation of crossovers (COs) between their DNA molecules, a process that is completed within the context of the SC.

The SC has long been recognized as a hallmark cytological feature of meiosis. It was discovered based on its highly-ordered structure and location at the interface between aligned chromosomes in EM images of nuclei at the pachytene stage of meiotic prophase (MOSES 1956; MOSES 2006). Each of the homologs is associated with one of the two lateral elements (LEs) of the SC, which are composed of cohesin complexes and other meiosis specific structural and regulatory proteins (reviewed in MLYNARCZYK-EVANS and VILLENEUVE 2010). The LEs are connected by a highly ordered latticework of transverse filaments, and often a pronounced central element, that comprise the central region of the SC. The protein components of the SC central region are very poorly conserved at the primary sequence level, but the major central region proteins identified from diverse species share in common extended regions of predicted coiled coil structure.

The SC has been demonstrated to have at least two conserved functions in meiotic prophase. First, the SC serves to stabilize and maintain tight associations along the lengths of aligned homologs (reviewed in MLYNARCZYK-EVANS and VILLENEUVE 2010). This is true both in organisms in which SC assembly is coupled to formation of recombination intermediates (e.g. budding yeast, mouse, and Arabidopsis) and in organisms in which formation of SC between homologs can occur independently of recombination (e.g. C. elegans and Drosophila). Second, SC central region proteins play a role in promoting maturation of recombination intermediates into crossover products (reviewed in DE BOER and HEYTING 2006). How the SC functions to promote CO formation is not well understood. Moreover, whether the SC might also have additional functions that help to ensure a successful outcome of meiosis has been a matter of debate.
In addition to its roles in stabilization of pairing and promoting CO formation, the SC has also been proposed to function in inhibiting CO formation (EGEL 1978; EGEL 1995; MAGUIRE 1988). This idea of the SC playing an inhibitory role in recombination dates almost as far back as the discovery of the SC itself. Finding a highly ordered structure with a zipper-like appearance extending along the length of each homolog pair naturally gave rise to speculation that it might play a role in the phenomenon of crossover interference, defined as the ability of a (nascent) CO to inhibit the formation of other COs nearby on the same chromosome pair (HILLERS 2004; MULLER 1916). It was variously proposed either that the SC might serve as a conduit of information along a chromosome pair (e.g. undergoing a distance-dependent “change in state” to inhibit COs) or that SC polymerization might itself confer CO inhibition (EGEL 1978; MAGUIRE 1988; SYM and ROEDER 1994).

Early analysis of the budding yeast mutants lacking Zip1, a major structural component of the SC central region, initially seemed to support the idea that the SC central region played a key role in CO interference, as zip1 mutants formed COs at 30-50% of wild-type levels and the residual COs did not display interference (SYM and ROEDER 1994). However, these data were subsequently reinterpreted by postulating that the major interference-sensitive meiotic CO pathway is eliminated in the zip1 mutant, and that the residual COs form by an alternative pathway that is not subject to interference (DE LOS SANTOS et al. 2003; ZALEVSKY et al. 1999). According to this two-pathway view, the lack of interference in the zip1 mutant can be readily explained without invoking a role for Zip1 in the interference mechanism per se. Conversely, Page and Hawley found that Drosophila females expressing a mutant form of the fly SC central region protein C(3)G retained substantial interference between residual COs despite exhibiting incomplete synapsis, implying that complete SC formation was not required for CO interference (PAGE and HAWLEY 2001). In light of these and other findings (e.g. BORNER et al. 2004; FUNG et al. 2004), the idea that the SC might play a role in inhibiting CO formation had fallen from favor.

In this study, we revisit a potential role for the SC central region in inhibiting CO formation, using the *C. elegans* experimental system. Several features make this an interesting system for investigating factors that promote and/or inhibit COs during meiosis. First, essentially all COs in *C. elegans* depend on conserved meiotic CO-promoting machinery (i.e. Msh4 and Msh5) and on SC central region proteins (SYP-1,-2,-3 and -4), so analysis is generally
not complicated by residual COs forming by alternative pathways (Colaiacovo et al. 2003; Kelly et al. 2000; MacQueen et al. 2002; Smolikov et al. 2007a; Smolikov et al. 2009; Zalevsky et al. 1999). Second, C. elegans hermaphrodites exhibit robust CO control, with COs usually being limited to one per homolog pair per meiosis (Hammarlund et al. 2005; Hillers and Villeneuve 2003; Nabeshima et al. 2004). Consequently, circumstances that give rise to double crossover (DCO) meiotic products can be inferred to represent impairment of mechanisms that normally inhibit CO formation. Finally, COs are distributed non-uniformly along the lengths of the chromosomes, with each chromosome containing broad domains of relatively high CO frequency flanking a more central domain where CO frequency is low (Barnes et al. 1995; Brenner 1974; Rockman and Kruglyak 2009), providing an opportunity to evaluate how factors that promote and/or inhibit COs contribute to this landscape.

Our strategy was to use RNAi to reduce the levels of wild-type SYP-1 protein without eliminating synapsis entirely, and then to examine the effects on CO frequency and distribution. This approach has indeed revealed a role for SC central region protein SYP-1 in mechanisms that normally limit the number of COs/homolog pair. Further, it has also revealed a role for the SC central region in determining CO distribution, presumably by enabling formation of COs in chromosome regions distant from the dominant site of synapsis initiation. Finally, our experimental design also afforded the opportunity to evaluate spatial distribution of the SC in the context of limiting amounts of a key central region component. This analysis has provided additional insight into the process of SC assembly and the role of cis-acting meiotic pairing centers in this process.

**MATERIALS AND METHODS**

**Genetic methods**

**Worm strains**

N2 Bristol wild type strain
AZ212 unc-119(ed3) ruIs32[unc-119(+)] pie-1::GFP::H2B] III
AZ244 unc-119(ed3) III; ruIs57[unc-119(+)] pie-1::GFP::tubulin]
EU1067 unc-119(ed3) ruIs32[unc-119(+)] pie-1::GFP::H2B] III; ruIs57[unc-119(+)] pie-1::GFP::tubulin] (provided by Bowerman lab)
AV335  emb-27(g48) II; unc-119(ed3) ruIs32[unc-119(+) pie-1::GFP::H2B] III; ruIs57[unc-119(+) pie-1::GFP::tubulin]

CB4856  Hawaiian wild type strain

**RNA interference**

*E. coli* HT115 cells containing clone DE3 from the Ahringer Lab RNAi feeding library (Fraser et al. 2000; Kamath et al. 2003) (carrying a PCR fragment corresponding to a portion of *syp-1/F26D2.2* cloned into the L4440 vector) or the control empty vector were cultured in 3 ml LB with 50 μg/ml ampicillin at 37°C overnight. Saturated cultures were used to seed two NGM plates containing 100 μg/ml ampicillin and 1mM IPTG; plates were left overnight at room temperature. After worms were put on the plates, plates were kept at 20 °C.

For the crossover analysis, AZ212 hermaphrodites (N2 background) were crossed with Hawaiian CB4856 males on RNAi feeding plates for 1.5 days, and heterozygous F1 hermaphrodites were plated individually (on RNAi plates) at the late L4 stage. F1s were transferred to the new plates 24 hrs later and allowed to lay eggs for 12 hrs; the F1 worms were then removed from the plates and fixed as in (Villeneuve 1994) for DAPI analysis. All viable F2 progeny arising from the eggs laid during this 12 hr time window (24 to 36 hrs after L4) were picked and lysed for PCR. To ensure that the F2 hermaphrodites had not mated with sibling males (which would compromise genotyping), F2 worms were picked at the L3 or L4 stage; when necessary, worms were plated individually and allowed to mature until gender could be scored.

For cytological analysis, AZ212 hermaphrodites and Hawaiian males were crossed on RNAi feeding plates as above, but F1 hermaphrodites were plated as a group at the L4 stage.

**Single nucleotide polymorphism genotyping**

Markers used, corresponding primer sequences and overall SNP genotyping strategy followed the general protocol of (Hammarlund et al. 2005). Heterozygote F1 hermaphrodites from the cross AZ212 x Hawaiian were plated individually at the L4 stage and F2 worms were picked into PCR tubes containing 10μl lysis buffer. Tubes were placed on dry ice for 30 min, then incubated at 65°C for 1 hr and 95°C for 15 min. After lysis, 10μl of water was added to each tube. PCR reactions were performed using either Qiagen Master Mix or Taq polymerase (Takara)
cat#1647679). PCR reaction conditions were: 95°C for 2 min, 35 cycles of 95°C for 40 sec, 58 or 60°C for 40 sec and 72°C for 40 sec, and 72°C for 10 min. PCR products were digested with DraI and separated by electrophoresis through 2% agarose or 4% GenePure 3:1 agarose gels to genotype SNP markers.

Each F2 hermaphrodite genotyped carries two different meiotic products, in most cases derived from two independent meioses. This method reliably detects the majority of DCOs products, but as discussed in detail by Hammarlund et al. (Hammarlund et al. 2005), it underestimates the total number of DCOs because a subset of DCO products cannot be identified unambiguously. For example, the genotypes of some individuals carrying one DCO product and one NCO product will be indistinguishable from those of some individuals carrying two SCO products. 13 hermaphrodites from the control data set and 6 hermaphrodites from the syp-1 RNAi data set had this class of genotype; for Table 3, these were conservatively classified as having two independent SCO products. It is likely that this SCO assignment is correct for the control data set, as no unambiguous DCOs were detected among controls. For the syp-1 RNAi analysis, however, 10 unambiguous DCOs were detected; thus, one or more syp-1 RNAi F2 hermaphrodites in this genotypic category may have harbored a DCO chromosome.

Whereas most F2 hermaphrodites genotyped in this study carried products of two independent meioses (i.e., one derived from oocyte meiosis and one derived from spermatocyte meiosis), a subset of F2 hermaphrodites from the syp-1 RNAi data set were likely derived from diplo-X gametes produced by meiosis I non-disjunction of an achiasmate X chromosome pair. Such worms would carry 2 NCO chromosomes, one of each parental haplotype, resulting in heterozygosity for all SNP markers (Het-all genotype). Occurrence of such events is inferred from the fact that there was a highly significant overrepresentation of the Het-all genotype among syp-1 RNAi F2 hermaphrodites carrying 2 NCO chromosomes (Chi-square test, p = 0.0001); thus there is strong statistical evidence that a subset of meiotic products were derived from E0 tetrads (see below). In contrast, control F2s carrying 2NCO chromosomes had a distribution of Het-all and homozygous parental genotypes that was consistent with expectations for independent inheritance of the two X chromosomes (p = 0.9).

Estimating the incidence of NCO products from E0 tetrads
For part of the analysis of CO interference presented in Table 4, we wished to exclude from consideration NCO products that had been derived from E₀ meiotic tetrads (in which a homolog pair undergoes zero COs, yielding 4 NCO chromatids). To this end, we estimated the number of NCO products derived from meiotic tetrads that had undergone one or more COs, as follows:

An E₁ tetrad (in which a homolog pair undergoes one CO) gives 2 SCO chromatids and 2 NCO chromatids, or a ratio of 1SCO: 1NCO meiotic products. There are 3 classes of E₂ tetrads, defined by the number of chromatids involved in the CO events; assuming no chromatid interference, these collectively yield 1DCO: 2SCO: 1NCO meiotic products. From measured numbers of DCO (10) and SCO (73) products, one can infer the number of NCO (63) products that came from meioses in which one or more COs had occurred between the leftmost and rightmost markers assayed. In addition, we made a correction to account for the fraction of meioses in which a CO occurred in the unassayed portion of the chromosome. This estimate was based on the fact that *C. elegans* chromosomes, including the X chromosomes, are divided into domains within which the frequency of CO per Mb is relatively uniform. As the domain boundaries defined by (ROCKMAN and KRUGLYAK 2009) correspond closely to the positions of markers F and D of the present study, we used the number of CO in the H-F interval to estimate the number of COs in the unassayed portion of the Left domain (13), and we used the number of COs in the D-A interval to estimate the number of COs in the unassayed portion of the Right domain (6). Taking all of these factors into account, we estimated the total number of NCO meiotic products derived from crossover tetrads to be 101, and the total number of NCO products derived from E₀ tetrads to be 79.

**Cytological methods**

*Immunofluorescence analysis*

Worms were maintained on RNAi feeding plates until dissection. For Figure 1A and 1C, worms were dissected at 20-21 hr post L4; for all other immunofluorescence panels, worms were dissected at 44 hr post L4. Dissection of gonads, fixation, immunostaining were done as in (MARTINEZ-PEREZ and VILLENEUVE 2005). Primary antibodies used were rabbit anti-SYP-1 (1:250) (MACQUEEN *et al.* 2002), rabbit anti-RAD-51 (1:50) (COLAIACOVO *et al.* 2003), guinea pig anti-HIM-8 (1:500) (PHILLIPS *et al.* 2005). Secondary antibodies used (1:400) were Alexa Fluor 488 α-rabbit and Alexa Fluor 555 α-guinea pig.
S-phase labeling of X chromosomes

Injection of fluorescent nucleotides was performed as in (JARAMILLO-LAMBERT et al. 2007), with the following modifications. Young adult worms (24 hrs post L4) from control or syp-1 RNAi plates were microinjected in their anterior gonad arms, approximately midway between the distal tip and the turn, with a small volume (sufficient to fill approximately one quarter of the gonad) of 1 mM Alexa Fluor 647-OBEA-dCTP (Invitrogen); worms were recovered and maintained on RNAi plates until they were dissected and fixed for immunofluorescence at 20 hrs post injection, a time at which nuclei with meiotic S-phase labeled X chromosomes had progressed to the mid-pachytene stage of meiotic prophase. Because the X chromosomes replicate late in S phase (JARAMILLO-LAMBERT et al. 2007), nuclei in which the X chromosomes were specifically labeled during meiotic S phase enter meiotic prophase prior to other S-phase labeled nuclei and are thus found near the proximal front of labeled nuclei as they progress through meiotic prophase. A field of view containing the proximal front of labeled nuclei was imaged as described below, and nuclei within this field that contained a single chromosome pair labeled along its length with Alexa Fluor 647 were chosen for analysis. For the analysis of SYP-1 distribution in syp-1 RNAi germ cells, nuclei from seven different gonads were examined; control nuclei were from two different gonads.

Cytological measurements

Stacks of two-dimensional images were acquired using the DeltaVision microscopy system (Applied Precision), images were deconvolved using softWoRx software, and three-dimensional images were constructed from these Z stacks. The lengths of SYP-1 stretches and X chromosomes were measured in 3D using Priism software (Modeling-Interactive segmentation-3D model function). This function sums the lengths of lines connecting a series of successive points marking the trajectory of the X chromosome (identified by Alexa Fluor 647 fluorescence and HIM-8 staining) and associated SYP-1 stretches. In this analysis, the left end of the chromosome was identified by its associated HIM-8 focus. Distances between SYP-1 and HIM-8 were measured using the Measurement-Distance function of the Priism software package.
RESULTS

Partial depletion of SYP-1 by RNAi: Our goal at the outset of this work was to test the hypothesis that the SC central region plays a role in limiting the number of crossovers per chromosome pair. Because SC central region proteins (e.g. SYP-1, -2, -3, or -4) are required to promote crossover formation, however, mutants that completely lack the SYP proteins lack the ability to make crossovers and are thus unsuitable for this purpose. We therefore sought to define feeding RNAi conditions under which the SYP-1 protein was reduced in abundance but not eliminated. We used the incidence of achiasmate chromosomes in DAPI-stained oocytes at diakinesis (the last stage of meiotic prophase; Figure 1A) as a readout of RNAi efficacy. Since wild-type oocytes contain six DAPI-stained bivalents (homolog pairs attached by chiasmata) and sypr-1 null mutants have 12 DAPI-stained univalents (individual achiasmate chromosomes) (MacQueen et al. 2002), a mixture of bivalents and univalents is indicative of partial loss of SYP-1 function.

During the course of these experiments, we found that the efficacy of syp-1 RNAi was highly dependent on the genotype of the worms used (Table 1). Whereas feeding RNAi initiated at the L1 larval stage in the AV335 strain (which carries integrated transgenes expressing GFP:histone H2B and GFP:tubulin and a temperature-sensitive mutation affecting the anaphase promoting complex, all in the standard wild-type Bristol N2 genetic background (Wignall and Villedeneuve 2009)) was highly effective at eliciting a strong syp-1 phenotype, syp-1 RNAi performed using identical conditions (in side-by-side experiments) was almost completely ineffective in Bristol N2 worms (Table 1a). Strains carrying different subsets of the components of the AV335 strain showed different degrees of syp-1 RNAi efficacy, with the AZ212 strain (which carries the GFP:histone transgene alone (Praffit et al. 2001)) giving a robust syp-1 RNAi response comparable to that seen in AV335.

Because recombination analysis requires heterozygosity for multiple single nucleotide polymorphism (SNP) markers distributed along the lengths of the chromosomes, we next examined syp-1 RNAi efficacy in the germ lines of heterozygous F1 worms generated by crossing AZ212 hermaphrodites with males from the Hawaiian wild-type strain CB4856 (Table 1b). These F1 worms exhibited weaker syp-1 RNAi phenotypes than their hermaphrodite parents, presumably reflecting intermediate depletion of SYP-1. For all experiments described below, F1 worms heterozygous for SNP markers were generated by crossing AZ212 hermaphrodites with
CB4856 males, either on syp-1 RNAi plates or on control plates seeded with bacteria containing the empty RNAi vector. F1 hermaphrodites were either dissected and processed for immunofluorescence or plated for recombination analysis (see below).

Immunostaining of dissected gonads confirmed partial depletion of SYP-1 under our experimental conditions. SYP-1 protein was readily detected in the germline nuclei of most syp-1 RNAi worms, but the amount of SYP-1 detected was substantially reduced (Figure 1B, Figure S1). Interestingly, the residual SYP-1 protein present was not uniformly distributed among the chromosomes. Instead, most meiotic prophase nuclei in syp-1 RNAi germ lines exhibited long SYP-1 stretches associated with a subset of the chromosomes, while the remainder of the chromosomes lacked detectable SYP-1, suggesting that some chromosome segments were synapsed and others were not. This distribution of SYP-1 protein is indicative of cooperative assembly of the SC central region. Incomplete synapsis in syp-1 RNAi germ lines was accompanied by persistence of the clustered configuration of chromosomes that is normally associated with the onset of pairing (Figure 1B, Figure S1). Further, RAD-51 foci (which mark the sites of double-strand break-dependent recombination intermediates (ALPI et al. 2003; COLAIACOVO et al. 2003)) persisted at high levels well into the late pachytene region of syp-1 RNAi germ lines (Figure 1C, Figure S2). Both of these features are common characteristics of mutants in which some or all chromosome pairs fail to assemble the SC central region.

**Altered distribution and regulation of crossovers following partial depletion of SYP-1:** For analysis of meiotic crossing over, syp-1 RNAi F1 hermaphrodites were plated singly at the L4 stage, and 24 hrs later these individual F1 hermaphrodites were transferred to fresh plates and allowed to lay eggs for a 12 hr period; the F1 worms were then removed from the plates and fixed and stained with DAPI. Plates from the 12-hr egg lay window that had abundant dead eggs and yielded 10 or fewer viable F2 progeny (indicating effective RNAi in the F1 parent) were included in the crossover analysis. All viable F2 worms from these plates were genotyped for eight single nucleotide polymorphism (SNP) markers distributed along the length of the X chromosome, spanning approximately 85% of the X chromosome genetic map. The results of the crossover analysis are presented in Figure 2 and Tables 2, 3 and 4. Several features of the data are notable:
First, we found that the distribution of COs along the length of the X chromosome differed significantly between syp-1 RNAi and control worms (Figure 2A, Table 2). The genetic maps derived from the control and syp-1 RNAi data (Figure 2A) reveal a substantial bias for COs to occur on the left half of the chromosome following partial depletion of SYP-1. The frequency of COs on the left half of the chromosome in syp-1 RNAi was not significantly different from that in control worms (p = 0.36), whereas the frequency of COs on the right half of the chromosome in syp-1 RNAi worms was reduced to less than half of the control frequency (p < 0.0001), indicating that the CO deficit primarily reflected a loss of right-half COs. Further, the analyses presented in Table 2 indicate that the observed leftward shift in CO distribution in the syp-1 RNAi worms is highly significant. In Table 2A, the chromosome was bisected into two intervals, Left and Right, with respect to the SNP marker closest to the center of both the physical and genetic maps. In Table 2B, the chromosome was divided into three domains, termed Left, Center and Right, using SNP markers that correspond closely to the known boundaries between previously-defined large chromosomal domains within which recombination rates have been demonstrated to be roughly constant (ROCKMAN and KRUGLYAK 2009). Both analyses indicated a highly significant difference between the syp-1 RNAi and control data with respect to the distribution of COs among these intervals, clearly indicating a bias favoring COs near the left end and disfavoring COs near the right end of the X chromosome following partial depletion of SYP-1.

Second, although the overall frequency of X chromosome crossovers was reduced in syp-1 RNAi worms to 77% of the control frequency, the incidence of meiotic products with two crossovers (DCO products) was substantially elevated (Table 3). Whereas no DCOs were detected in controls, DCO products represented 12% of the total crossover products of the syp-1 RNAi worms. (This is likely an underestimate of the DCO incidence, as certain classes of progeny carrying DCO products would not be unambiguously distinguished by the methods used; see Materials and Methods.) This extremely significant increase in the occurrence of DCO products (p < 0.0001) indicates that partial depletion of SYP-1 impairs mechanisms that normally limit crossovers to one per homolog pair per meiosis.

Figure 2B depicts the SNP genotypes of the DCO chromosomes detected in this analysis. Examination of the positions of the CO events in the DCO meiotic products did not reveal any
differences when compared with either SCO products or total CO events. Further, no obvious pattern was evident with regard to spacing between coincident COs.

Finally, we used two different approaches to analyze the data for evidence of effects of \textit{syp-1 RNAi} on crossover interference (Table 4). We first used the classical “coefficient of coincidence” (C) parameter to assess interference between COs in intervals corresponding to the left (H-E) and right (E-A) halves of the chromosome. Whereas the data for control worms indicated complete interference (C= 0), the C value for the full \textit{syp-1 RNAi} data was 0.7, suggesting that interference might be impaired. Because the C value provides no information regarding statistical significance, we also used the Fisher exact test to evaluate whether COs in the left interval occurred independently of COs in the right interval (Table 4). For control worms, there was an extremely significant departure from expectations based on independent occurrence of COs in the two intervals (p < 0.0001), indicating robust CO interference. For the \textit{syp-1 RNAi} data set, the data are consistent with independent occurrence of CO in the two intervals (p = 0.68), further supporting impairment of CO interference.

We considered the possibility that population heterogeneity in the \textit{syp-1 RNAi} data might be responsible for the apparent loss of interference observed. Specifically, we reasoned that meiotic products arising from non-exchange (E\textsubscript{0}) tetrads (which do not occur in wild type meiosis) would represent a potential source of population heterogeneity in the \textit{syp-1 RNAi} data; these would presumably reflect meioses in which the X chromosome pair either lacked SYP-1 completely or had insufficient SYP-1 to promote CO formation. Thus, we used the incidence of DCO and SCO products to estimate the fraction of NCO products arising from E\textsubscript{0}, E\textsubscript{1} and E\textsubscript{2} tetrads (see Materials and Methods), in turn generating “corrected values” for interference analysis that only considered meiotic products derived from exchange tetrads. Using these corrected values, we calculated a p value of 0.08, which still does not reflect a significant departure from expectations for independent occurrence of CO in the two intervals. Thus, even after accounting for the potential confounding effects of population heterogeneity, our data suggest that partial depletion of SYP-1 impairs CO interference on the \textit{C. elegans} X chromosome.

**Synapsis occurs preferentially on the X chromosomes in the context of limiting SYP-1:** The bias in the distribution of COs on the X chromosomes in \textit{syp-1 RNAi} worms prompted us to
investigate the spatial distribution of SYP-1 protein as a potential factor contributing to this bias. To this end, we devised a method for assessing the distribution of SYP-1 protein along the X chromosome that exploits the recent discovery that the X chromosome replicates late in meiotic S phase, out of phase with the autosomes (Jaramillo-Lambert et al. 2007). This property makes it possible to deliver a pulse of fluorescently labeled nucleotides (by microinjection into the germ line), allow labeled nuclei to progress into meiotic prophase, and subsequently identify germ cell nuclei in which the label had been incorporated throughout the length of a single chromosome pair, the X chromosomes (Jaramillo-Lambert et al. 2007). We combined this S-phase X-chromosome labeling strategy with immunofluorescence using antibodies against the SYP-1 and HIM-8 proteins. HIM-8 is a DNA binding protein that localizes in a bright focus near the left end of the X chromosome at a specialized cis-acting domain known as the meiotic pairing center (Phillips et al. 2005), thus serving as an orientation marker to discriminate between the left and right ends of the chromosome. Gonads were fixed for immunofluorescence 20 hr after injection of nucleotide, since nuclei containing X chromosomes specifically labeled during meiotic S phase had reached the mid-pachytene stage in control germ lines fixed at this time point.

Use of this X-chromosome labeling strategy following partial depletion of SYP-1 by RNAi revealed a strong preference for SC central region proteins to associate with X chromosomes vs. autosomes. In control germ lines, pachytene nuclei with meiotic S-phase labeled X chromosomes consistently exhibited robust SYP-1 localization at the interfaces between all chromosome pairs (Figure 3A). In the syp-1 RNAi germ lines, in contrast, the comparable population of nuclei with meiotic S-phase labeled X chromosomes either showed no detectable SYP-1 (3/45) or had SYP-1 stretches on only a subset of their chromosomes (42/45) (Figure 3A, B). Strikingly, SYP-1 was associated with the X chromosome pair in 33/33 nuclei that contained from 2 to 4 SYP-1 stretches. The X chromosomes accounted for 35% of the SYP-1-associated chromosome pairs in these nuclei, despite representing only 1/6 of the total chromosome pairs and only 18% of the total physical length of the genome. Moreover, in 7 of the 9 nuclei in which SYP-1 was associated with only a single chromosome pair, that chromosome pair was the X chromosomes. These data demonstrate that SYP-1 preferentially associates with the X chromosomes when it is present in limiting amounts. This finding indicates that, relative to the autosomes, the X chromosomes have a higher affinity for SYP.
proteins, a higher probability SC nucleation, and/or a higher processivity of SC central region assembly.

**Distribution of SYP-1 along the X chromosomes in the context of a limiting amount of SYP-1:** S-phase X-chromosome labeling in combination with HIM-8 and SYP-1 immunofluorescence allowed us to assess the distribution of SYP-1 protein along the length of the X chromosomes following partial depletion of SYP-1 (Figure 4A, B). In some nuclei, SYP-1 protein was detected along nearly the whole length of the X chromosome, whereas in other nuclei, a shorter SYP-1 stretch extended over a much smaller portion of the chromosome length. Further, in some cases apparent gaps in the SYP-1 staining along the X chromosome pair suggested that regions of synapsis were sometimes interrupted by regions that either lacked SC central region entirely or contained an extremely low level of SYP-1 protein that was below the threshold for cytological detection; in either case, these gaps indicate discontinuities in the SC structure. Such discontinuities were detected in 10/40 X chromosome pairs from *syp-1 RNAi* germ lines, while none were detected in control germ lines (0/18; \( p = 0.02 \)). Figure 4B shows a graphical representation of SYP-1 distribution along the lengths of each of the labeled X chromosome pairs examined. Each X chromosome pair is represented by a horizontal bar, with white corresponding to regions where SYP-1 was not detected and black corresponding to regions associated with SYP-1. Distances from the chromosome ends to the ends of the SYP-1 stretches and the positions of discontinuities in the SYP-1 stretches are represented as percent of total chromosome length, with the left end of the chromosome identified by its associated HIM-8 focus.

Analysis of this data set revealed a significant bias for association of SYP-1 with the left half of the chromosome. This bias was demonstrated using three different approaches for analyzing the data. First, we evaluated the 24 X chromosome pairs exhibiting incomplete synapsis by comparing the left and right ends of the chromosomes with respect to distance from the chromosome end to the nearest end of a SYP-1 stretch. (Incomplete synapsis was defined as having SYP-1 associated with less than 60% of the chromosome length and/or having a gap interrupting the SYP-1 stretch; 100% of control chromosomes were considered fully synapsed based on these criteria.) The mean fractional distance from the left end of the chromosome to the SYP-1 stretch (15% of chromosome length ± 2% [SEM]) was significantly shorter than the mean
fractional distance from the right end (29 ± 4%), as indicated by a p value of 0.015 (two-tailed Mann-Whitney test). Further, this left-end bias was also observed for the subset of chromosomes for which SYP-1 was associated with less than 50% of the chromosome length (20 ± 2% from the left end vs. 39 ± 2% from the right end; p = 0.015).

Second, for each chromosome pair, we assessed the fraction of the SYP-1 length present on that specific chromosome pair that was associated with each half of the chromosome. Comparison of the left and right halves of the chromosome with respect to this parameter revealed a very significant bias for the left half of the chromosome to have a larger fraction of the SYP-1: for the chromosome pairs with incomplete synapsis, the mean fraction of SYP-1 associated with the left half was 62% (± 4% SEM) compared with 38% (± 4%) for the right half, and the Mann-Whitney test indicated that this left-half bias was very significant (two-sided p value = 0.0019). This tendency for the left half of the chromosome to harbor the larger fraction of SYP-1 was even more pronounced for the subset of chromosomes for which SYP-1 was associated with less than 50% of the chromosome length (68 ± 7% for left vs. 32 ± 7% for right; p = 0.0051) and was also detected for the full set of chromosomes analyzed (58 ± 3% for left vs. 42 ± 3% for right; p = 0.0018), but was not observed for chromosome pairs exhibiting complete synapsis (48.5 ± 2% for left vs. 51.5 ± 2% for right). The observation that the left half of the X chromosome tends to harbor a higher proportion of SYP-1 than the right half following syp-1 RNAi parallels the observation that the left half of X also harbors a higher proportion of the crossovers. This in turn suggests that the relative amount of SYP-1 on each half of the chromosome in a given meiotic nucleus directly affects the probability of the crossover occurring in each chromosome half.

Finally, we considered the fractions of X chromosomes that lacked SYP-1 on the left 25% vs. the right 25% of the chromosome length. Once again, the left and right portions of the X chromosome differed significantly with respect to this parameter: while 4/40 chromosomes lacked SYP-1 on the left 25% of chromosome length, 15/40 chromosomes lacked SYP-1 on the right 25% (p = 0.0075).

We also measured the distances between the HIM-8 foci and the nearest SYP-1 stretch on the X chromosomes. HIM-8 - SYP-1 distances in the syp-1 RNAi data set were significantly larger than distances in the control data set (p< 0.0001). Whereas control measurements ranged from 0 to 0.25 μm, with a mean distance of 0.04 μm, syp-1 RNAi distances ranged from 0 to 0.63
μm, with a mean of 0.27 μm and with more than half of the distances exceeding the maximum distance observed in the control data set.

**DISCUSSION**

**Synapsis functions both in promoting and inhibiting crossing over:** Previous analysis of mutants lacking SC central region proteins in several different experimental systems had identified a conserved role for the SC central region in promoting conversion of initiated recombination events into interhomolog crossovers (de Boer and Heyting 2006). Whether the SC central region plays additional roles that inhibit CO formation has been a matter of controversy.

A difficulty in addressing this issue stems from limitations of available experimental tools. Null mutants that completely lack SC central region proteins may lack the very events whose regulation is under investigation. In some organisms (e.g. C. elegans or Drosophila, mutations that eliminate such components eliminate nearly all crossovers (MacQueen et al. 2002; Page and Hawley 2001)). Although non-null mutations affecting these proteins can be used to investigate CO regulation, the fact that such mutations result in proteins with abnormal structure complicates interpretation of such experiments. In other organisms (e.g. S. cerevisiae (Chen et al. 2008; Sym and Roeder 1994)), null mutations that eliminate SC central region proteins significantly reduce but do not eliminate COs; however, the residual COs that form in these organisms may arise by alternative pathways that are not governed by the same mechanisms that regulate SC-dependent COs during normal meiosis.

In the current study, we circumvent these problems by using RNAi to reduce, but not eliminate, the pool of SC central region proteins in C. elegans. Because all known SC central region proteins in this organism (SYP-1, -2, -3, and -4) are interdependent for localization and stability (Colaiacovo et al. 2003; Smolikov et al. 2007b; Smolikov et al. 2009), partial depletion of SYP-1 by RNAi allowed us to create a situation in which reduced levels of structurally normal SC central region proteins were available for assembly. Under these conditions, we found that SC formed on a subset of chromosome segments, making it possible to test whether the SC central region might play a role in inhibiting COs in the context of an organism in which essentially all meiotic COs are normally SC-dependent.
The results of this analysis clearly demonstrate that SC central region proteins play a role in inhibiting the formation of multiple COs on *C. elegans* X chromosomes. DCO meiotic products were not detected among the CO products of control worms, consistent with the findings of several previous studies. In contrast, DCO products represented at least 12% of CO products produced following partial reduction of SYP-1 protein levels. We conclude from this analysis that SYP proteins contribute to mechanisms that limit the number of COs per homolog pair. Below we consider several possible mechanisms by which SYP-1 could function to limit CO number.

One possibility, suggested by our observation of discontinuities in SYP-1 staining in 25% of X chromosome pairs in *syp-1* RNAi worms, but never in control worms, is that continuity of the SC central region is important for CO inhibition. A requirement for SC continuity could reflect a role for the SC central region in communication along a chromosome between an incipient CO and other sites of recombination intermediates. Alternatively, or in addition, the SYP protein complex could itself undergo a change in state that requires continuity for propagation. Under this scenario, a nascent crossover would trigger conversion of SYP complexes, or some other feature of meiotic chromosome structure (*e.g.* the chromosome axis), into a conformation that is no longer competent to support crossover formation. These types of mechanisms for CO inhibition would fall under the umbrella of “CO interference” as traditionally defined.

A related possibility, also suggested by the observed discontinuities in SC structure, is that continuity of SC structure may be relevant to a distinct manifestation of CO regulation referred to as “obligate chiasma” (*Jones* 1984). In many organisms (including mice and *C. elegans*), many chromosome pairs average only a single CO event per meiosis, yet chromosome pairs that lack a crossover (and the consequent chiasma) are extremely rare. This implies the existence of a cellular mechanism that can detect the presence of a chromosome pair lacking a (nascent) CO and respond in a manner that ensures the formation of a crossover. If a continuous stretch of SC were an important feature used by the obligate chiasma mechanism to recognize a chromosome “unit”, it is possible that two stretches of SC separated by a discontinuity might be perceived by the cell as two separate chromosome units, each of which warrants a CO.

It is also possible that the increased incidence of DCOs might be a consequence of an increase in DSB formation in response to incomplete synapsis. A recent study quantifying DSB
formation during *C. elegans* meiosis provided evidence that many chromosome pairs apparently receive only a single DSB and showed that increasing DSB levels can increase COs, suggesting that DSBs are a limiting factor for COs in this system (METS and MEYER 2009). In our analysis of *syp-1 RNAi* germ lines (Figure S2), we detected peak levels of RAD-51 foci that significantly exceeded the number of DSBs estimated to form during wild-type meiosis (METS and MEYER 2009). Although we cannot exclude other possible explanations for elevated RAD-51 foci (see legend to supplemental Figure S2), our data are consistent with an increase in DSB formation following partial depletion of SYP-1. These data and other findings suggest that germ cells may have the capacity respond to incomplete synapsis *per se*, or to the presence of chromosomes and/or SC stretches lacking incipient COs, by prolonging the opportunity for DSB formation (ALPI et al. 2003; CARLTON et al. 2006; MACQUEEN et al. 2005; NABESHIMA et al. 2004). (Indeed, this capacity could be part of the mechanism that ensures the aforementioned obligate chiasma.) An inherent feature of this scenario is that complete SC formation would shut down formation of the DSBs that serve as the initiating events of meiotic recombination, and that cessation of DSB formation would consequently prevent excess COs.

In summary, our data clearly establish a role for SC central region protein SYP-1, and presumably completion of SC assembly, not only in promoting the formation of COs but also in limiting their numbers. The CO-inhibitory function of the SC could reflect a role in inhibiting CO repair downstream of DSB formation, in limiting DSB formation, or both. However, it remains an open question whether this demonstrated CO-inhibitory function implies a direct contribution of the SC central region to the phenomenon of CO interference as traditionally defined.

In light of our discovery of a CO-inhibitory role for the SC central region in *C. elegans*, it is interesting to note that a recent global analysis of the meiotic recombination landscape in yeast also revealed an unanticipated role for Zip1, the primary SC central region component, in inhibiting COs in the vicinity of centromeres (CHEN et al. 2008). Given these demonstrations of CO-inhibitory roles for major building blocks of the SC central region, it is worth revisiting the basis of the argument that the yeast SC central region does not play a role in CO interference. This argument was based primarily on the conclusions that CO/NCO differentiation of recombination intermediates has already occurred prior to synapsis and occurs normally in the absence of synapsis (reviewed in BISHOP and ZICKLER 2004). One piece of evidence leading to
this conclusion came from the analysis of recombination intermediates and products analyzed at an engineered recombination hotspot with an extremely high incidence of DSBs (BORNER et al. 2004). In a zip-1 mutant, formation and progression of CO intermediates were severely perturbed but NCO products at the assay locus formed at normal levels and with normal kinetics, leading to the conclusion that intermediates had been designated for the CO and NCO pathways even in the absence of Zip1. While it may be valid to conclude that commitment to the CO pathway had occurred at the assay locus, however, the experimental design did not address whether CO/NCO designation had been executed chromosome-wide. It is possible that features that lead to unusually high levels of DSBs may also render the assay locus especially CO-prone and relatively immune to inhibition by interference mechanisms in the first place. Without a means to assess the status of CO/NCO designation at other sites, this study cannot exclude the possibility that Zip1 might also have a CO-inhibitory role. The second set of evidence came from a separate study examining the cytological distribution of Zip2 foci as a surrogate for COs, finding that these foci, like COs, exhibit nonrandom distribution in spacing along chromosomes (FUNG et al. 2004). As 1) Zip2 foci appear prior to synapsis and 2) Zip2 foci also exhibit a nonrandom distribution in a zip1 mutant, the authors inferred that CO interference does not depend on synapsis. This conclusion was based on several assumptions: 1) that the positions of Zip2 foci before and after synapsis were the same, i.e., that “synapsis initiation sites” are equivalent to CO sites; and 2) that the positions of Zip2 foci assayed in wild type and the zip1 mutant are the same. However, a recent detailed analysis of SC assembly provided strong evidence that Zip2 complexes move at the leading edge of SC polymerization during SC assembly, invalidating these prior assumptions (TSUBOUCHI et al. 2008). Based on all of these considerations, the possibility that Zip1 might function in CO interference in yeast remains viable.

SC assembly and role of meiotic pairing centers: In addition to demonstrating a role for SYP-1 in limiting CO formation, this work has provided several insights into the process of SC assembly. First, our cytological analysis has clearly demonstrated the cooperative nature of SC assembly, which had been suggested by previous reports (COUTEAU et al. 2004; NABESHIMA et al. 2004). We created a situation in which there is a reduced pool of structurally normal SC precursors that is quantitatively insufficient to achieve full synapsis, thereby requiring SC
assembly to occur in the context of a limiting concentration of SYP-1 protein. Under these conditions, we found that robust SC stretches form but are associated with only a subset of chromosome segments. This localization pattern is clearly indicative of a cooperative assembly process and suggests that nucleation is rate-limiting. That is, our observations are consistent with the interpretation that reducing the abundance of SYP-1 lowers the probability of nucleating SC central region assembly, but that once nucleation occurs, loading of more SYP-1 protein is favored.

Second, our data provide new information regarding the function of specialized cis-acting chromosome domains known as meiotic pairing centers (PCs) in promoting SC assembly (MacQueen et al. 2005; McKim et al. 1993; Villeneuve 1994). Repeated DNA elements and associated Zn finger proteins concentrated at these PCs mediate attachment of chromosomes to the cytoskeletal motility apparatus via association with nuclear envelope (NE)-spanning protein complexes (Penkner et al. 2007; Penkner et al. 2009; Phillips and Dernburg 2006; Phillips et al. 2009; Phillips et al. 2005; Sato et al. 2009). PCs have been demonstrated to play at least two distinct roles in promoting synapsis of homologous chromosomes (MacQueen et al. 2002; MacQueen et al. 2005; Villeneuve 1994). First, PCs promote local associations between homologs that are stable even in the absence of synapsis. Second, they play a separable role in promoting SC assembly, and a variety of data support a model in which SC assembly is nucleated in the vicinity of the PCs (which are located near one end of each chromosome) and continues processively toward the opposite chromosome end. Our analysis of synapsis in the context of limiting SYP-1 allows refinement of our understanding of the relationship between PCs and SC assembly. On one hand, the left end bias for SYP-1 association with the X chromosome supports the idea that PCs promote synapsis most strongly in their own vicinity. On the other hand, we observed discontinuities in SYP-1 staining along some X chromosome pairs, which may reflect SC stretches separated by gaps. The presence of such gaps suggests that nucleation can also occur at additional sites distant from the PC (although we cannot exclude the possibility that gaps may have arisen subsequent to initial assembly). Further, clear gaps between HIM-8 foci (which mark the X chromosome PCs) and SYP-1 stretches on the X chromosomes strongly suggest that PCs and their associated binding proteins do not act as a seed upon which SC assembly is directly nucleated. Rather, our data support a model in which PCs promote synapsis more indirectly, likely at least in part through their synapsis-independent
pairing stabilization activity: by virtue of their mediating close associations between homologs prior to synapsis, nucleation and extension of SC assembly between paired chromosome axes is most likely to occur in close proximity to the PCs. We are currently conducting a high temporal resolution time-course analysis of early meiotic prophase events that will test these ideas directly.

Finally, we found that X chromosomes were more proficient than autosomes at assembling SC in the context of limiting amounts of SYP-1. This finding contributes to and extends a growing body of evidence that C. elegans X chromosomes possess unique properties during meiosis. While all chromosomes exhibit PC-mediated NE associations that connect them to the cytoskeletal motility apparatus during a transient period that is critical for achieving correct pairing and synapsis, the X chromosomes have recently been shown to retain their NE connections and, presumably, their mobility over a much longer fraction of meiotic prophase (Penkner et al. 2009; Sato et al. 2009). Further, in a variety of mutants defective for different components of the meiotic machinery, X chromosomes have been found to be more successful than autosomes at pairing, synapsis, and/or recombination (Couteau et al. 2004; Couteau and Zetka 2005; Jantsch et al. 2007; Martinez-Perez and Villeneuve 2005; Nabeshima et al. 2004; Smolikov et al. 2008). In these prior studies, it was not possible to distinguish whether the X was more proficient at assembling SC per se, or whether better synapsis and/or recombination was a secondary consequence of more robust X pairing under the mutant conditions. The strong preferential association of SYP-1 with X in the current study is not readily explained by differences in pairing proficiency, however, since the X and autosomes exhibit comparable levels of synapsis-independent pairing in the absence of SYP-1 (Macqueen et al. 2002). Thus, our findings imply that paired X chromosomes are inherently more proficient than paired autosomes for loading of SC central region proteins. One factor that could potentially contribute to differential SC assembly is chromosome compaction. The X chromosomes and autosomes display differences in chromatin composition and cytological appearance that suggest a greater compaction for the X (Bender et al. 2004; Kelly et al. 2002). Moreover, direct measurements of chromosome axis lengths for the X chromosome and chromosome I during the pachytene stage indicate a 50% higher degree of compaction for the X chromosome than for chromosome I (Mets and Meyer 2009). This higher degree of chromosome compaction may reflect an underlying difference in chromosome axis organization that increases the X
chromosome’s affinity for SC central region proteins in a way that facilitates nucleation and/or propagation of synapsis.

**CO-promoting and CO-inhibiting roles of SYP-1 collaborate to shape the CO landscape:**
One of the most striking features of our data was the shift in distribution of COs along the length of the X chromosome, with COs occurring preferentially in the left and middle of the chromosome and depleted from the right side of the chromosome following partial depletion of SYP-1. An altered distribution of COs could potentially be an indirect response to impaired synapsis on other chromosomes, as there is prior evidence that asynapsis of the X chromosomes may affect CO distribution on synapsed autosomes (Carlton et al. 2006). In the current analysis, however, the leftward shift in CO distribution on X was shown to correlate with a similar bias in the spatial distribution of the SYP-1 protein along the length of the X chromosome. This correspondence between SC distribution and CO distribution supports the conclusion that SYP-1 acts locally in promoting CO formation, *i.e.*, SYP-1 protein must be present at the site of a recombination intermediate to achieve a crossover, and thus COs will form only where SYP-1 is present. This local requirement likely reflects a role for SYP-1 in recruiting other essential CO-promoting factors.

The inference that SYP-1 acts locally in promoting COs is bolstered by a complementary finding from a previous study analyzing CO regulation in worms carrying the *him-3(me80)* mutation, which reduces the stability of chromosome axis protein HIM-3 (Nabeshima et al. 2004). *him-3(me80)* worms similarly exhibited an elevated incidence of DCOs on the X chromosomes (suggesting that integrity of chromosome axis structure also contributes to mechanisms that limit CO number), but CO distribution along the X chromosome length was not altered. Nevertheless, as in our *syp-1 RNAi* analysis, the cytological distribution of SYP-1 protein in the *him-3(me80)* mutant paralleled its CO distribution: although autosomal synopsis was severely impaired, the X chromosomes were usually fully paired and synapsed, with robust localization of SYP-1 along their entire lengths. Thus, SYP-1 distribution and CO distribution along the X chromosomes behaved concordantly in both of these studies, reinforcing the conclusion that SC central region proteins act locally in their CO-promoting capacity.

A corollary to the requirement for SYP proteins to act locally to promote COs is that extensive cooperative assembly of the SC is needed in order to achieve COs in chromosome
regions distant from predominant sites of synapsis initiation. Because these distant COs in turn can impose CO interference, a consequence of the CO-promoting function of SC polymerization is CO inhibition elsewhere on the same chromosome, including at the site at which synapsis initiated. This need not necessarily imply a direct role for the SC central region in conferring CO inhibition, however. Instead, it could be an indirect consequence of the fact that SC assembly allows the formation of incipient CO events that are capable of eliciting inhibition; inhibition could then occur through interference mechanisms that ultimately operate independently of the SC central region, e.g. through communication along chromosome axes.

Collectively, our data support a model in which SC central region proteins play several roles that together shape the crossover landscape: local CO-promoting activity at CO sites, polymerization to enable CO formation distant from sites of synapsis initiation, and CO-inhibitory role(s) that limit CO number. Consolidation of these distinct yet interconnected roles in a single structure makes it possible to reshape the CO landscape by regulating SC assembly, a feature that may have important implications for chromosomal and organismal evolution.

ACKNOWLEDGEMENTS
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LITERATURE CITED


SATO, A., B. ISAAC, C. M. PHILLIPS, R. RILLO, P. M. CARLTON et al., 2009 Cytoskeletal forces span the nuclear envelope to coordinate meiotic chromosome pairing and synapsis. Cell 139: 907-919.


FIGURE LEGENDS

Figure 1. Partial depletion of SYP-1 by RNAi. A) DAPI-stained chromosomes in oocytes at diakinesis, the last stage of meiotic prophase. Wild type oocyte, six bivalents; syp-1 null mutant oocyte, 12 univalents; syp-1 RNAi oocyte, mixture of bivalents and univalents. B)
Immunolocalization of SYP-1 protein in nuclei from the mid-pachytene regions of germ lines from control and syp-1 RNAi worms. SYP-1 is localized along the full lengths of all chromosome pairs in the control pachytene nuclei. In the syp-1 RNAi nuclei, SYP-1 stretches are associated with only a subset of chromosomes and appear less uniform along their lengths than SYP-1 stretches in control nuclei. C) Immunolocalization of DNA strand exchange protein RAD-51 in nuclei from the mid-pachytene (left) and late pachytene (right) regions of control and syp-1 RNAi germ lines; persistence of RAD-51 foci into the late pachytene region of the syp-1 RNAi germ line reflects reduced SYP-1 function. Scale bar = 5μm.

Figure 2. Altered CO distribution and increased incidence of DCO meiotic products following partial depletion of SYP-1. A) Genetic maps of the X chromosome, derived from SNP marker analysis of crossover frequencies in control (top) and syp-1 RNAi worms (bottom). B) SNP genotypes of DCO chromosomes detected in male (pale blue) and hermaphrodite (pink) progeny. Blue indicates the Bristol N2-derived allele, yellow indicates the Hawaiian-derived allele. For six of the seven DCOs detected in hermaphrodites, the intervals containing the two COs could be deduced unambiguously from the genotype of the hermaphrodite, as the other X chromosome present in those hermaphrodites was an NCO chromosome. For worm 13-3, the position of one of the two COs on the DCO chromosome was ambiguous (occurring either in interval G-F or interval F-E, as indicated by blue/yellow diagonal hatching) because the other X chromosome in that hermaphrodite also had a CO in the G-E interval.

Figure 3. Preferential association of SYP-1 with the X chromosomes. A) Images of pachytene region nuclei from control and syp-1 RNAi worms in which the X chromosomes were specifically labeled by S-phase incorporation of fluorescently labeled nucleotides (red) and immunostained to detect X-chromosome pairing center binding protein HIM-8 (green) and SYP-1 (white). Scale bar = 2μm. B) Pie chart depicting the fractions of nuclei assayed that contained the indicated numbers of SYP-1 stretches associated with X chromosomes and/or autosomes.

Figure 4. Distribution of SYP-1 along labeled X-chromosome pairs. A) Example images of pachytene region nuclei from syp-1 RNAi germ lines, exhibiting a range of SYP-1 localization patterns. Top, SYP-1 extends along the full length of the X chromosome pair. Middle, SYP-1 is
detected in a short stretch adjacent to the HIM-8 focus. Bottom, SYP-1 stretch associated with the X chromosomes is interrupted by a gap. Scale bar = 2μm. B) Graphical representation of the distribution of SYP-1 along the lengths of labeled X chromosome pairs. Each X chromosome pair is represented by a horizontal bar, with white corresponding to regions where SYP-1 was not detected and black corresponding to regions associated with SYP-1. The left end of the chromosome, which harbors the X-PC, was identified by its associated HIM-8 focus (not depicted). Distances from chromosome ends to the ends of the SYP-1 stretches and the positions of discontinuities in SYP-1 stretches are represented as percent of total chromosome length.
Table 1. Efficacy of *syp-1* RNAi

<table>
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<tr>
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<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>n</th>
<th>Mean</th>
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<tbody>
<tr>
<td>a. Oocytes scored in worms exposed to feeding RNAi beginning at L1 stage</td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>N2</td>
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<td>b. Oocytes scored in F1 progeny of indicated cross; feeding RNAi initiated when parents were at L4 stage</td>
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<tr>
<td>AZ212 x Haw</td>
<td>45.4</td>
<td>15.7</td>
<td>14.0</td>
<td>7.5</td>
<td>9.4</td>
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<td>c. Oocytes scored in the subset of F1 worms used for crossover analysis; feeding RNAi initiated when parents were at L4 stage</td>
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<td>AZ212 x Haw*</td>
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</table>

*The oocytes scored for part c were from the F1 worms whose progeny were genotyped for the recombination analysis. All F2 worms genotyped were from plates on which abundant embryos laid by the F1 hermaphrodite (during the 12 hr egg-laying window) gave rise to less than 10 viable F2 progeny; a high proportion of inviable embryos was used as an indicator of effective *syp-1* RNAi. 20% of the viable F2 progeny of these F1s were males, indicative of X chromosome mis-segregation resulting from SYP-1 depletion.
### Table 2. Spatial Distribution of Crossovers

#### A. Division of chromosome into 2 intervals (left, right)

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<th>Right interval (E-A)</th>
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<td></td>
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<td>Number of COs</td>
<td>cM</td>
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<td>(% of total COs)</td>
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<td>(% of total COs)</td>
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<tr>
<td>Control</td>
<td>62</td>
<td>20.5</td>
<td>76</td>
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</tr>
<tr>
<td></td>
<td>(45%)</td>
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<td>(55%)</td>
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<tr>
<td>syp-1 (RNAi)</td>
<td>63</td>
<td>24.0</td>
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<td></td>
<td>(68%)</td>
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<td>(32%)</td>
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#### B. Division of chromosome into 3 domains (left, center, right)

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<th>Right domain (D-A)</th>
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<td>cM</td>
<td>Number of COs</td>
<td>cM</td>
</tr>
<tr>
<td></td>
<td>(% of total COs)</td>
<td></td>
<td>(% of total COs)</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>33</td>
<td>10.9</td>
<td>50</td>
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<tr>
<td></td>
<td>(24%)</td>
<td>(36%)</td>
<td>(36%)</td>
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<td>12.9</td>
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</tr>
<tr>
<td></td>
<td>(37%)</td>
<td>(44%)</td>
<td>(19%)</td>
<td></td>
</tr>
</tbody>
</table>

*Chi-square test for independence
Table 3. Depletion of SYP-1 reduces overall CO frequency, but elevates the occurrence of Double Crossovers

<table>
<thead>
<tr>
<th>Meiotic products</th>
<th>Non-CO (NCO)</th>
<th>Single CO (SCO)</th>
<th>Double CO (DCO)</th>
<th>N</th>
<th>CO frequency (%)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>DCO ratio (%)&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>164</td>
<td>138</td>
<td>0</td>
<td>302</td>
<td>45.7</td>
<td>0</td>
</tr>
<tr>
<td><em>syp-1 (RNAi)</em></td>
<td>180</td>
<td>73</td>
<td>10&lt;sup&gt;c&lt;/sup&gt;</td>
<td>263</td>
<td>35.4</td>
<td>12</td>
</tr>
</tbody>
</table>

<sup>a</sup> (Total number of crossover events [SCO + 2DCO] / number of meiotic chromosomes analyzed) x 100

<sup>b</sup> (Number of DCO / number of SCO + number of DCO) x 100

<sup>c</sup> This number of DCO products likely represents an underestimate of DCO incidence, as certain classes of progeny carrying DCO products would not be unambiguously distinguished by the methods used; see Materials and Methods.

The distribution of NCO, SCO and DCO classes among total meiotic products differs significantly between *syp-1 RNAi* and control (P< 0.0001, Chi-square test for independence). Further, the distributions between SCO and DCO classes among total CO products also exhibit an extremely significant difference (p < 0.0001, Fisher exact test).
Table 4. Analysis of Crossover Interference

<table>
<thead>
<tr>
<th>Meiotic products with crossover(s) present in:</th>
<th>Coefficient of coincidence (^{a})</th>
<th>(P)-value (^{b})</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>syp-1 (RNAi) Corrected (^{d})</td>
</tr>
<tr>
<td>Left interval (H-E) only</td>
<td>62</td>
<td>54</td>
</tr>
<tr>
<td>Right interval (E-A) only</td>
<td>76</td>
<td>24</td>
</tr>
<tr>
<td>Both</td>
<td>0</td>
<td>5(^{c})</td>
</tr>
<tr>
<td>Neither</td>
<td>164</td>
<td>180</td>
</tr>
</tbody>
</table>

\(^{a}\) (Number of observed meiotic products with COs in both L and R intervals)/(Number expected), where expected = (frequency of COs in L interval x frequency of COs in R interval).

\(^{b}\) \(P\)-value from Fisher’s exact test assessing the probability of obtaining the observed data set assuming independent behavior of L and R intervals.

\(^{c}\) For syp-1 (RNAi), five of the 10 DCO products had one CO in L and one in R; three had 2 COs in L, and two had 2 COs in R. For these calculations, we specifically considered the frequency of DCO products involving both intervals.

\(^{d}\) For syp-1 (RNAi) Corrected”, we hypothesized that the original syp-1 (RNAi) data set included meiotic products derived from two populations of meiotic tetrads: tetrads in which at least one CO had occurred (E\(_{1}\) and E\(_{2}\)) and tetrads that lacked a CO (E\(_{0}\)). The number indicated in the “neither” class for the “corrected” data is an estimate of the number of NCO products derived from CO tetrads; the number of NCO products estimated to have been derived from E\(_{0}\) tetrads were excluded (see Materials and Methods).
FIGURE 3

Panel A shows images of control and syp-1(RNAi) conditions. The images are stained with DAPI, Xchr, HIM-8, and SYP-1. The images depict different combinations of chromosomal elements.

Panel B is a pie chart indicating the distribution of various chromosomal configurations:
- no SYP-1
- only X
- X + 1 autosome
- X + 2 autosomes
- X + 3 autosomes
- only 1 autosome
FIGURE 4

A

<table>
<thead>
<tr>
<th></th>
<th>DAPI</th>
<th>Xchr</th>
<th>HIM-8</th>
<th>Xchr</th>
<th>HIM-8</th>
<th>SYP-1</th>
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<tr>
<td>long</td>
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B

<table>
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</thead>
<tbody>
<tr>
<td>syp-1(RNAi)</td>
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