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A Link between Meiotic Prophase Progression and Crossover Control

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Introduction

Meiosis ensures the reductive division of a diploid genome into haploid complements. Proper meiotic segregation depends on pairing, synapsis, and crossing over between homologous chromosomes. In addition to promoting genetic diversity [1,2], crossing over enables the bi-orientation of homologous chromosomes at metaphase I by establishing physical connections (chiasmata) between homologs [3]. Crossover recombination is therefore essential for proper meiotic chromosome disjunction.

The very low frequency of achiasmate chromosomes at metaphase I implies that a specific mechanism ensures the placement of at least one crossover per chromosome, called the “obligate crossover.” In principle, a minimum crossover number of one could be achieved with an unregulated, random process, if the number of crossovers was sufficiently high. However, most organisms have far too few crossovers for a Poisson process to ensure that each chromosome receives at least one [4].

In addition to this “obligate crossover” phenomenon, exchanges are also subject to genetic interference. This term describes the observation that crossovers are spaced farther apart from each other than would be expected if they occurred independently. In Caenorhabditis elegans, interference is extremely potent, limiting the number of crossovers per chromosome to exactly one. It has been proposed that these two facets of crossover control, genetic interference and the obligate crossover, are mechanistically linked [5,6]. A high rate of crossover attempts could give rise to a low but nonzero number of crossovers on all chromosomes if interference prevented most crossover attempts from being realized.

The mechanisms governing crossover control are not well understood. In Saccharomyces cerevisiae, recombination events destined to give rise to crossovers can be identified very early in meiotic prophase [7], but it is not known whether these events display interference when they first appear. The chromosomal localization of synopsis initiation complexes containing Zip2 and Zip3 shows a pattern consistent with interference, even in the absence of synopsis or crossover formation [8], supporting an early imposition of interference. Recent work has suggested that intact meiotic chromosome axes are necessary to mediate the high level of interference seen in C. elegans [9–11], but we still know very little about the mechanism by which this control is mediated.

Recent work in our laboratory has characterized the effects of two different mutations that specifically prevent synopsis of the X chromosomes, and thereby severely inhibit the formation of crossovers on one chromosome pair. Asynapsis can be caused by deletion of the X chromosome Pairing Centers, cis-acting sites that are required to stabilize homolog pairing and initiate synopsis [12]. Mutations in him-8, which encodes a protein that specifically binds to the X chromo-
Synopsis

Meiosis is a specialized cell division and an essential component of sexual reproduction. During meiotic prophase, each chromosome must pair with its unique homologous partner and undergo crossing over (genetic exchange) to segregate properly. A major mystery is how the molecular events of meiotic recombination are coupled to the large-scale dynamics of chromosome synapsis. This work reveals a link between the large-scale regulation of chromosome organization and the distribution of crossover events on the chromosomes. In *C. elegans*, defects in chromosome pairing or synapsis result in an extension of a normally transient stage of meiotic prophase. This study finds that this extension is associated with dysregulation of crossovers, so that more than the usual number of crossovers occur, and their distribution is shifted along the chromosomes. These observations contribute to our understanding of crossover control, which normally ensures accurate transmission of genetic information from parent to progeny.

Results

Defects in X Chromosome Synapsis Result in a Global Delay in Nuclear Reorganization during Meiotic Prophase

To estimate the length of time each nucleus spends in a given meiotic substage, whole gonads of age-matched animals (20 h post-L4) were stained with DAPI and imaged by wide-field optical sectioning microscopy and image deconvolution. The *C. elegans* gonad contains nuclei at all stages of meiotic prophase arranged in a spatiotemporal gradient. Each gonad contains between 400 and 1,000 nuclei, with an average of 655 nuclei per gonad. Every nucleus in each imaged gonad was classified into one of four meiotic prophase substages based on criteria illustrated in Figure 1. Previous work has documented the appearance of nuclei in the premeiotic germline and the “transition zone,” which corresponds to the stages of leptotene and zygotene, where pairing and synapsis are initiated. Transition zone nuclei have a distinct polarized appearance, with the chromosomes and the large nucleolus each displaced toward one side [14]. We further divided the pachytene stage into discrete early and late pachytene substages. Early pachytene nuclei retain a polarized appearance, but most of the chromosomes are clearly separated and synapsed. Late pachytene onset is defined by the disappearance of the polarized configuration of chromosomes. Although there is a general progression from one substage to the next as distance from the distal end of the gonad arm increases, we note that the physical range of each substage overlaps with adjacent stages. To obtain an accurate measure of stage duration, we carried out a systematic classification of all nuclei by stage.

The fraction of nuclei that were classified as premeiotic and transition zone was not altered by mutations that perturb meiotic synapsis or crossing over, including *spo-11(ok79), msh-5(me23), him-8(mn253), rad-51(g8701),* and *meDf2* (unpublished data). However, *him-8* and *meDf2* caused a clear shift in the relative numbers of early and late pachytene nuclei (Figure 2), suggesting that this particular transition is subject to regulation. As shown in Figure 2, *him-8* animals possess a significantly larger proportion of early pachytene nuclei, relative to wild-type animals, indicating that the exit from the earlypachytene stage is delayed in these mutants. *meDf2* gonads had an appearance qualitatively similar to that of *him-8* gonads (unpublished data). Chromosome synapsis occurs normally between autosomes in early pachytene in *him-8* (Figure 2, inset) and *meDf2* hermaphrodites, while the X chromosomes are unsynapsed. Therefore, a single pair of unsynapsed X chromosomes is sufficient to delay the early pachytene–late pachytene transition.
Persistence of RAD-51 Foci Correlates to the Delay in Chromosome Reorganization

We utilized immunofluorescence to determine whether the increase in early pachytene duration is accompanied by persistence of the recombination protein RAD-51 [15] on the chromosomes. Figure 3 displays a wild-type gonad (top), in which RAD-51 foci first appear in the transition zone, peak in early pachytene, and then largely disappear upon entry into late pachytene. In contrast, gonads from him-8, msh-5, and him-8(msh-5) hermaphrodites (Figure 3, middle and bottom) contain RAD-51 foci throughout the delayed early pachytene stage as well as roughly halfway through late pachytene. We performed quantitation of RAD-51 localization in wild-type and mutants (Figure 3) and found that RAD-51 foci achieve higher absolute numbers, and persist through later meiotic substages, when all three mutant conditions were compared to wild-type. Significantly, in nuclei from the extended early pachytene region of him-8 mutants, RAD-51 foci remain on the autosomes as well as the X chromosome (Figure 4). This demonstrates that recombination intermediates are more prevalent at later stages on autosomes, despite the availability of a synapsed homolog that should provide a template for recombinational repair. This observation suggested that the failed synapsis of the X chromosomes might influence the dynamics of recombination on the autosomes.

Delays in Meiotic Progression Require Components of the Crossover Machinery

To investigate the possible relationship between persistent recombination intermediates and the delay in chromosome reorganization we observed in him-8 and meDf2 mutants, we tested whether the delay required components that are important for recognizing DNA damage or processing double-strand breaks. We assessed whether such mutations...
affected the delay by staging entire gonads and comparing the ratio of early pachytene to late pachytene nuclei. Data are shown in Figure 5.

It has been speculated that delays in reorganization of nuclear morphology might represent a response to unrepaired DNA damage, and we investigated this by crossing a *hus-1* mutation into *meDf2* and *him-8* mutants. Most mutations that disrupt the meiotic DNA damage checkpoint in *C. elegans* are also associated with defects in telomere maintenance and fecundity [17,18], but the *hus-1(op241)* point mutation, which we used, specifically abrogates its role in the meiotic DNA damage checkpoint. This *hus-1* mutation had no effect on the duration of polarized morphology in *meDf2* and *him-8* hermaphrodites, suggesting that the DNA damage checkpoint is dispensable for the delay in meiotic progression.

In contrast, mutations in genes required for the formation of double-strand breaks and crossovers did suppress the delay. Null alleles of *spo-11(ok79)* and *msh-5(me23)*, as well as a temperature-sensitive allele of *him-14(it44)* (the *C. elegans* Msh4 ortholog) at the restrictive temperature all restored the wild-type ratio of early to late pachytene nuclei in the presence of unsynapsed X chromosomes. These results indicate that the presence of a molecular species that requires double-strand breaks and the Msh4/5 complex is required to maintain the early, polarized morphology of post-transition zone nuclei. Although HIM-14 and MSH-5 are required for crossover recombination, we currently do not have enough information to conclude that it is specifically the formation of crossovers, rather than another recombination intermediate involving the Msh4/5 complex, that

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**Figure 3.** Progression of RAD-51 Focus Formation and Removal in Wild-Type and Meiotic Mutants

(A) From top to bottom, gonads from wild-type (N2), *him-8*, *msh-5*, and *him-8 msh-5* worms are shown. In wild-type (top), RAD-51 foci appear in the transition zone and disappear in early pachytene. In all mutant conditions, RAD-51 focus formation begins in the transition zone, but persists throughout early pachytene, only disappearing at the very end of the gonad in late pachytene. Scale bar, 50 μm.

(B) Quantitation of RAD-51 focus formation in wild-type and mutant conditions. Gonads were automatically divided into six equally sized regions, and nuclei assigned to each region based on their location. Graphs display box-whisker plots of focus numbers. The x axis indicates bins of equal length along the gonad; the y axis indicates the number of RAD-51 foci observed in a nucleus. The center horizontal line of each box indicates the median value; the box top and bottom indicate the first and third quartile values; the lines above and below the boxes extend to the entire range of measurements. Number of nuclei observed for each case are indicated at upper right.

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removes the block to meiotic progression caused by asynaptic chromosomes. Further characterization of the Msh4/5 complex in the *C. elegans* germline is likely to shed light on this question.

Unlike mutations in *spo-11*, *him-14*, and *msh-5*, a null mutation in *rad-51* (lg8701) did not suppress the delay. This is surprising, as RAD-51 plays an early and essential role in double-strand break processing and is thought to mediate the assembly of

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**Figure 4.** RAD-51 Foci Perdure on Synapsed Autosomes and Unsynapsed X Chromosomes in Extended Early Pachytene

Three different nuclei from the extended early pachytene region of *him-8* gonads are shown, one on each row. Immunofluorescence of SYP-1, HTP-3, and RAD-51 is shown in columns A, B, and C; DAPI counterstaining of chromosomes is shown in column D; the colors of each component in the merged image in column E are indicated by colored circles below (green, SYP-1; red, HTP-3; blue, RAD-51; DAPI staining is not shown in the merged image). Chromosomes containing HTP-3 but not SYP-1 are the unsynapsed X chromosomes (arrowheads). RAD-51 foci are visible on both the X chromosomes and the autosomes. Scale bar, 5 μm.

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**Figure 5.** Ratios of Early Pachytene to Late Pachytene Nuclei in Wild-Type and Various Mutant Backgrounds

Above each genotype analyzed, the mean early:late ratios are plotted; error bars indicate the standard error of the mean. The number of gonads scored (top row) and the total number of nuclei scored (bottom row) are indicated below the genotype.

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recombination complexes, yet these results indicate that HIM-14/MSH4 and MSH-5 may play a RAD-51–independent role in mediating the delay. To test this possibility directly we used RNAi to inhibit rad-51 expression in him-8 and him-8 msh-5 mutants. Like the rad-51 mutation, RNAi-mediated knockdown of rad-51 did not suppress the extended early pachytene zone in him-8 mutants. In agreement with previous studies and our own analysis of rad-51 mutants, reduction of rad-51 function by RNAi did not in itself result in an extension of early pachytene. However, msh-5(me23) did suppress the delay in him-8 mutants even when rad-51 expression was inhibited through RNAi (unpublished data). Thus, we conclude that the HIM-14/MSH-5 complex is required to mediate the delay in early pachytene and that they must interact with the chromosomes in a rad-51–independent manner. This is consistent with previous observations that RNAi-mediated inhibition of rad-51 had a different effect in msh-5 hermaphrodites than in wild-type animals [16].

Globally Altered Recombination Patterns on the Autosomes Result from Asynapsis of the X Chromosome

Our observation that asynapsis of the X chromosomes had a global effect on chromosome organization and on the abundance and timing of RAD-51 foci suggested that the autosomes might be affected by asynapsis of the X chromosomes, although their segregation is not markedly impaired [19]. Prior evidence has indicated that him-8 can alter recombination in specific autosomal intervals. Specifically, crossover frequencies in specific intervals on Chromosomes I and III increase significantly in a him-8(mn253) mutant [20].

To test whether this effect is caused specifically by the him-8 mutation, or results from chromosome asynapsis, we measured recombination in the dpy-1 – textit{unc-36} region on Chromosome III in both him-8 and meDf2. In both him-8 and meDf2, we observed an increase in genetic distance between dpy-1 and unc-36 similar to that observed previously. The distance increased from 17 centimorgans in wild-type (n = 1301) to 28 centimorgans (n = 1494) in him-8, and in meDf2 the distance increased to 29 centimorgans (n = 963) (Figure 6A).

We therefore conclude that the increase in genetic distance was not caused specifically by the him-8 mutation, but by failure of the X chromosomes to synapse.

The increase in recombination between dpy-1 and unc-36 could reflect either a shift in the distribution of crossovers to favor the specific interval at the expense of other regions on the

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**Figure 6. Crossover Alteration in X Chromosome Asynapsis Backgrounds**

Two genotypes (him-8, meDf2) were assayed for recombination by genetic crossing and SNP mapping. (A) The genetic distance between two visible markers on chromosome III was assayed by genetic crosses. Map distance increased from 17 centimorgans in N2, to 29 centimorgans in both him-8 and meDf2. 

(B) Single-nucleotide polymorphism mapping of chromosomes II, III, and V. Five SNP markers were used, resulting in four intervals across the chromosome in which recombination could be assayed (x axis). The relative physical length of each region is shown by the distance between gray bars in the graph background.

(C) Physical and genetic locations of single-nucleotide polymorphisms analyzed. The horizontal bars represent the physical length of the chromosomes (II, III, and V), with polymorphisms indicated above, proportional to their physical distance. Below each bar the polymorphisms are traced to a horizontal dashed line representing the interpolated genetic distance between them, also indicated numerically in centimorgans. Labels for each interval, numbered 1–12, correspond between B and C.

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Chromosome III. An alternative possibility is that autosomal recombination is globally increased in the presence of unsynapsed X chromosomes. To distinguish between these possibilities, and to examine the consequences of X asynapsis on other chromosomes, we measured the global number and distribution of crossovers on autosomes by single nucleotide polymorphism (SNP) mapping. Five primer pairs amplifying SNP-containing regions were chosen from the Washington University Genome Sequencing Center collection [21] to span the majority of each chromosome analyzed (Figure 6B). To generate worms for mapping experiments, meDf2 and him-8(mn253) worms were repeatedly backcrossed to the Hawaiian strain CB14856 (Figure 6C) until all SNP markers converted to the Hawaiian alleles.

The genetic map lengths of Chromosomes II, III, and V increased in the X asynapsis mutants (Table 1), revealing a global increase in genetic exchange. Significantly, double crossovers were observed on Chromosome III in him-8, and on Chromosomes III, IV, and V in meDf2 (Table 1), whereas no instances of double crossovers were detected for wild-type chromosomes, in accordance with many previous observations [9,10,22]. All mapping experiments that indicated double crossovers were verified by repeating the PCR and digestion on the same DNA sample. The presence of asynaptic chromosomes in a nucleus, therefore, impairs normal crossover interference on the other chromosomes.

<table>
<thead>
<tr>
<th>Chromosome</th>
<th>N2</th>
<th>him-8</th>
<th>meDf2</th>
</tr>
</thead>
<tbody>
<tr>
<td>II</td>
<td>0.50(0), n = 43, 26.0%*</td>
<td>0.63(0), n = 19, 8.9%</td>
<td>0.50(0), n = 34, 22.8%</td>
</tr>
<tr>
<td>III</td>
<td>0.45(0), n = 43, 67.3%</td>
<td>0.64(2), n = 45, 2.5%</td>
<td>0.69(2), n = 26, 2.8%</td>
</tr>
<tr>
<td>IV</td>
<td>0.47(0), n = 42, 60.6%</td>
<td>n.d.</td>
<td>0.62(2), n = 21, 7.3%</td>
</tr>
<tr>
<td>V</td>
<td>0.41(0), n = 43, 85.8%</td>
<td>0.52(0), n = 25, 30.1%</td>
<td>0.86(1), n = 25, 2.9%</td>
</tr>
</tbody>
</table>

*Data shown as observed genetic map length, followed by number of detected double crossovers (in parentheses), followed by the number of chromosomes examined for each condition, followed by the percent likelihood of observing a map length equal to or greater than the given value if there were only a single crossover.

Discussion

We have shown that the failure of a single pair of chromosomes to synapse during meiotic prophase has far-reaching effects on the other chromosomes in the nucleus. The C. elegans Msh4/5 complex is involved in imposing a delay in the exit from early pachytene. The delay is associated with increased number and perdurance of RAD-51 foci, and increased autosomal recombination. While this study has used alterations of the X chromosome pairing center [25,13] to cause asynapsis of a single chromosome, earlier studies of other meiotic mutants such as syb-1 and syb-2 in C. elegans have reported an increase in the extent of the transition zone [14,26]. A simple explanation is that meiotic progression is delayed on a per-nucleus basis until key events such as recombination have been completed. The apparent action of this checkpoint between early and late pachytene suggests that this transition is a target for cell cycle regulatory machinery.

Based on observations that the number of RAD-51 foci in wild-type hermaphrodites peaks at early pachytene, and then steeply declines [15], and that RAD-51 foci persist longer when early pachytene is delayed (this study), we propose that there is a critical time period in early pachytene during which chromosomes can initiate and/or complete crossover recombination events, and that this period lapses upon entry into late pachytene. Therefore, nuclei that linger in early pachytene, i.e., nuclei containing unsynapsed chromosomes, have more opportunities for exchange to occur, through either initiation of new recombination events or conversion of existing recombination intermediates into crossovers. Our observation that this delay depends on the Msh4/5 complex strongly hints that the regulation of crossover recombination is linked to meiotic progression in wild-type meiosis. This hypothesis shares key features with a previous model proposing a “window of opportunity” during which double-strand breaks can be made on chromosomes [27]. Our results suggest that there may also be a “window of opportunity” for crossover formation, and that this window corresponds to the cytological appearance of polarized meiotic nuclei.

The mechanism by which the early, polarized pachytene configuration of chromosomes is converted into the late, dispersed configuration is not known. It has been proposed that recombinational events on chromosomes can affect their large-scale structure [28]. Alternatively, synopsis itself may be involved in driving the transition, as previously proposed. Further studies involving direct measurements of the physical properties of meiotic chromosomes may shed light on this question.

We have shown that meiotic progression delay in him-8 and meDf2 mutants requires HIM-14 and MSH-5, but not RAD-51. This implies that HIM-14 and MSH-5 (the C. elegans Msh4/5 complex) can act independently of RAD-51 to delay prophase progression, an unexpected result. It was previously shown that C. elegans MSH-5 can interact with meiotic chromosomes in the absence of RAD-51 [16]; we propose that in the absence

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of RAD-51, double-strand breaks are nevertheless processed into an intermediate that is bound by the Msh4/5 complex.

Previous work showed persistence of RAD-51 foci in the msh-5 background [15]; we have shown here that foci also persist in him-8 and meDf2. Loss of msh-5 causes a return to wild-type meiotic progression, but also compromises RAD-51 focus removal. Since in no case do we observe RAD-51 focus removal from early pachytene nuclei before the transition to late pachytene, we conclude that the transition from early to late pachytene is necessary but not sufficient to enable removal of RAD-51 foci. If this is correct, then RAD-51 foci persist in him-8 nuclei due to the failure to make the transition, and in him-8 msh-5 nuclei due to the lack of MSH-5 protein.

Double crossovers were observed on autosomes in X asynapsis mutants, indicating that crossover interference is compromised. If the observed increase in autosomal recombination were brought about simply by an increase in the number of crossovers on chromosomes, we would expect with perfect sampling to observe double crossovers at a frequency equal to half of the increase in recombination. Our observations showed two out of 95 autosomes in him-8, and five out of 89 in meDf2 with double crossovers, whereas none were detected for the 262 wild-type chromosomes we analyzed. Since previous studies of wild-type C. elegans meiosis have not detected double crossovers on autosomes, and only a very small number on the X chromosome [9,22], the modest level of autosomal double crossovers seen here shows a significant departure from the X chromosome [9,22], the modest level of autosomal double crossovers on autosomes, and only a very small number on the studies of wild-type C. elegans for the 262 wild-type chromosomes we analyzed. Since previous Drosophila melanogaster, as a consequence of suppressed crossovers on one chromosome leads to alterations in meDf2 in him-8, showed two out of 95 autosomes in half of the increase in recombination. Our observations sampling to observe double crossovers at a frequency equal to of crossovers on chromosomes, we would expect with perfect nation were brought about simply by an increase in the number compromised. If the observed increase in autosomal recombi-

The interchromosomal effect, wherein prevention of crossovers on one chromosome leads to alterations in crossing over on other chromosomes, was first described in Drosophila melanogaster, as a consequence of suppressed exchange on specific chromosomes due to structural heterozygosity [29–31]. A similar phenomenon called the intrachromosomal effect, wherein alterations to part of a chromosome increase the probability of crossover exchange on the unaltered part, has been documented in C. elegans [32], yeast [33], and Drosophila [34]. Our results show that global cytological differences in nuclear morphology and recombination protein dynamics correlate with conditions in which the interchromosomal effect is observed, supporting an earlier model [35] that the effect is due to a global delay in progression through meiosis, which lengthens the window of time during which recombination events can take place.

We have shown that the presence of unsynapsed chromosomes leads to both a delay in the normal progression of meiotic nuclear reorganization and to an increase in the amount of crossover recombination on the normally synapsed autosomes. The persistence of polarized chromosome morphology, and of RAD-51 foci on autosomes after they would normally have been cleared, suggests a global response of all the chromosomes triggered by asynapsis of a single chromosome pair. Two known meiotic checkpoints can be triggered by unsynapsed chromosomes in C. elegans: a DNA damage checkpoint [36] and the recently described asynapsis checkpoint [37]. However, the extension of the early pachytene region in him-8 or meDf2 mutants does not depend on hus-1 (Figure 5), which is required for the DNA damage checkpoint; nor does it require the presence of an unsynapsed pairing center, which is required for the asynapsis-specific pathway. Thus, this delay in morphology may reflect a distinct meiotic checkpoint mechanism. The suppression of early pachytene extension by him-14 and msh-5 suggests that the delay is triggered by unresolved crossover intermediates.

![Figure 7. Model of Meiotic Progression in Wild-Type and X Asynapsis Mutants](https://example.com/figure7.png)

Top: in the wild-type situation, after initial pairing completes in the transition zone (TZ), multiple recombination events are initiated (red) on chromosomes as they adopt the early pachytene (EP) configuration. When all chromosomes have received a crossover (blue), recombination intermediates no longer inhibit forward progression in meiotic prophase, and chromosomes enter late pachytene (LP) and lose their polarized configuration. Bottom: in him-8 and meDf2 mutants, recombination events are initiated normally on both synapsed autosomes and the unsynapsed X chromosomes. The failure of recombination intermediates to resolve leads to a delay in the normal progression of meiosis, during which either additional recombination intermediates (arrow) can be initiated, or existing recombination intermediates can persist without being removed. Some proportion of these extra events may also lead to crossovers. Eventually, all recombination intermediates are cleared from both synapsed and unsynapsed chromosomes, and the polarized configuration is lost.

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whose formation is dependent on the Msh4/5 complex. In this model (Figure 7), the presence of unrepaired Msh4/5-containing intermediates triggers a checkpoint mechanism that acts to ensure the obligate crossover by delaying meiotic progression from early to late pachytene until a crossover has formed. This delay results in higher than normal accumulation of recombination intermediates, which may allow supernumerary crossovers to form on the synapsed chromosomes by overwhelming the normal operation of crossover interference. We conclude that this instance of the interchromosomal effect in C. elegans is best explained as an effect of the disruption of the normal timing of meiotic prophase events, and is not due to a direct, mechanistic effect of unynapped chromosomes on autosomes. Since recombination failure has the potential to lead to aneuploidy and developmental defects, especially in humans (reviewed in [38]) it is critical to understand the feedback between molecular events on chromosomes and cell cycle progression in the germline. We have shown that one aspect of this feedback in C. elegans is linked to the process of crossover formation.

Materials and Methods

Genetic mapping. Marker pairs dpy-1(e1) and unc-36(ec251), in strain SP402, were crossed into medI2; mnD666 and him-8(mn253) animals. These markers were then made heterozygous by crossing to medI2; mnD666 or him-8(mn253) males. F1 hermaphrodite progeny of this cross were allowed to self and their progeny scored for R, the fraction of recombinant (Dpy non-Unc and Unc non-Dpy) progeny. Map distances (p) were calculated using the equation \( p = 1 - \sqrt{1 - 2R} \) [59]. Genetic map lengths for SNP mapping experiments were calculated based on the fraction of recombinant chromosomes out of the total observed. Significance was assessed by estimating via simulation (program code available on request) the probability of observing a map length equal to or greater than a given value on each chromosome we analyzed, given the total chromosome length and the locations of SNP markers.

Creating X annysm mutants in the Hawaiian background. Male him-8(mn253) and medI2 worms were crossed to strain CB4856; cross progeny were picked to single plates and allowed to self. Ten hermaphrodites from the F2 generation were picked to single plates and scored for >30% male self-progeny. After five backcrosses, the strains were checked by single-worm PCR for the conversion of N2 to Hawaiian SNPs. The lack of unc2 (N2) bands in all regions under consideration indicated backcrossing had gone to completion.

DNA preparation and PCR. To generate DNA for SNP analysis, single hermaphrodites heterozygous for Hawaiian and N2 markers were test-crossed to males of strain PD4792 homozygous for N2 SNPs. These males contained the insertion mls11, enabling the identification of cross progeny by GFP fluorescence in the pharynx. Extensive testing showed no abnormal chromosome segregation in worms carrying one copy of mls11 (unpublished data). Cross progeny were allowed to self 2–3 generations, at which point DNA was prepared. As a control, SNP analysis was also performed on single N2(CB4856) mosaic male worms. These worms were generated by crossing N2 males to single N2(CB4856) heterozygotes, which were generated by crossing CB4856 males to single N2 hermaphrodites. To prepare DNA, single animals were frozen in 4°C for 1 h, followed by 15 min at 95°C. Each lysate was mixed with 100 µL of a PCR mix (13 mM Tris-HCl [pH 8.3], 65 mM KCl, 3.2 mM MgCl2, 0.26 mM mixed dNTPs, 0.07 units Taq Polymerase), and 20 µL aliquots of this mix were transferred into five separate wells of a 96-well plate. Following this, the appropriate primers were separately added to each well (2.5 µL of a combined 4.4 mM forward primer, 4.4 µM reverse primer mix). The SNP-containing regions were amplified via touchdown PCR (10 cycles of a 67°C to 62°C touchdown: 94°C 15s, 67°C [−0.5°C/cycle] 30s, 72°C 45s; 18 standard cycles: 94°C 15s, 62°C 25s, 72°C 45s). Next, a digest solution (3.0 µL of 10× restriction enzyme buffer [New England Biolabs, Ipswich, Massachusetts, United States], 0.3 µL of 10 mg/mL BSA [if needed by estimate], four units of restriction enzyme, and water to 10 µL) was added directly to the PCR solutions, which were then incubated at the appropriate temperature for at least 2 h.

Imaging. Gonads were dissected from worms in 1× egg buffer (25 mM HEPES [pH 7.4], 118 mM NaCl, 48 mM KCl, 2 mM MgCl2, 2 mM CaCl2). For antibody staining, gonads on slides were fixed in 1% formaldehde for 5–10 min, frozen on dry ice, and transferred to ethanol at −20. Slides were washed 5× for 10 min in 1× PBST (PBS with 0.1% Tween-20), blocked with 10 mg/mL BSA in PBST for 1–2 h, then incubated with an antibody against mouse α-NOP-1 overnight at room temperature. Slides were washed 3× in 1× PBST, then secondary antibody (Cy3-labeled goat α-mouse; Invitrogen, Carlsbad, California, United States) was applied in PBST for 1 h at room temperature. Three more washes in PBST were carried out, with the second wash containing 10 µg/mL 4′,6-diamidino-2-phenylindole (DAPI) to stain DNA. Slides were mounted in glycerol with 1% n-propyl gallate as an antifade agent. Slides were imaged with a DeltaVision microscope system (Applied Precision, Issaquah, Washington, United States) using a 100×, 1.4NA lens (Olympus, Tokyo, Japan). Images were deconvolved with the conservative algorithm from the Prism suite [40]. Nuclei were scored by the appearance of chromosomes and the position of the nucleolus relative to the nuclear envelope, and labeled using the PickPoints program in the Prism software suite. Custom image processing programs, written in C (source code available upon request), were used to composite separate three-dimensional stacks into TIFF mosaic projections.

RNAi inhibition of rad-51 expression. To inhibit rad-51 expression by RNA interference, hermaphrodites were injected with 0.5 µg/ml dsRNA complementary to the rad-51 coding sequence. The template for in vitro transcription of this RNA was the rad-51 clone from the Ahringer Lab RNAi library [41]. Heterozygous him-8(mls11 or him-8 msh-5) animals were injected and these P0s transferred to individual plates. Homozygous him-8 or him-8 exttmsh-5 progeny were selected based on the absence of myn-2:GFP expression in worms whose myn-2:GFP- sisters produced only inviable eggs, indicating effective inhibition of rad-51 expression. These him-8(+), xithim-8 msh-5/+ or +/+ siblings were also dissected, fixed, and stained with DAPI and showed univalent chromosomes and the presence of chromosome fragments at diakinesis, identical to the defects reported in both rad-51(lg8701) and rad-51(RNAi) hermaphrodites.

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Author contributions. PMC and AFC conceived and designed the experiments. PMC, APF performed the experiments. PMC, APF, and AFC analyzed the data. PMC and AFD wrote the paper.

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References


