The interaction of crossover formation and the dynamic architecture of the synaptonemal complex during meiosis

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During meiosis, chromosomes pair with their homologous partners, and a structure known as the synaptonemal complex (SC) assembles between them. Evidence indicates that this material plays a role in regulating crossover recombination between homologs, although this remains controversial. Here we use three-dimensional STochastic Optical Reconstruction Microscopy (3D-STORM) to interrogate the molecular architecture of the SC in intact germline tissue from *Caenorhabditis elegans*, and analyze its ultrastructure during meiotic progression. Using a probabilistic mapping approach, we determine the position and orientation of the four known SC structural proteins. We report that a marked structural transition occurs within this material upon crossover designation. We also identify a mutation that disturbs both SC ultrastructure and crossover interference, the widespread mechanism that results in non-randomly wide spacing of crossovers between the same chromosome pair. Together with other evidence, our findings suggest that the SC is an active material, and that structural transitions may directly contribute to chromosome-wide crossover regulation.

The synaptonemal complex (SC) is a protein structure that assembles between homologous chromosomes during meiotic prophase, stabilizing their parallel alignment [1]. The SC appears as a periodic ladder-like structure in electron micrographs [2]. Recent studies have revealed that this material shows dynamic, liquid-like behavior [3-7]. The SC is required for and appears to regulate meiotic recombination [3, 8-13]. Despite its central role in meiosis, the molecular organization of the SC remains poorly characterized.

In *C. elegans*, the SC assembles between homologs even in the absence of meiotic recombination [14]. Four structural proteins essential for SC assembly have been identified: SYP-1, SYP-2, SYP-3, and SYP-4 [15-18]. Limited information about their organization within the SC has been obtained through protein-protein interaction analysis and immuno-electron microscopy [19]. Super-resolution microscopy has emerged as a powerful tool to investigate the organization of macromolecular assemblies, including the SC [20, 21]. We recently described the molecular architecture of meiotic chromosome axes, which form the substrate for SC assembly, based on 3D-STORM [22, 23] in intact *C. elegans* gonads [24]. Here, we apply this approach to analyze the organization of the SC during meiotic prophase.

Results

Using antibodies that bind either short peptides within SC proteins or epitope tags inserted by genome editing, we localized the SYP proteins along paired meiotic chromosomes using 3D-STORM and averaging methods (Extended Data Fig. S1). The orientation of the SC in our images was defined by labeling a reference protein in the chromosome axes, HIM-3 [25]. For positional mapping of SC proteins, we analyzed regions that were observed in frontal view (Fig. 1b, c). We rotated all images about the optical axis (z -axis) such that the chromosomes are extended along the y -axis, with the SC spanning the distance between the homologs on the x -axis (Extended Data Fig. S1b-c, methods). Taking advantage of the well-characterized progression of meiotic stages within the germline of adult *C. elegans*, we compared SC organization before, during and after the designation of crossover sites (Fig. 1a).

Dynamic architecture of the synaptonemal complex in *C. elegans*

The width of the SC in x in *C. elegans*, as measured from electron micrographs, is approximately 96 nm [3]. An antibody recognizing the C-terminal peptide of SYP-1 was resolved as two parallel strands, located at 42.3 ± 0.8 nm and 42.0 ± 1.2 nm off-center in early and late pachytene, respectively (Fig. 1b, m). This epitope thus lies near the outer edges of the SC and within a few nanometers of HIM-3, the most proximal known component of the chromosome axis (Table S1)[24]. Intriguingly, in cross-sectional views (xz -view, Fig. 1c) we observed striking differences in its distribution in z between early and late pachytene SCs: While the C-terminus of SYP-1 was confined near the central plane in early pachytene, it was widely distributed in z in late pachytene (Fig. 1d, e, m, center and Table S1).

We then asked whether the N-terminus of SYP-1 is similarly relocalized during meiotic progression. Tagging the N-terminus of SYP-1 disrupts SC assembly ([26] and data not shown), but we identified a poorly conserved region close to the N-terminus in which insertion of an HA epitope did not impair function (Table S2). Interestingly, this epitope was confined to a thin plane in the center of the SC along both the x and z axis in both early and late pachytene SCs (Fig. 1d, e, m, top). Our data support a transverse orientation for SYP-1, as previously reported [19], with its N-terminus at the center and C-terminus near the axes. Thus, SYP-1 appears to be an ortholog of “transverse filament” proteins from other organisms [27-31]. Importantly, these localization data are inconsistent with a uniform growth in thickness of the SC through e.g.

lateral stacking of SYP-1 molecules during meiotic progression, which has previously been suggested based on observations of increased fluorescence intensity during late pachytene in budding yeast [4] and *C. elegans* [5]. The distinct localization patterns of N- and C-terminal epitopes in early and late SCs along the *z* axis suggest a more complex reorganization of SYP-1 during meiosis.

To further probe the orientation of SYP-1 within the SC, we mapped the N-terminal localization events to the C-terminal distributions, assuming a rigid, rod-like conformation (Fig. S2a-b, see methods). Our approach indicated that SYP-1 lies nearly parallel to the SC in early pachytene, but is diagonally oriented in late pachytene (Fig. 2a-b).

We next asked how the organization of other SC components was affected by meiotic progression. We mapped the C-termini of SYP-2, SYP-3-HA and SYP-4-HA, the N-terminus of GFP-SYP-3[32], and an epitope inserted into the middle of SYP-4 (Table S2, Fig. 1d, e, m and Extended Data Fig. S3). The results are summarized in Table S1. We observed consistent differences between early and late pachytene for most epitopes, particularly for the C-terminus of SYP-4, which moves from a central plane in early pachytene to a splayed orientation, protruding above and below the central plane of the SC, in late pachytene (Fig. S2c and S3, Table S1). Similarly, epitopes on SYP-2 and SYP-3 were more central in early pachytene than late pachytene (Fig. 1d,e,m, S2c and Fig. S3). We further found that SYP-3 localized at (N) or close to (C) the midline of the SC in *x*, suggesting a head-to-head arrangement of SYP-3 molecules (Fig. S3 and Table S1). These findings are inconsistent with one conclusion of a previous study, that the C-terminus of SYP-3 lies in proximity to the axes [19]. This discrepancy may reflect the challenges of determining the orientation of the SC in samples prepared for immuno-EM. Overall, 3D-STORM imaging and probabilistic modeling provide new insights into SC ultrastructure and reveal a marked reorganization of SC components during meiotic progression (Fig. S2c).

Crossover formation is accompanied by a change in synaptonemal complex organization

Programmed double-strand breaks (DSBs) are generated during early prophase. A subset eventually gives rise to crossover (CO) recombination events. In *C. elegans* each pair of homologous chromosomes undergoes only a single CO, while all other breaks are repaired to yield noncrossover products. Some of the key signaling molecules that regulate CO site selection

localize to the SC [3, 11-13, 33, 34], and the integrity of this structure is important for CO regulation [9]. Previous work from our group revealed that the SC behaves as a liquid crystal[3], suggesting that diffusing signals and/or structural transitions could be propagated through this material [35]. Interestingly, recent evidence suggests that the central region of the SC grows in length and/or thickness throughout the pachytene stage of meiotic prophase, and that SC proteins may become more stably associated with this structure upon crossover formation [4-7, 9]. Thus, we wondered whether the dramatic reorganization of the SC during meiotic prophase might be linked to crossover formation and might provide a structural basis for the SC's role in CO regulation. We therefore first asked whether the timing of SC reorganization coincides with CO designation. In *C. elegans*, designated crossovers can be visualized as bright GFP-COSA-1 foci, which are first detected in mid-pachytene [36] (Fig. 1a). To test whether the structural transition within the SC coincides with the appearance of bright GFP-COSA-1 foci, we imaged nuclei at mid-pachytene within intact gonads. We inferred the crossover designation status of individual nuclei based on the detection of bright COSA-1 foci and classified them as either mid(-) pachytene or mid(+) pachytene. Throughout mid-pachytene, SYP-1 retains its transverse orientation and both N- and C-termini of SYP-1 are widely distributed in *z* (Fig. 1f,g,m and Table S1). This is consistent with a lateral stacking of SYP-1 molecules in parallel orientation, as observed in early pachytene (Fig. 2a,b). However, the distance of the C-termini of SYP-1 from the central axis of the SC in frontal view was drastically lower in mid(+) pachytene nuclei compared to SCs in mid(-) pachytene nuclei.

This indicates that the designation of COs coincides with a conformational switch within the SC. To take an unbiased approach to analyze the dynamics of SC organization during meiotic prophase, we employed principal component analysis (PCA) of SYP-1 and SYP-2 distributions (see methods). The first dimension in the PCA, indicative of the main changes within the SC across different stages during meiotic prophase, corresponds to the transition from early to late pachytene SCs (Fig. 2c). Mid(+) pachytene SCs are similar to late pachytene SCs along the first principal component, while mid(-) SCs are in between early and late pachytene SCs, indicating that the SC is reorganized upon CO designation.

In *C. elegans*, designation of COs triggers the asymmetrical remodeling of chromosome axes and the SC to create two domains on either side, known as the long and the short arm. Downstream steps in this differentiation require the recruitment of the Polo-like kinase PLK-2 to

the SC [5, 37, 38]. To assess whether the changes we observe in SC ultrastructure are dependent on crossover designation and/or the subsequent remodeling processes, we determined the organization of the SC in *zhp-3(jf61)* null mutants, which lack crossovers [39], and in *plk-2(ok1936)* null mutants, which are proficient for CO designation but show pronounced delays in downstream remodeling and arm differentiation [5, 7, 37, 40], although they do eventually segregate most chromosomes properly [38]. We found that SCs in late pachytene in *zhp-3(jf61)* mutants resembled those in early pachytene nuclei in wild-type hermaphrodites, while SCs at late pachytene in *plk-2(ok1936)* mutants are very similar to wild-type (Fig. 1h,i,n, 2 and Fig. S3c,e). Thus, structural reorganization of the SC requires CO designation but is independent of PLK-2. We note that PLK-2 also plays an early role in homologous pairing and synapsis [37, 40], and our super-resolution images reveal severe defects in SC architecture in *plk-2(ok1936)* null mutants despite the overall similarity to wild-type late pachytene SCs (Fig. 1i, n). Similarly, while the global SC architecture in *zhp-3(jf61)* mutants resembled early pachytene SCs in wild type, the SC increased in thickness along the optical axis during pachytene in *zhp-3(jf61)* oocytes, based on the spatial distribution of SYP-1 (Fig. 1h, n), consistent with the continuous incorporation of SC subunits even in the absence of crossover formation [5]. In summary, our data indicate that the SC grows in thickness from early to late pachytene independent of crossovers, while the reorganization of SC ultrastructure requires and coincides with CO designation.

Defects in SC organization are linked to defects in crossover interference

The SC plays a critical role in regulating the number and distribution of CO recombination events [3, 8, 9, 12, 13]. We therefore wondered whether reorganization of the SC is linked to CO regulation. Fortuitously, in our efforts to isolate a functional C-terminally tagged *syp-4* allele, we identified an unusual, partially functional allele, *syp-4(ie25)* (Fig. 3a). In *syp-4(ie25)* homozygotes, we observed timely homologous synapsis, as determined by the length of the transition zone, which corresponds to the leptotene and zygotene stages of meiosis. Six bivalents were consistently observed at diakinesis, indicative of CO formation on all six chromosome pairs (Fig. 3b, c, e). However, super-resolution microscopy indicated that the architecture of the SC was drastically altered in *syp-4(ie25)* late pachytene after crossover formation (Fig. 1l and 2, Extended Fig. S3). SYP-1 lies in a highly tilted orientation (Fig. 2a,b) and the overall conformation of the SC in *syp-4(ie25)* at late pachytene is clearly distinct from

any conformation in wild-type animals in our principal component analysis (Fig. 2c). In contrast, early pachytene SCs in *syp-4(ie25)* are more reminiscent of SC conformation of prior to CO formation in wild-type animals (Fig. 1k and 2). These data suggest a strong defect in SC organization in *syp-4(ie25)* that is exacerbated upon CO designation (Fig. 2c).

Notably, *syp-4(ie25)* mutant hermaphrodites exhibited high frequencies of chromosome missegregation, resulting in embryonic lethality ($38 \pm 4\%$ survival, compared to 104% in wild-type) and male self-progeny ($6 \pm 1\%$ compared to 0.1% in wild-type, Table S2), albeit less severe than in a *syp-4(tm2713)* deletion allele (2.5% viability and 40% males) [18]. Importantly, crossover formation was severely impacted in *syp-4(ie25)*: We observed a slight delay in meiotic progression, indicated by a modest extension of CHK-2 kinase activity [41] (Fig. 3d) and an accumulation of RAD-51 foci, which mark unrepaired recombination intermediates (Extended Data Fig. S4). Moreover, the structure of bivalents at diakinesis was altered in *syp-4(ie25)* oocytes (Fig. 3f), indicating that crossover formation might be affected [42, 43].

To better characterize the effects of *syp-4(ie25)*, we imaged GFP-COSA-1 to quantify designated crossover sites at late pachynema [36]. Wild-type animals display a single bright GFP-COSA-1 focus on each of the 6 chromosome pairs, even when the number of DSBs is markedly increased [36, 42]. In contrast to the 6 ± 0.2 (s.d.) GFP-COSA-1 foci we observed in wild-type and heterozygous *ie25/+* animals, we observed 10.9 ± 1.7 GFP-COSA-1 foci at late pachytene in homozygous *syp-4(ie25)* hermaphrodites (Fig. 3g, h). This suggested that crossover interference, which in wild-type animals acts over distances longer than any single chromosome [9, 42], is greatly decreased in *syp-4(ie25)* homozygotes. To test this hypothesis, we use two different metrics to quantify the crossover interference strength, the gamma factor [44] and the coefficient of coincidence [45]. Each of these approaches confirmed that crossover interference is severely reduced or absent in *syp-4(ie25)* mutants (Fig. 3i,j).

To verify that GFP-COSA-1 foci indeed correspond to designated crossover sites in wild-type and *syp-4(ie25)* animals, we also measured crossing-over genetically using whole genome sequencing (see methods). This confirmed that crossovers in *syp-4(ie25)* homozygotes match the number of GFP-COSA-1 foci. Thus, *syp-4(ie25)* mutants are proficient for synapsis, yet they exhibit drastic alterations in SC architecture and cannot impose normal crossover interference.

Crossover interference acts along the physical length of the synapsed chromosomes, and in some organisms chromosomes with longer axes undergo more crossovers than chromosomes

with shorter axes [46-49]. Therefore, we next asked whether the axis length is increased in *syp-4(ie25)* mutants. Interestingly, axes in *syp-4(ie25)* mutants are shorter than in wild-type animals. Thus, changes in axis length cannot account for the observed increase in COs in *syp-4(ie25)* mutants. We hypothesize that the increase in CO number may be caused by the defects in SC organization in *syp-4(ie25)* mutants.

Partial depletion of SC proteins in *C. elegans* by RNAi can result in a slight increase in the number of crossovers, to about 7 GFP-COSA-1 foci per nucleus [9]. Thus, we tested whether the defect in crossover regulation in *syp-4(ie25)* can be attributed to reduced levels of SC proteins. The abundance of an epitope-tagged HA-SYP-1 was not altered by the *syp-4(ie25)* mutation (Fig. S5a). However, the immunofluorescence signal of SYP-1 and SYP-2 was reduced in *syp-4(ie25)*, although staining appeared contiguous along the chromosomes (Fig. S5b, c). This indicates the *syp-4(ie25)* mutation impairs assembly of SYP proteins between chromosomes. In contrast, we also isolated a mutant, *syp-4(ie27)* (Fig. S6a), which reduced expression levels of SYP-4-HA to about 30% of wild-type (Fig. S6c). Both the transition zone and CHK-2 active zone were extended in *syp-4(ie27)* single mutants, indicating that completion of synapsis and CO designation were delayed (Fig. 3c-d, Fig. S6b, d). We also observed a slight increase in the number of GFP-COSA-1 foci to 7 ± 1.1 (Fig. S6e, Fig. 3g), which is comparable to results obtained for partial depletion of SYP-1, -2 or -3 by RNAi [9], but distinct from our results for *syp-4(ie25)*. This indicates that the defects in crossover interference in *syp-4(ie25)* are not a mere consequence of reduced protein expression.

To test whether the defects in SC ultrastructure can account for the effects of *syp-4(ie25)* on crossover regulation, we investigated the role of *syp-4(ie25)* in the regulation of COSA-1 foci within aggregates of SC proteins known as polycomplexes. These bodies self-assemble prior to or after synapsis in many organisms, including in early meiotic nuclei in *C. elegans*. Large polycomplexes also assemble in meiotic nuclei in worms lacking the axis protein HTP-3, and show periodic striations in orthogonal directions that correspond precisely to the width and longitudinal banding of the SC [3, 50]. Intriguingly, we have found that polycomplexes in *htp-3* null mutants recapitulate key aspects of the spatial patterning of CO proteins normally seen along SCs, although these mutant animals lack DSBs and meiotic recombination intermediates. Specifically, at late pachytene, most polycomplexes display a single COSA-1 focus (1.1 ± 0.2 ; Fig. 4a, b) [3]. When we combined the *syp-4(ie27)* mutation, which reduces expression of SYP-

4, with $\Delta htp-3$, this resulted in smaller polycomplexes, but they showed an identical number of GFP-COSA-1 foci at late pachytene (1.1 ± 0.2) (Fig. 4a, b). By contrast, when *syp-4(ie25)* was combined with $\Delta htp-3$, multiple GFP-COSA-1 foci (2.1 ± 0.8) were associated with each polycomplex (Fig. 4a, b), mirroring the ~2-fold increase in GFP-COSA-1 foci observed along bona fide SCs in *syp-4(ie25)* mutants.

We thus interrogated the organization of polycomplexes in *syp-4(ie25)* by EM. While structures resembling the electron-dark lateral and brighter, striated central regions were observed in $\Delta htp-3$ *syp-4(ie25)*, the overall organization was dramatically altered: polycomplexes appeared to be internally fragmented, rather than maintaining constant orientation over hundreds of nm, as in “normal” polycomplexes [3] (Fig. 4c), and the distance between parallel electron-dark bands was much narrower (46.3 ± 1.2 nm, vs 97.6 ± 1.5 nm in $\Delta htp-3$ polycomplexes, Fig. 4c). This likely reflects differences in organization and/or orientation of SYP proteins within polycomplexes. Overall, the disorganized structure of polycomplexes in $\Delta htp-3$ *syp-4(ie25)* in EM is reminiscent of the architecture of SCs in *syp-4(ie25)* determined by 3D-STORM. These findings indicate that structural defects within polycomplexes are sufficient to alter special patterning of CO proteins, analogous to the structurally altered SCs that assemble in *syp-4(ie25)* mutants.

Discussion

Together these findings establish that the correct organization of the synaptonemal complex is required for spatial patterning of crossovers, and further corroborate previous findings that the SC is important for crossover interference. They further suggest that CO regulation may involve transduction of structural information through this liquid crystalline material. This idea is reminiscent of a “beam-film” model for crossover interference, which proposes that crossovers relieve local stress along a beam-like material [49, 51]. This mechanical stress has been proposed to be imposed by chromatin structure and/or tethering of chromosomes; however, our findings reinforce previous studies that implicated the SC as a crucial mediator of crossover patterning [8, 9, 12]. In particular, we have reported that polycomplexes comprised of SC central region proteins alone can mediate spatial patterning of GFP-COSA-1 foci even in the absence of association with chromatin or axis proteins, and polycomplexes in *syp-4(ie25)* mutants exhibit both defects in their internal organization and altered patterning of GFP-COSA-1

272 foci. Interestingly, forces applied to nematic liquid crystalline materials such as the SC [3] can
273 perturb the orientation of molecules within the liquid crystal [35]. Therefore, we speculate that
274 the reorganization of proteins within the SC that occurs concomitant with CO designation may
275 reflect a mechanical signaling component of CO interference.

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Figures

Figure 1: Super-resolution microscopy reveals the dynamic three-dimensional organization of the synaptonemal complex. (a) In the *C. elegans* gonad, nuclei (representative DAPI images) are arranged in a gradient of premeiotic and meiotic stages. Using highly inclined illumination

(red), we imaged SCs in the bottom section of nuclei adjacent to the coverslip (gray) in early, mid- and late pachytene (rectangles). Mid(-) (cyan) and mid(+) (red) pachytene correspond to nuclei without and with bright GFP-COSA-1 foci in the same field of view, respectively. (b) Measurements based on averaged STORM images indicate that the C-terminus of SYP-1 lies close to the chromosome axes. Colors denote localizations in z from -150 to 150 nm (color scale bar). (c) The schematic depicts the SC, which forms a ladder-like structure approximately 100-nm wide (purple), which links the axes (black) of paired homologous chromosomes (white rods) in the frontal view (left). To visualize the 3D architecture of the SC, we show cross-sectional views of the SC as depicted (right). (d-l) Averaged cross-sectional views of STORM images for the N-terminus of HA-SYP-1 (top row), the C-terminus of SYP-1 (center row), and C-terminus of SYP-2 (bottom row), for wild-type SCs in early (d, blue closed spheres in m), late (e, purple closed spheres in m), mid(-) (f, cyan open squares in m), and mid(+) (g, red closed squares in m) pachytene and for *zhp-3(jf61)* (h, green triangles, late pachytene in n), *plk-2(ok1936)* (i, pink diamonds in n, late pachytene) and *syp-4(ie25)* (k, black closed triangles for early pachytene orange crosses in late pachytene in n). (m, n) Histograms of localization events in frontal view (x , left) and across the SC (z , right) are fitted with 1 or 2 Gaussians. The results of the fits are summarized in Extended Data Table S1.

Figure 2: The synaptonemal complex undergoes a structural transition upon crossover designation. (a) The stochastic nature of STORM does not allow us to image the N- and C-terminus of a specific single molecule at the same time but will yield population averages for the localization of individual domains. We therefore used a probabilistic mapping approach to reconstruct the orientation of SYP-1 molecules for each condition and genotype. The resultant models of SYP-1 (black) are shown with C-termini depicted as cubes and N-termini as spheres. A proxy for chromatin (gray areas) is added for visualization purposes. Models are rendered using POV-ray (v3.7.0). Models show changes in the orientation of SYP-1 upon crossover formation. (b) These changes are revealed by the distributions of angles of SYP-1 with respect to the central plane of the SC in cross-sectional views. Cumulative distribution functions are shown. Gray horizontal lines mark median (50%) and 90th percentile values. (c) A principal component analysis (see methods) summarizes the SC organization at different conditions and

genotypes. The progression of SC reorganization in wild-type animals throughout pachytene is highlighted by a gray dashed line. Dimension 1 scales with SC reorganization upon crossover formation (colored arrow). We note that mid(-) SCs prior to designation of COs marked by bright GFP-COSA-1 foci are closer to late pachytene than early pachytene. This suggests that some aspects of the transition in SC reorganization may occur even prior to the appearance of GFP-COSA-1 foci and/or nuclei designated mid(-) pachytene may include false negatives given that our field of view in *z* is limited to 1 μ m slices and does not contain whole nuclei. SC organization in late pachytene *zhp-3(jf61)* mutants that lack CO formation is similar to wild-type early pachytene, while SCs in *plk-2(ok1936)* null mutants resemble wild-type SCs. By contrast, SCs in *syp-4(ie25)* mutants are distinct in both early and late pachytene.

Figure 3: Crossover interference is diminished in *syp-4(ie25)* mutants. (a) The C-terminal amino-acid sequences of wild-type SYP-4 and SYP-4(*ie25*) are indicated. (b) Fluorescence micrographs of DAPI-stained gonads from wild-type (*syp-4-ha(ie29)*, top) and *syp-4(ie25)* mutants (bottom). Insets depict single oocyte nuclei, revealing 6 bivalents in both wild-type and *syp-4(ie25)*. (c) The *syp-4(ie25)* mutation or epitope tagging of *syp-4* alone (*ie29*) did not perturb the length of the transition zone (solid lines in b), but this length was significantly increased in *syp-4-ha(ie27)* mutants, indicative of a defect in synapsis. (d) Immunofluorescence detection of a CHK-2-dependent phosphopeptide provides a more consistent assay for meiotic progression. In animals homozygous for the *syp-4-HA* allele *ie29*, this “CHK-2 active” zone comprises 45% of the length of the germline containing leptotene, zygotene and pachytene stages of meiosis. This fraction was extended to 56% and 77% in *ie25* and *ie27*, respectively. Boxplots in c and d show mean $\pm$ s.d (whiskers show minima and maxima). (e) Fluorescence micrographs reveal robust synapsis for *ie29* (top) and *ie25* (bottom). (f) The cruciform bivalent structure, as illustrated in the cartoon (top) is disrupted in *ie25*, likely reflecting the occurrence of multiple crossovers per

chromosome pair. Immunostaining of HTP-3 (axis; green) and SYP-4 (SC; magenta) is shown (e-f). (g) The number of crossovers per nucleus as estimated from whole genome sequencing (black/white boxplots, mean±s.e., whiskers are extreme values) correspond to the number of GFP-COSA-1 foci (green dots and boxplots, mean±s.d.). (h) Fluorescence micrographs show a marker for designated crossovers, GFP-COSA-1 (yellow), and SYP-4 (blue). (i) To measure crossover interference strength, we fit the cumulative distribution function of inter-GFP-COSA-1 distances in *syp-4(ie25)* mutants (orange crosses) using a gamma function[44]. $\gamma=1$ denotes no interference (gray), $\gamma=37$ corresponds to interference measured for wild-type animals[9] (purple dashed line), while interference in *syp-4(ie25)* is severely reduced to $\gamma=3.5$ (orange line, $n=41$ chromosomes). (j) Likewise, interference strength can be described by the coefficient of coincidence[45], which denotes the ratio of the observed number of 2 COs (GFP-COSA-1 foci) at a given distance to the expected number assuming random CO distribution. In wild-type animals, the coefficient of coincidence of GFP-COSA-1 foci is 0 corresponding to complete interference (purple dashed line; $n=88$ chromosomes), while it is approximately 1 in *syp-4(ie25)* mutants (orange, $n=41$), indicating no interference (gray line). (k) Chromosome axes lengths are shorter in *syp-4(ie25)* mutants compared to wild-type axes in mid(-)-pachytene prior to designation of CO sites (left) and comparable in late pachytene (right). Boxplots show mean±s.d and whiskers are extreme values.

Figure 4: Irregular organization and an increase in GFP-COSA-1 foci in *syp-4(ie25)* polycomplexes. (a) Fluorescence micrographs show that the number of GFP-COSA-1 foci (yellow) is limited to 1 or 2 per polycomplex (SYP-2, blue) in wild-type conditions ($\Delta htp-3$) and in the presence of *syp-4(ie27)* but is elevated in *syp-4(ie25)*. DAPI is shown in gray. (b) Quantification of GFP-COSA-1 foci. Boxplots show mean $\pm$ s.d. and whiskers are extreme values. (c) Representative electron micrographs show that the SC-like structure of polycomplexes ($\Delta htp-3$, 97 nm spacing of parallel, electron dark regions) is disturbed in $\Delta htp-3$ *syp-4(ie25)*, which are disorganized and 46 nm apart.

Table S1: Summary of localizations of SC components determined by STORM. Positions are distances of the maxima to the center of the SC (wt = wild-type; early = early pachytene; mid(-) = mid pachytene nuclei without bright GFP-COSA-1 foci; mid(+) = mid pachytene nuclei with bright GFP-COSA-1 foci). HWHM denotes the half width at half maximum of the distributions (Fig. 1m, gray lines)

domain	condition	x [nm]		z [nm]		total length analyzed [nm] (# images)
		position	HWHM	position	HWHM	
HA-SYP-1	wt, early	0	28.3 ± 1.8	0	33.1 ± 2.7	3438 (8)
	wt; mid(-)	0	27.5 ± 1.6	0	65.4 ± 2.8	3056 (5)
	wt; mid(+)	0	29.4 ± 2.6	0	69.7 ± 3.5	3992 (6)
	wt, late pachytene	0	27.0 ± 1.6	0	29.1 ± 3.7	4780 (9)
	zhp-3(jf61)	0	42.7 ± 2.5	0	46.9 ± 2.9	3579 (7)
	plk-2(ok1936)	0	25.5 ± 1.5	0	52.0 ± 3.9	4136 (10)
	syp-4(ie25); early	0	22.1 ± 1.4	0	28.6 ± 1.2	4351 (10)
	syp-4(ie25); late	0	19.8 ± 0.8	0	20.5 ± 1.6	3114 (6)
SYP-1(C)	wt, early	42.3 ± 0.8	22.8 ± 0.9	0	37.1 ± 2.3	4092 (7)
	wt; mid(-)	40.8 ± 1.3	28.2 ± 0.6	0	68.1 ± 4.3	3184 (7)
	wt; mid(+)	35.9 ± 0.9	31.2 ± 1.0	0	78.7 ± 2.8	3503 (7)
	wt, late pachytene	42.0 ± 1.2	28.5 ± 1.0	0	62.7 ± 3.2	5312 (9)
	zhp-3(jf61); late	41.0 ± 1.3	23.6 ± 1.1	0	48.2 ± 4.3	1851 (8)
	plk-2(ok1936); late	36.3 ± 1.4	39.6 ± 3.6	0	67.0 ± 2.5	5741 (9)
	syp-4(ie25);	38.5 ± 2.5	24.9 ± 0.8	0	43.2 ± 3.8	2768 (5)

	early					
	syp-4(ie25); late	30.5 ± 1.5	29.1 ± 1.8	0	54.0 ± 2.2	4848 (6)
SYP-2(C)	wt, early	0	27.0 ± 2.0	0	38.3 ± 3.7	5866 (10)
	wt; mid(-)	16.3 ± 7.5	27.1 ± 2.7	0	27.6 ± 1.1	8437 (9)
	wt; mid(+)	20.6 ± 6.3	26.5 ± 3.6	0	27.7 ± 1.4	8615 (12)
	wt, late pachytene	18.9 ± 2.1	21.6 ± 1.5	0	49.5 ± 5.0	4573 (9)
	wt, late (HA)	20.9 ± 0.9	20.0 ± 1.3	0	39.9 ± 2.6	2958 (8)
	zhp-3(jf61); late	0	36.1 ± 4.3	0	38.6 ± 3.8	3746 (6)
	plk-2(ok1936); late	0	37.1 ± 2.0	0	47.4 ± 2.3	8206 (9)
	syp-4(ie25); early	0	32.4 ± 2.2	0	73.6 ± 8.8	3172 (7)
	syp-4(ie25); late	0	28.9 ± 2.7	0	33.4 ± 3.2	3604 (8)
GFP-SYP-3	wt, early	0	23.3 ± 0.8	0	40.0 ± 4.5	1784 (4)
	wt, late pachytene	0	18.5 ± 1.4	0	63.2 ± 6.2	2238 (6)
SYP-3-HA	wt, early	12.3 ± 1.8	20.3 ± 1.2	0	27.1 ± 2.4	4653 (10)
	wt, late pachytene	16.6 ± 4.7	29.0 ± 2.3	0	48.1 ± 5.9	2764 (7)
	zhp-3(jf61); late	14.2 ± 2.0	24.4 ± 1.4	0	28.5 ± 2.2	2723 (6)
SYP-4-intFlag	wt, early	0	25.8 ± 0.9	0	43.3 ± 5.3	3462 (9)
	wt, late pachytene	0	30.3 ± 4.3	0	49.8 ± 4.6	2524 (4)
SYP-4-HA	wt, early	14.2 ± 2.0	23.0 ± 1.6	0	38.6 ± 2.4	5355 (10)

	wt, late pachytene	0	36.8 ± 1.1	33.1 ± 11.9	51.5 ± 8.0	5264 (9)
SYP-4(<i>ie25</i>)	syp-4(<i>ie25</i>); late	0	29.1 ± 1.1	0	54.9 ± 5.6	4611 (9)
HIM-3	wt, early	33.5 ± 5.2	43.4 ± 2.3	n.d.	n.d.	2548 (6)
	wt, mid(-)	41.4 ± 2.2	46.7 ± 1.8	n.d.	n.d.	3661 (7)
	wt, mid(+)	41.8 ± 1.9	45.1 ± 1.2	n.d.	n.d.	2817 (7)
	wt, late pachytene	47.3 ± 1.5	30.2 ± 1.2	n.d.	n.d.	4411 (11)
	zhp-3(<i>jf61</i>); late	45.9 ± 1.6	49.0 ± 2.3	n.d.	n.d.	6969 (11)
	plk-2(<i>ok1936</i>); late	41.5 ± 3.8	53.9 ± 4.1	n.d.	n.d.	4769 (8)
	syp-4(<i>ie25</i>); early	45.3 ± 3.6	48.9 ± 2.7	n.d.	n.d.	3900 (7)
	syp-4(<i>ie25</i>); late	35.8 ± 3.8	45.9 ± 3.1	n.d.	n.d.	2926 (6)

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Table S2: List of Strains used in this study and their characterization. *p*-Values for deviation from wild-type are calculated using Mann-Whitney tests.

name	genotype	egg viability [%] (<i>p</i> -value)	males [%] (<i>p</i> -value)	# of eggs
N2	<i>wild-type</i>	104±6	0.08±0.20	4773
GFP-COSA-1 [36]	<i>mels8</i> II	107±4	0.00±0.00	1362
mEos-HIM-3 [24]	<i>mels8</i> II; <i>him-3(ie33)</i> IV	n.d.	n.d.	
mMaple-HIM-3 [24]	<i>mels8</i> II; <i>him-3(ie34)</i> IV	n.d.	n.d.	
HA-SYP-1	<i>mels8</i> II; <i>him-3(ie33)</i> IV; <i>syp-1(ie40[syp-1(1-47)::HA::syp-1(48-489)])</i> V	100±4 (0.886)	0.00±0.00 (0.452)	840
SYP-2-HA	<i>mels8</i> II; <i>him-3(ie33)</i> IV; <i>syp-2(ie99[syp-2::HA])</i> V	101±6 (0.394)	0.00±0.00 (1.00)	1405
GFP-SYP-3	<i>syp-3(ok758)</i> I; <i>ieSi11 [syp-3p::syp-3::EmGFP::syp-3-3'UTR + unc-119(+)]</i> II; <i>him-3(ie33)</i> IV	83±10 (0.031)	0.87±0.59 (0.045)	1143
SYP-3-HA	<i>syp-3(ie42[syp-3::HA])</i> I; <i>mels8</i> II; <i>him-3(ie33)</i> IV	95±4 (0.041)	1.80±0.60 (0.004)	1457
SYP-4-HA(<i>ie29</i>)	<i>syp-4(ie29[syp-4::HA])</i> I; <i>mels8</i> II; <i>him-3(ie34)</i> IV	100±2 (0.150)	0.72±0.41 (0.052)	1336
SYP-4-HA(<i>ie29</i>)	<i>syp-4(ie29[syp-4::HA])</i> I; <i>mels8</i> II	105±3 (0.686)	0.00±0.00 (1.000)	951
SYP-4-intFlag	<i>syp-4(ie30[syp-4(1-315)::2xFlag::syp-4(319-605)])</i> I; <i>mels8</i> II; <i>him-3(ie33)</i> IV	98±3 (0.032)	1.91±0.59 (0.010)	1350
SYP-4(<i>ie25</i>)	<i>syp-4(ie25[syp-4ΔC::3xFlag])</i> I; <i>mels8</i> II	38±4 (0.008)	5.97±1.25 (0.010)	1024
SYP-4-HA(<i>ie27</i>)	<i>syp-4(ie29[syp-4::HA::mod3'UTR])</i> I; <i>mels8</i> II	80±9 (0.002)	1.58±1.58 (0.010)	992
Δ <i>htp-3</i> [3]	<i>htp-3(tm3655)</i> I; <i>mels8</i> II	n.d.	n.d.	
Δ <i>htp-3 syp-4(ie25)</i>	<i>htp-3(ie100) syp-4(ie25)</i> I; <i>mels8</i> II	n.d.	n.d.	

<i>Δhtp-3 syp-4(ie27)</i>	<i>htp-3(ie101) syp-4(ie27) I; mels8 II</i>	n.d.	n.d.	
SYP-4(ie25)	<i>syp-4(ie25[syp-4ΔC::3xFlag] I; mels8 II; him-3(ie33) IV</i>	n.d.	n.d.	
SYP-4(ie25); HA-SYP-1	<i>syp-4(ie25) I; mels8 II; him-3(ie33) IV; syp-1(ie40) V</i>	n.d.	n.d.	
<i>zhp-3(jf61)</i>	<i>zhp-3(jf61) I; mels8 II; him-3(ie33) IV</i>	n.d.	n.d.	
<i>zhp-3(jf61); HA-SYP-1</i>	<i>zhp-3(jf61) I; mels8 II; him-3(ie33) IV; syp-1(ie40) V</i>	n.d.	n.d.	
<i>zhp-3(jf61) SYP-3-HA</i>	<i>syp-3(ie102[syp-3::HA]) zhp-3(jf61) I; mels8 II; him-3(ie33) IV</i>	n.d.	n.d.	
<i>plk-2(ok1936); HA-SYP-1</i>	<i>plk-2(ok1936) I; mels8 II; him-3(ie33) IV; syp-1(ie40) V</i>	n.d.	n.d.	

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Extended Data Figure S1: Experimental strategy for mapping the architecture of the synaptonemal complex. (a) The resolution in conventional fluorescence micrographs is insufficient to visualize the structure of the axis or SC (HIM-3, magenta, left) and SC components (SYP-2-HA, green, center) are co-localized (merge, right). (b) The structure of the SC is resolved by STORM super-resolution microscopy. Colors denote localization in z (left and center). Localization of HIM-3 (magenta, right) is used to determine the orientation of the SC (SYP-2-HA(C), wild-type, late pachytene in green). White boxes in b show regions magnified in other panels. (c) For further analysis, individual stretches in frontal view (right), which are characterized by clearly separated HIM-3 axes, are straightened and aligned in x and z to generate the averaged image (left). (d) The results are highly reproducible between different preparations and different antibodies (SYP-2(C) peptide antibody, black, and SYP-2-HA epitope antibody, green) (Table S2). Note that the widths of the distributions are narrower for the monoclonal HA antibody compared to the polyclonal SYP-2(C) peptide antibody.

Extended Figure S2: Dynamic orientation of SYP-1 molecules. (a) To generate a model of SYP orientation within the SC, individual datapoints (magenta circle, center) in the measured distributions of the N- (blue, left) and C-terminus (gray, left) of a given component (here: SYP-1 in wild-type late pachytene) are randomly mapped to a localization event within the same percentile $\pm 7.5\%$ tolerance of the C-terminal distribution in x and z (purple, center). Mapped events resulting in the shortest or longest 5% of antibody to antibody distances (red, right) are disregarded. (b) The boxplots show means $\pm$ s.d. of the distances between N- and C-terminal antibodies as estimated by the probabilistic mapping approach. Whiskers are extremes and the black line shows the mean across all genotypes and conditions. (c) The resultant model of SC organization generated by mapping of N- and C-terminal distributions of SYP proteins is shown for early (left) and late (right) pachytene in wild-type animals. C-termini are cubes and N-termini

418 (central domain for SYP-4) are spheres. The localization of HIM-3 in x is used as a proxy for
419 axis localization. Models are rendered using POV-ray (v3.7.0).

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Extended Data Figure S3: Dynamic organization of SYP-3 and SYP-4 within the SC. (a-b) Averaged STORM images show cross-sectional views of SYP-3 (a) and SYP-4 (b) domains as indicated in wild type at early (blue) and late pachytene (purple) and *zhp-3(jf61)* (orange) and *syp-4(ie25)* (green) mutants in late pachytene. Colors denote localization in *z*. The distributions of localization events in frontal (*x*), and vertical (*z*) views are analyzed in (c). The results are summarized in Extended Data Table S2.

Extended Data Figure S4: Double-strand break formation is extended in *syp-4(ie25)*. (a)

Immunofluorescence images show DAPI (upper) and RAD-51 (lower), which marks double-strand breaks. While the RAD-51 zone (gray line) is normally limited to a short region in early/mid-pachytene in wild-type (top), it is markedly elongated in *syp-4(ie25)* (bottom). (b)

shows the average number of RAD-51 foci per nucleus (boxes are mean $\pm$ s.e.) as a function of the relative length of the gonad from the distal tip to the end of pachynema of wild-type (purple solid line, $n=1672$ nuclei from (7) gonads), *syp-4(ie29)* (red dashed line, $n=1407$ (6)), *syp-4(ie25)* (green dot-dashed line, $n=1440$ (6)) and *syp-4(ie27)* (blue dotted line, $n=1162$ (4)), respectively.

Extended Data Figure S5: Abundance of SC proteins in *syp-4(ie25)*. (a) The expression level of HA-SYP-1 (magenta) is identical in wild-type and *syp-4(ie25)* strains as shown by a Western Blot. Tubulin (green) is shown as a reference. (b) By contrast, the levels of immunofluorescence staining for SYP-1 (left) and SYP-2 (center) are drastically reduced in *syp-4(ie25)* SCs (bottom) compared to wild-type (*syp-4-ha(ie29)*, right). To ensure identical handling, gonads from both genotypes were stained in a single reaction and their genotype was determined by anti-Flag staining (right), which marks *syp-4(ie25)* but not *syp-4(ie29)*. (c) Boxplots show mean $\pm$ s.d. (whiskers are extreme values) of fluorescence intensities of late pachytene SCs (*ie29*: $n=339$, *ie25*: $n=209$).

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a *syp-4-ha(ie29)* CCTCGTTCTTTAACTTTTACCCCTACGATGTCCAGATTATGCTTAGaaaattatcatgta
syp-4-ha(ie27) CCTCGTTCTTTAACTTTTACCCCTACGATGTCCAGATTATGCTTAGaaaattatcatgta
HA-tag Stop
syp-4-ha(ie29) ttattcagctcttgtatcatttgtatc-----
syp-4-ha(ie27) ttattcagctcttgtatcatttgtatcataatctgagggcggaaacagcacctcgttcttt
3'UTR insertion
syp-4-ha(ie29) -----gtttaatgtca
syp-4-ha(ie27) aacttttagaaaattatcatgtattattcagctcttgtatcatttgtatcgtttaatgtca

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464 **Extended Data Figure S6: The *syp-4-ha(ie27)* mutation phenocopies partial RNAi of other**

465 **SC components.** (a) The mutation *syp-4-ha(ie27)* (bottom) is constituted by an insertion (cyan)

466 in the 3' untranslated region (lower case letters) of the *syp-4* gene, but the coding sequence

467 (capital letters) is identical to *syp-4-ha(ie29)* (top), which is phenotypically wild-type. (b) A

468 DAPI stained fluorescence micrographs show that meiotic progression is delayed in *syp-4-*

469 *ha(ie27)* as indicated by an elongated transition zone (gray line) but can form 6 bivalents (inset)

470 indicating largely successful homologous pairing and crossover formation. (c) A western blot

471 shows that the level of SYP-4-HA (top) is reduced to ~ 30 % in *syp-4-ha(ie27)* compared to *syp-*

472 *4-ha(ie29)*. Tubulin is shown as a reference (bottom). (d) Synapsis is delayed in *syp-4-ha(ie27)*

473 as shown by the incomplete overlap of immunofluorescence images of an axial protein (HTP-3,

474 green) and SC proteins (SYP-4-HA, magenta). (e) As has been described for partial RNAi

475 depletion of SYP-1, -2 and -3[9], a reduction in SYP-4 expression level results in a slight

476 increase in the number of GFP-COSA-1 (yellow) foci to about 7 foci/nucleus (DAPI is shown in

477 blue).

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Methods

Worm strains and transgenes

A complete list of *C. elegans* strains used in this study can be found in Table S1. All strains were cultured at 20°C using standard methods[52]. GFP-SYP-3[32] was inserted by MosSCI[53] and crossed into *syp-3(ok758)*. Other tags were inserted using CRISPR-Cas9 genome editing as described in[24]. Briefly, Cas9 and gRNA were delivered by microinjection, either encoded on a plasmid[54] or as *in vitro* preassembled Cas9-ribonucleoprotein complexes. Repair templates for small epitope tags were codon optimized for *C. elegans* [55] and synthesized as “Ultramers” by IDT.

Immunofluorescence

Immunofluorescence of dissected gonads from young adults 24 h post L4 was performed as described[56] with modifications described in[24]. The following primary antibodies, all of which have been previously described, were used: goat anti-SYP-1 (1:500, affinity purified)[37], mouse anti-HA (1:500, monoclonal 2-2.2.14, Thermo Fisher Scientific), rabbit anti-SYP-2 (1:500, affinity purified)[16], mouse anti-GFP (1:500, monoclonal 7.1 and 13.1, Roche), mouse anti-Flag (1:500, monoclonal M2, Sigma-Aldrich), chicken anti-HTP-3 (1:500)[57], rabbit anti-HIM-3 (1:500, SDQ4713 ModENCODE project)[58], rabbit anti-HIM-8pT64 (1:2000)[41], and rabbit anti-RAD-51 (1:500)[37]. Secondary antibodies raised in donkeys were fluorescently labelled with Alexa Fluor® 488, Cy3 or Alexa Fluor® 647 (1:500, Jackson ImmunoResearch and Invitrogen). Gonads were mounted in ProLong Gold antifade mountant (ThermoFisher Scientific) and epifluorescence images were acquired on a DeltaVision Elite microscope (Applied Precision) using a 100× N.A. 1.4 oil-immersion objective.

For quantification of proteins levels by immunofluorescence, imaging was performed on a Marianas spinning-disk confocal microscope (3i) with a 100x 1.46 NA oil immersion objective. 3D stacks of wild-type (*syp-4-ha(ie29)*) and *syp-4(ie25)* animals were taken from the same slide and quantified in Fiji. SC containing voxels are identified by thresholding the SYP-2 and SYP-4-3xFlag channels for *syp-4(ie29)* and *syp-4(ie25)*, respectively. Images shown in Fig. S6 are background subtracted maximal intensity projections with equally scaled intensity values.

STORM and PALM imaging

Super-resolution imaging of dissected, immuno-stained intact germline tissue was carried out as described[24]. Targets for STORM were labeled with donkey or goat secondary antibodies labelled with Alexa Fluor® 647 (1:500, Jackson ImmunoResearch and Invitrogen). Subsequently, the fluorescently tagged internal reference protein, mEos2-HIM-3 or mMaple3-HIM-3[24] was imaged using PALM [59] [60]. For early pachytene images, mEos2-HIM-3 was co-stained with a rabbit anti-HIM-3 antibody and a donkey-anti-rabbit secondary antibody (Jackson ImmunoResearch) labelled with NHS esters of CF568 to achieve a 2:1 dye-to-antibody ratio. Aligned and averaged images (Fig. S1) were used to generate histograms of localization events in x and z [24]. To systematically distinguish between mono- and bi-modal distributions, we evaluated fits with one and two Gaussians using an ANOVA test in R ($p < 0.05$). Standard deviations of fit parameters were estimated by a subsampling approach using subsets of half the number of individual SC stretches[24]. The results are summarized in Table S2.

Statistical analysis

Sample sizes were not predetermined, and experiments were not randomized. The investigators were not blinded to allocation during experiments and outcome assessment. For STORM experiments, the total lengths and number of stretches analyzed are summarized in Table S2.

Analysis of of SC organization

To derive a model for SC organization from our data, we asked which orientations of SC proteins were consistent with the observed distributions, assuming that each protein is a rigid rod. We first corrected the raw data by removing the extreme 2.5% of the localization events using squared Mahalanobis distances in R (version 3.4.2). We then mapped each localization event corresponding the n th-percentile in x and the m th-percentile in z of the N- (or C-) terminal distribution to a randomly selected localization event within the n th $\pm 7.5\%$ in x and m th $\pm 7.5\%$ in z of the corresponding C- (or N-) terminal distribution. This analysis reveals the distance between the N- and C-terminal antibodies used for imaging and the orientation of the protein within the SC. For further analysis, we remove outliers among the mapped localization events with extreme values corresponding to the lower and upper 2.5% of distances.

To visualize changes within SC organization, we performed principal component analysis of the widths, and – where applicable – positions off-center, for N- and C-terminal distributions of

SYP-1 and the C-terminal distribution of SYP-2, the orientations of SYP-1 molecules as characterized by the median and 90th percentile angles, and the average distance between N- and C-terminal antibodies using R (version 3.4.2). We excluded SYP-3 and SYP-4 from this analysis since experimental challenges prevented us from analyzing their localizations for all genotypes.

Recombination mapping

To map meiotic recombination events, the *syp-4(ie25)* allele (generated in Bristol N2 background) was introgressed into the divergent Hawaiian strain CB4856 by 8 sequential crosses and selection for *ie25*. Hawaiian and Bristol wild-type and *syp-4(ie25)* strains were crossed, and the hybrid F1 progeny were backcrossed to Hawaiian males. These cross-progeny, which have Hawaiian paternal chromosomes and recombined Bristol/Hawaiian maternal chromosomes, were plated individually, allowed to reproduce for one generation, and the genomic DNA of their pooled progeny was extracted by phenol-chloroform extraction. Illumina sequencing libraries were prepared as described in [61] and sequenced as 50 bp single reads on a HiSeq2500 Illumina sequencer at the Vincent J. Coates Genomics Sequencing Laboratory at UC Berkeley, with a coverage of 2-4x per individual. Reads were mapped to the genomes of Bristol (Wormbase release WS230) and Hawaiian[62] strains and genotypes called using the multiplexed shotgun genotyping (MSG) toolbox and default parameters[63]. Aligned sequences obtained by deep sequencing have been uploaded on the NCBI SRA database under accession number SRP126693. Following this analysis, crossovers in oogenesis are characterized by transitions from Hawaiian/Bristol heterozygous stretches to homozygous Hawaiian stretches, and the calculated number of crossovers per nucleus is twice the number of mapped crossovers in the oocytes.

Electron microscopy

High-pressure freezing, freeze-substitution, sample preparation, and microscopy was performed as described previously[3, 64, 65]. Images were acquired on a Tecnai 12 transmission electron microscope (120 kV, FEI, Hillsboro, OR) equipped with a Gatan Ultrascan 1000 CCD camera (Pleasanton, CA).

Immunoblotting

To compare expression levels of SYP proteins, 120 adult worms were lysed by boiling in 40 μ L Laemmli sample buffer (with β -mercaptoethanol) for about 5 min, until particulate matter was not detected using a dissection stereomicroscope. Samples of whole-worm lysates were run on a NuPage 4-12% polyacrylamide gradient gel and transferred to a PVDF membrane. Primary antibodies were rabbit (Pierce, PA1-985, used for HA-SYP-1) or mouse anti-HA (SYP-4-HA) and mouse anti-tubulin (EMD Millipore), each diluted 1:5,000. HRP-conjugated secondary antibodies (Jackson Laboratory) were detected with ECL reagents (Amersham). For SYP-4-HA blots, HA and tubulin were easily resolved and can be detected simultaneously. For HA-SYP-1, HA was detected by HRP-anti-rabbit/ECL and tubulin is detected by Cy3-conjugated anti-mouse secondary antibody (1:5,000). Images were recorded using a Chemidoc system (Bio-Rad) and quantified using Fiji.

Data Availability

NGS datasets generated during the current study are available on the NCBI SRA database under accession number SRP126693 (<https://www.ncbi.nlm.nih.gov/sra/SRP126693>). Any additional data are available upon reasonable request.

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Competing Interests

The authors declare no competing interests.

Author Contributions

SK and MW performed experiments and analyzed data. SK, MW, KX and AFD designed experiments and wrote the manuscript.

References

1. Zickler D, Kleckner N (1999) Meiotic chromosomes: integrating structure and function. *Annu Rev Genet* 33:603–754. doi: 10.1146/annurev.genet.33.1.603
2. Moses MJ (1968) Synaptonemal complex. *Annu Rev Genet* 2:363–412.
3. Rog O, Köhler S, Dernburg AF (2017) The synaptonemal complex has liquid crystalline properties and spatially regulates meiotic recombination factors. *Elife* 6:e21455. doi: 10.7554/eLife.21455

4. Voelkel-Meiman K, Moustafa SS, Lefrançois P, et al (2012) Full-length synaptonemal complex grows continuously during meiotic prophase in budding yeast. *PLoS Genet* 8:e1002993. doi: 10.1371/journal.pgen.1002993
5. Pattabiraman D, Roelens B, Woglar A, Villeneuve AM (2017) Meiotic recombination modulates the structure and dynamics of the synaptonemal complex during *C. elegans* meiosis. *PLoS Genet* 13:e1006670. doi: 10.1371/journal.pgen.1006670
6. Machovina TS, Mainpal R, Daryabeigi A, et al (2016) A Surveillance System Ensures Crossover Formation in *C. elegans*. *Curr Biol* 26:2873–2884. doi: 10.1016/j.cub.2016.09.007
7. Nadarajan S, Lambert TJ, Altendorfer E, 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:e23437. doi: 10.7554/eLife.23437
8. Hayashi M, Mlynarczyk-Evans S, Villeneuve AM (2010) The synaptonemal complex shapes the crossover landscape through cooperative assembly, crossover promotion and crossover inhibition during *Caenorhabditis elegans* meiosis. *Genetics* 186:45–58. doi: 10.1534/genetics.110.115501
9. Libuda DE, Uzawa S, Meyer BJ, Villeneuve AM (2013) Meiotic chromosome structures constrain and respond to designation of crossover sites. *Nature* 502:703–706. doi: 10.1038/nature12577
10. Wang K, Wang C, Liu Q, et al (2015) Increasing the Genetic Recombination Frequency by Partial Loss of Function of the Synaptonemal Complex in Rice. *Mol Plant* 8:1295–1298. doi: 10.1016/j.molp.2015.04.011
11. Woglar A, Villeneuve AM (2018) Dynamic Architecture of DNA Repair Complexes and the Synaptonemal Complex at Sites of Meiotic Recombination. *Cell* 173:1678–1691.e16. doi: 10.1016/j.cell.2018.03.066
12. Cahoon CK, Helm JM, Libuda DE (2019) Synaptonemal complex proteins direct and constrain the localization of crossover-promoting proteins during *Caenorhabditis elegans* meiosis. *bioRxiv* 523605. doi: 10.1101/523605
13. Zhang L, Köhler S, Rillo-Bohn R, Dernburg AF (2018) A compartmentalized signaling network mediates crossover control in meiosis. *Elife* 7:245. doi: 10.7554/eLife.30789

14. Dernburg AF, McDonald K, Moulder G, et al (1998) Meiotic recombination in *C. elegans* initiates by a conserved mechanism and is dispensable for homologous chromosome synapsis. *Cell* 94:387–398.
15. MacQueen AJ, Colaiácovo MP, McDonald K, Villeneuve AM (2002) Synapsis-dependent and -independent mechanisms stabilize homolog pairing during meiotic prophase in *C. elegans*. *Genes Dev* 16:2428–2442. doi: 10.1101/gad.1011602
16. Colaiácovo MP, MacQueen AJ, Martinez-Perez E, et al (2003) Synaptonemal complex assembly in *C. elegans* is dispensable for loading strand-exchange proteins but critical for proper completion of recombination. *Dev Cell* 5:463–474. doi: 10.1016/S1534-5807(03)00232-6
17. Smolikov S, Eizinger A, Schild-Prüfert K, et al (2007) SYP-3 restricts synaptonemal complex assembly to bridge paired chromosome axes during meiosis in *Caenorhabditis elegans*. *Genetics* 176:2015–2025. doi: 10.1534/genetics.107.072413
18. Smolikov S, Schild-Prüfert K, Colaiácovo MP (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. doi: 10.1371/journal.pgen.1000669
19. Schild-Prüfert K, Saito TT, Smolikov S, et al (2011) Organization of the synaptonemal complex during meiosis in *Caenorhabditis elegans*. *Genetics* 189:411–421. doi: 10.1534/genetics.111.132431
20. Schücker K, Holm T, Franke C, et al (2015) Elucidation of synaptonemal complex organization by super-resolution imaging with isotropic resolution. *Proc Natl Acad Sci* 112:2029–2033. doi: 10.1073/pnas.1414814112
21. Cahoon CK, Yu Z, Wang Y, et al (2017) Superresolution expansion microscopy reveals the three-dimensional organization of the *Drosophila* synaptonemal complex. *Proc Natl Acad Sci* 114:E6857–E6866. doi: 10.1073/pnas.1705623114
22. Rust MJ, Bates M, Zhuang X (2006) Sub-diffraction-limit imaging by stochastic optical reconstruction microscopy (STORM). *Nat Meth* 3:793–795. doi: 10.1038/nmeth929
23. Huang B, Wang W, Bates M, Zhuang X (2008) Three-dimensional super-resolution imaging by stochastic optical reconstruction microscopy. *Science* 319:810–813. doi: 10.1126/science.1153529

24. Köhler S, Wojcik M, Xu K, Dernburg AF (2017) Superresolution microscopy reveals the three-dimensional organization of meiotic chromosome axes in intact *Caenorhabditis elegans* tissue. *Proc Natl Acad Sci* 114:E4734–E4743. doi: 10.1073/pnas.1702312114
25. Zetka MC, Kawasaki I, Strome S, Müller F (1999) Synapsis and chiasma formation in *Caenorhabditis elegans* require HIM-3, a meiotic chromosome core component that functions in chromosome segregation. *Genes Dev* 13:2258–2270.
26. Gao J, Barroso C, Zhang P, et al (2016) N-terminal acetylation promotes synaptonemal complex assembly in *C. elegans*. *Genes Dev* 30:2404–2416. doi: 10.1101/gad.277350.116
27. Schmekel K, Meuwissen RL, Dietrich AJ, et al (1996) Organization of SCP1 protein molecules within synaptonemal complexes of the rat. *Exp Cell Res* 226:20–30. doi: 10.1006/excr.1996.0198
28. Sym M, Roeder GS (1995) Zip1-induced changes in synaptonemal complex structure and polycomplex assembly. *J Cell Biol* 128:455–466.
29. Higgins JD, Sanchez-Moran E, Armstrong SJ, et al (2005) The Arabidopsis synaptonemal complex protein ZYP1 is required for chromosome synapsis and normal fidelity of crossing over. *Genes Dev* 19:2488–2500. doi: 10.1101/gad.354705
30. Meuwissen RL, Offenberg HH, Dietrich AJ, et al (1992) A coiled-coil related protein specific for synapsed regions of meiotic prophase chromosomes. *EMBO J* 11:5091–5100.
31. Anderson LK, Royer SM, Page SL, et al (2005) Juxtaposition of C(2)M and the transverse filament protein C(3)G within the central region of *Drosophila* synaptonemal complex. *Proc Natl Acad Sci USA* 102:4482–4487. doi: 10.1073/pnas.0500172102
32. Rog O, Dernburg AF (2015) Direct Visualization Reveals Kinetics of Meiotic Chromosome Synapsis. *Cell Rep* 10:1639–1645. doi: 10.1016/j.celrep.2015.02.032
33. Reynolds A, Qiao H, Yang Y, et al (2013) RNF212 is a dosage-sensitive regulator of crossing-over during mammalian meiosis. *Nat Genet* 45:269–278. doi: 10.1038/ng.2541
34. Qiao H, Prasada Rao HBD, Yang Y, et al (2014) Antagonistic roles of ubiquitin ligase HEI10 and SUMO ligase RNF212 regulate meiotic recombination. *Nat Genet* 46:194–199. doi: 10.1038/ng.2858
35. de Gennes PG, Prost J (1993) *The Physics of Liquid Crystals*, 2nd ed. Clarendon Press, Oxford

36. Yokoo R, Zawadzki KA, Nabeshima K, et al (2012) COSA-1 reveals robust homeostasis and separable licensing and reinforcement steps governing meiotic crossovers. *Cell* 149:75–87. doi: 10.1016/j.cell.2012.01.052
37. Harper NC, Rillo R, Jover-Gil S, et al (2011) Pairing centers recruit a Polo-like kinase to orchestrate meiotic chromosome dynamics in *C. elegans*. *Dev Cell* 21:934–947. doi: 10.1016/j.devcel.2011.09.001
38. Sato-Carlton A, Nakamura-Tabuchi C, Chartrand SK, et al (2018) Phosphorylation of the synaptonemal complex protein SYP-1 promotes meiotic chromosome segregation. *The Journal of Cell Biology* 217:555–570. doi: 10.1083/jcb.201707161
39. Jantsch V, Pasierbek P, Mueller MM, et al (2004) Targeted gene knockout reveals a role in meiotic recombination for ZHP-3, a Zip3-related protein in *Caenorhabditis elegans*. *Mol Cell Biol* 24:7998–8006. doi: 10.1128/MCB.24.18.7998-8006.2004
40. Labella S, Woglar A, Jantsch V, Zetka M (2011) Polo kinases establish links between meiotic chromosomes and cytoskeletal forces essential for homolog pairing. *Dev Cell* 21:948–958. doi: 10.1016/j.devcel.2011.07.011
41. Kim Y, Kostow N, Demburg AF (2015) The Chromosome Axis Mediates Feedback Control of CHK-2 to Ensure Crossover Formation in *C. elegans*. *Dev Cell* 35:247–261. doi: 10.1016/j.devcel.2015.09.021
42. Hillers KJ, Villeneuve AM (2003) Chromosome-wide control of meiotic crossing over in *C. elegans*. *Curr Biol* 13:1641–1647. doi: 10.1038/nrg1210
43. Nabeshima K, Villeneuve AM, Colaiácovo MP (2005) Crossing over is coupled to late meiotic prophase bivalent differentiation through asymmetric disassembly of the SC. *J Cell Biol* 168:683–689. doi: 10.1083/jcb.200410144
44. Broman KW, Weber JL (2000) Characterization of human crossover interference. *Am J Hum Genet* 66:1911–1926. doi: 10.1086/302923
45. Muller HJ (1916) The mechanism of crossing-over IV. *American Naturalist* 50:421–434.
46. Drouaud J, Mercier R, Chelysheva L, et al (2007) Sex-specific crossover distributions and variations in interference level along *Arabidopsis thaliana* chromosome 4. *PLoS Genet* 3:e106. doi: 10.1371/journal.pgen.0030106

47. Petkov PM, Broman KW, Szatkiewicz JP, Paigen K (2007) Crossover interference underlies sex differences in recombination rates. *Trends Genet* 23:539–542. doi: 10.1016/j.tig.2007.08.015
48. Mets DG, Meyer BJ (2009) Condensins Regulate Meiotic DNA Break Distribution, thus Crossover Frequency, by Controlling Chromosome Structure. *Cell* 139:73–86. doi: 10.1016/j.cell.2009.07.035
49. Zhang L, Liang Z, Hutchinson J, Kleckner N (2014) Crossover Patterning by the Beam-Film Model: Analysis and Implications. *PLoS Genet* 10:e1004042. doi: 10.1371/journal.pgen.1004042
50. Goodyer W, Kaitna S, Couteau F, et al (2008) HTP-3 links DSB formation with homolog pairing and crossing over during *C. elegans* meiosis. *Dev Cell* 14:263–274. doi: 10.1016/j.devcel.2007.11.016
51. Kleckner N, Zickler D, Jones GH, et al (2004) A mechanical basis for chromosome function. *Proc Natl Acad Sci USA* 101:12592–12597. doi: 10.1073/pnas.0402724101
52. Brenner S (1974) The genetics of *Caenorhabditis elegans*. *Genetics* 77:71–94.
53. Frøkjær-Jensen C, Davis MW, Hopkins CE, et al (2008) Single-copy insertion of transgenes in *Caenorhabditis elegans*. *Nat Genet* 40:1375–1383. doi: 10.1038/ng.248
54. Dickinson DJ, Ward JD, Reiner DJ, Goldstein B (2013) Engineering the *Caenorhabditis elegans* genome using Cas9-triggered homologous recombination. *Nat Meth* 10:1028–1034. doi: 10.1038/nmeth.2641
55. Redemann S, Schloissnig S, Ernst S, et al (2011) Codon adaptation-based control of protein expression in *C. elegans*. *Nat Meth* 8:250–252. doi: 10.1038/nmeth.1565
56. Phillips CM, McDonald KL, Dernburg AF (2009) Cytological analysis of meiosis in *Caenorhabditis elegans*. *Methods Mol Biol* 558:171–195. doi: 10.1007/978-1-60761-103-5_11
57. Macqueen AJ, Phillips CM, Bhalla N, et al (2005) Chromosome sites play dual roles to establish homologous synapsis during meiosis in *C.-elegans*. *Cell* 123:1037–1050. doi: 10.1016/j.cell.2005.09.034
58. Gerstein MB, Lu ZJ, Van Nostrand EL, et al (2010) Integrative analysis of the *Caenorhabditis elegans* genome by the modENCODE project. *Science* 330:1775–1787. doi: 10.1126/science.1196914

59. McKinney SA, Murphy CS, Hazelwood KL, et al (2009) A bright and photostable photoconvertible fluorescent protein for fusion tags. *Nat Meth* 6:131–133. doi: 10.1038/nmeth.1296
60. Wang S, Moffitt JR, Dempsey GT, et al (2014) Characterization and development of photoactivatable fluorescent proteins for single-molecule-based superresolution imaging. *Proc Natl Acad Sci* 111:8452–8457. doi: 10.1073/pnas.1406593111
61. Meyer M, Kircher M (2010) Illumina sequencing library preparation for highly multiplexed target capture and sequencing. *Cold Spring Harb Protoc* 2010:pdb.prot5448. doi: 10.1101/pdb.prot5448
62. Thompson OA, Snoek LB, Nijveen H, et al (2015) Remarkably Divergent Regions Punctuate the Genome Assembly of the *Caenorhabditis elegans* Hawaiian Strain CB4856. *Genetics* 200:975–989. doi: 10.1534/genetics.115.175950
63. Andolfatto P, Davison D, Erezyilmaz D, et al (2011) Multiplexed shotgun genotyping for rapid and efficient genetic mapping. *Genome Res* 21:610–617. doi: 10.1101/gr.115402.110
64. McDonald KL, Webb RI (2011) Freeze substitution in 3 hours or less. *J Microsc* 243:227–233. doi: 10.1111/j.1365-2818.2011.03526.x
65. McDonald KL (2014) Rapid embedding methods into epoxy and LR White resins for morphological and immunological analysis of cryofixed biological specimens. *Microsc Microanal* 20:152–163. doi: 10.1017/S1431927613013846