Synapsis-Defective Mutants Reveal a Correlation Between Chromosome Conformation and the Mode of Double-Strand Break Repair During Caenorhabditis elegans Meiosis

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ABSTRACT

SYP-3 is a new structural component of the synaptonemal complex (SC) required for the regulation of chromosome synopsis. Both chromosome morphogenesis and nuclear organization are altered throughout the germlines of syp-3 mutants. Here, our analysis of syp-3 mutants provides insights into the relationship between chromosome conformation and the repair of meiotic double-strand breaks (DSBs). Although crossover recombination is severely reduced in syp-3 mutants, the production of viable offspring accompanied by the disappearance of RAD-51 foci suggests that DSBs are being repaired in these synapsis-defective mutants. Our studies indicate that once interhomolog recombination is impaired, both intersister recombination and nonhomologous end-joining pathways may contribute to repair during germline meiosis. Moreover, our studies suggest that the conformation of chromosomes may influence the mode of DSB repair employed during meiosis.

During meiosis, chromosomes undergo programmed DNA double-strand breaks (DSBs) generated by the conserved topoisomerase-like protein Spo11 (Bergerat et al. 1997; Keeney et al. 1997). The existence of a conserved enzymatic function set in place to generate DSBs in fungi, plants, and animals underscores the significance of undergoing recombination during meiosis. Specifically, the reciprocal exchange of DNA that occurs during crossover recombination, after formation of programmed meiotic DSBs, is important for genetic diversity given that meiosis results in the formation of haploid gametes required for sexual reproduction. However, crossover recombination is also essential to establish physical attachments between homologous chromosomes during prophase, allowing for their proper subsequent alignment and accurate segregation to opposite sides of the spindle at meiosis I.

The importance of meiotic DSB formation and crossover recombination have led to intense studying of the various components and mechanisms operating in this process (for reviews, see Paques and Haber 1999; Zickler and Kleckner 1999; Keeney 2001). However, far less is known about the effects of chromosome configuration in DSB repair. Therefore, we exploited the existence of two different syp-3 (synapsis defective) alleles with distinct differences both in the nuclear organization and conformation of chromosomes throughout prophase to investigate how changes in chromosome configuration interface with the progression of meiotic recombination.

In an accompanying article (Smolikov et al. 2007, this issue), we report the identification of syp-3, which encodes for a structural component of the central region of the synaptonemal complex (SC) in Caenorhabditis elegans. The SC is a proteinaceous structure that forms between paired and aligned homologous chromosomes during meiosis from yeast to mammals. It is composed of a pair of lateral elements assembled along the axes of a pair of homologous chromosomes, interconnected by transverse filaments that form the central region of this structure (Page and Hawley 2004). Our analysis of syp-3 mutants revealed that SYP-3 restricts the loading of central region components of the SC promoting their assembly only in an appropriate context, bridging the axes of paired homologs. As a result, SYP-3 is required to stabilize homologous pairing interactions and for chiasma formation. However, we also uncovered differences in chromosome organization between a syp-3 null (ok758) and C-terminal truncation (me42) mutants. Specifically, in the syp-3 null mutant, as in syp-1 and syp-2 null mutants (MacQueen et al. 2002; Colaiácovo et al. 2003), SC formation is not observed by transmission electron microscopy (TEM), central region components SYP-1 and SYP-2 do not associate onto chromosomes, and chromosomes fail to disperse upon entrance into pachytene, remaining instead in a clustered organization.

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Reduced crossover recombination in syp-3 mutants

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Recombinant progeny</th>
<th>Total progeny</th>
<th>Map distance (cM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>+/- (ok758 or +); dpy-3 unc-3/++</td>
<td>405 hermaphrodites</td>
<td>1407 hermaphrodites</td>
<td>35</td>
</tr>
<tr>
<td>+/- (me12 or +); dpy-3 unc-3/++</td>
<td>238 hermaphrodites</td>
<td>901 hermaphrodites</td>
<td>32</td>
</tr>
<tr>
<td>ok758/ok758; dpy-3 unc-3/++</td>
<td>1 hermaphrodite</td>
<td>290 hermaphrodites</td>
<td>&lt;0.3</td>
</tr>
<tr>
<td>0 males</td>
<td></td>
<td>137 males</td>
<td></td>
</tr>
<tr>
<td>me42/me42; dpy-3 unc-3/++</td>
<td>2 hermaphrodites</td>
<td>300 hermaphrodites</td>
<td>&lt;0.8</td>
</tr>
<tr>
<td>2 males</td>
<td></td>
<td>165 males</td>
<td></td>
</tr>
</tbody>
</table>

Recombination analysis was performed as in Kelly et al. (2000).

Materials and Methods

Genetics: C. elegans strains were cultured at 20°C under standard conditions as described in Brenner (1974). Bristol N2 worms were utilized as the wild-type background. The following mutations and chromosome rearrangements were used: Riddle et al. (1997); Dernburg et al. 1998; MacQueen et al. 2002; Colaiacovo et al. 2003; Martin et al. 2005; this article:

LG I: syp-3(me42, ok758), kT2[bl-4(e937) qf48] (I;III)
LG III: lg-4(ok716)
LG IV: sbo-11(ok729), nT1 [unc-7(n754) let-7(m435)] (IV;V)
LG V: syp-2(ok307), syp-1(me17)
LG X: dpy-3(e27) unc-3(e151)

DAPI analysis and immunostaining: DAPI staining, immunostaining, and analysis of stained meiotic nuclei were carried out as in Colaiacovo et al. (2003) and as in Rogers et al. (2002). Antibodies were used at the following dilutions: α-RAD-51 (1:100) and α-REC-8 (1:100). Secondary antibodies used were: Cy3 anti-rabbit (Jackson Immunocohemicals) at 1:200 and Alexa 488 anti-mouse (Molecular Probes, Eugene, OR) at 1:400.

RNA interference: Production of double-stranded RNA for rec-8(RNAi) and the RNAi procedure were carried out as described in Colaiacovo et al. (2003). REC-8 depletion was assayed by REC-8 antibody staining of whole mounted gonads and monitoring for oocytes carrying 24 univalents at diakinesis.

Time-course analysis for RAD-51 foci: Quantitation of RAD-51 foci was performed as described in Colaiacovo et al. (2003), except that all seven zones composing the divided germline were quantitated. The average number of nuclei scored per zone (n) for wild type, syp-3(ok758), syp-3(me42), syp-3(me42); syp-2, syp-3(ok758); rec-8(RNAi), syp-3(me42); rec-8(RNAi); lig-4(ok716), syp-3(me42); lig-4(ok716), syp-3(me42); rec-8(RNAi); lig-4(ok716) were: zone 1 (n = 123), zone 2 (n = 128), zone 3 (n = 115), zone 4 (n = 135), zone 5 (n = 109), zone 6 (n = 90), and zone 7 (n = 79).

Results

SYP-3 is required for meiotic crossover recombination: Cytological analysis of germline chromosome morphology in both syp-3 mutants revealed that in addition to altered chromosome morphogenesis and nuclear organization earlier in prophase, both carried 12 univalents instead of 6 bivalents in diakinesis oocytes (Smolikov et al. 2007 [accompanying article]). Therefore, we examined whether this lack of chiasmata results from an inability to form crossovers. We measured crossover frequencies for a genetic interval spanning 80% of the X chromosome and observed a reduction to <0.9 and 2.5% of the wild-type level in ok758 and me42, respectively (Table 1).

Formation and repair of DSBs in syp-3 mutants: The lack of chiasmata and the severely reduced levels of crossover recombination observed in the syp-3 mutants could reflect either an inability to form meiotic DSBs or an inability to repair DSBs as crossovers. To distinguish between these possibilities, we utilized an antibody against RAD-51 (a protein involved in DNA strand exchange during DSB repair; Sung 1994) and examined the levels of RAD-51 foci in nuclei throughout meiotic prophase (Figures 1 and 2). We observed that levels of RAD-51 foci rose upon entrance into transition zone (zones 2 and 3)
in both syp-3 mutants with wild-type kinetics, indicating that SYP-3 is not required for the initiation of meiotic recombination.

Analysis of nuclei in early and mid-pachytene (zones 4 and 5) revealed that levels of RAD-51 foci were higher at these stages in syp-3(ok758) than in wild type and remained elevated throughout late pachytene. While only 13% of nuclei still bear more than one RAD-51 focus by late pachytene (zone 7) in wild type, 61% carry more than one focus in syp-3(ok758). Furthermore, RAD-51 foci persisted in nuclei upon entrance into diplotene (one to two foci per nucleus), a stage at which RAD-51 foci are no longer observed in wild type. This result suggests that SYP-3 is required for the proper progression of recombination. Meanwhile, in syp-3(me42), both the levels of RAD-51 foci throughout prophase and the kinetics of foci formation and disappearance closely paralleled those observed in wild type. Moreover, RAD-51 foci were not present in diplotene nuclei in syp-3(me42). This suggests that DSBs are being repaired with near-wild-type efficiency in syp-3(me42), although repair is not resulting in crossovers.

We also examined the levels of germ cell apoptosis observed in syp-3 mutants (Table 2). Previous studies have shown that late pachytene nuclei undergo apoptosis triggered by unrepaird meiotic DSBs that are sensed as DNA damage (Gartner et al. 2000). Utilizing the levels of germ cell corpses observed in late pachytene as
a readout, we observed a 4.5-fold increase in syp-3(ok758) compared to either age-matched wild-type worms or spo-11 worms where the initiation of meiotic DSB formation is abrogated. This increase is similar to that observed in syp-1 and syp-2 mutants, where levels of RAD-51 foci also remain elevated throughout late pachytene (MacQueen et al. 2002; Colaiácovo et al. 2003). Meanwhile, levels of germ cell apoptosis were not as significantly increased in syp-3(me42) compared with either wild type or spo-11. This suggests that in syp-3(ok758), DSBs are either failing to be repaired or are engaging in aberrant intermediates leading to germ cell apoptosis via the activation of a pachytene DNA damage checkpoint. In contrast, DSB repair is more effective in syp-3(me42), resulting in lower levels of germ cell corpses.

To further examine the correlation between DSB repair, chromosome configuration, and structural constraints, we assessed progression of meiotic recombination via immunostaining of RAD-51 in syp-3(me42); syp-2 double mutants (Figures 1 and 2). In contrast to syp-3(me42) single mutants, in syp-3(me42); syp-2 double mutants, chromosomes remain in a clustered organization instead of redispersing prematurely and SYP proteins no longer associate with chromosomes, similar to syp-1, syp-2, and syp-3 null mutants (Figure 2; MacQueen et al. 2002; Colaiácovo et al. 2003; Smolíkov et al. 2007, accompanying article). Whereas in syp-3(me42), disappearance of RAD-51 foci occurs with near wild-type kinetics, in syp-3(me42); syp-2, DSB repair is no longer completed by late pachytene (zone 7), with RAD-51 foci persisting through early diplotene (one to three foci per nucleus). Moreover, levels of RAD-51 foci per nucleus are elevated and resemble those observed for either syp-2 or syp-3(ok758). These results suggest that either progression into a dispersed configuration and/or SYP-1 and SYP-2 loading, even if along unsynapsed chromosomes, can promote DSB repair throughout early and mid-prophase.

**TABLE 2**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Mean no. of germ cell corpses ± SE</th>
</tr>
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<tbody>
<tr>
<td>Wild-type</td>
<td>1.81 ± 0.12</td>
</tr>
<tr>
<td>spo-11(ok79)</td>
<td>1.6 ± 0.22</td>
</tr>
<tr>
<td>syp-1(me17)</td>
<td>8.94 ± 0.67</td>
</tr>
<tr>
<td>syp-3(ok758)</td>
<td>8.25 ± 0.43</td>
</tr>
<tr>
<td>syp-3(me42)</td>
<td>2.54 ± 0.21</td>
</tr>
</tbody>
</table>

Germ cell corpses were scored in adult hermaphrodites 20 hr post-L4 as in Kelly et al. (2000). Between 32 and 134 gonad arms were scored for each genotype. Statistical comparisons between genotypes were conducted using the two-tailed Mann-Whitney test. syp-3(ok758) differed significantly from wild type ($P = <0.0001$) and from spo-11 ($P = <0.0001$) but did not differ significantly from syp-1(me17) ($P = 0.6254$). Meanwhile, syp-3(me42) differed significantly from both syp-1(me17) and syp-3(ok758) ($P = <0.0001$, $P = 0.0001$); however, it also differed, albeit to a much lesser extent, from either spo-11 ($P = 0.0061$) or wild-type ($P = 0.0002$).
Evidence that both intersister recombination and NHEJ contribute to meiotic DSB repair when interhomolog recombination is impaired: The eventual disappearance of RAD-51 foci in \textit{syp-3} mutants coupled with the production of viable progeny (~4%; Smolikov \textit{et al.} 2007, accompanying article) suggests that DSB repair occurs in \textit{syp-3} mutants despite the absence of stable pairing between homologous chromosomes. Thus, we investigated the contribution of sister chromatid-directed repair by examining the consequences of depleting the meiotic cohesin REC-8 in \textit{syp-3} mutants.

Depletion of REC-8 via RNAi in an otherwise wild-type background leads to the formation of 24 chromatid-sized DAPI-stained bodies and occasional fragments in diakinesis oocytes due to the inability of maintaining the attachments between the 12 pairs of sister chromatids (Figure 3; Pasierebek \textit{et al.} 2001). REC-8 depletion in either \textit{syp-3} mutant did not alter the temporal progression of the nuclear reorganization characteristic of either single \textit{syp-3} mutant. However, it resulted in the formation of 24 DAPI-stained bodies in addition to severe chromosome fragmentation. Some chromosome clumping was also observed in \textit{syp-3(ok758); rec-8(RNAi)}). These results suggest that repair is occurring between sister chromatids rather than between homologous chromosomes in the \textit{syp-3} single mutants. Moreover, quantitative analysis of RAD-51 foci in \textit{syp-3(me42); rec-8(RNAi)} reveals significantly lower levels of RAD-51 foci during mid- and late pachytene in this background compared to \textit{syp-3(ok758); rec-8(RNAi)} ($P < 0.0001$ for zone 6 by the two-tailed Mann-Whitney test, 95% C.I.; Figure 1 and supplemental Figure 1 at http://www.genetics.org/supplemental/). Specifically, 83–94% of all nuclei in mid- through late pachytene (zones 5–7) carry seven or more RAD-51 foci in \textit{syp-3(ok758); rec-8(RNAi)}). This is similar to the 90–97% observed in \textit{rec-8(RNAi)} (data not shown) but clearly different from the only 19–60% observed in \textit{syp-3(me42); rec-8(RNAi)} throughout the same region. This last observation suggests that in addition to sister chromatid-mediated repair, chromosomes might be able to engage in additional modes of repair when they are dispersed as opposed to restrained in a clustered organization during early or mid-prophase.

To examine the contribution of NHEJ to repair in the \textit{syp-3(me42)} mutants, we observed the levels of RAD-51 foci throughout meiotic prophase in a \textit{syp-3(me42); lig-4} double mutant (ligase IV is involved in religating DNA ends during NHEJ; Figures 1 and 2). The levels of RAD-51 foci in \textit{syp-3(me42); lig-4; rec-8(RNAi)} increased significantly in late pachytene compared to \textit{syp-3(me42); rec-8(RNAi)} mutants ($P < 0.0001$ for all pairwise combinations in zone 6, two-tailed Mann-Whitney test, 95% C.I.; Figure 1 and supplemental Figure 1 at http://www.genetics.org/supplemental/). Moreover, RAD-51 foci (one to three per nucleus) were still apparent in mid-diakinesis oocytes in \textit{syp-3(me42); lig-4; rec-8(RNAi)} background, levels of RAD-51 foci increased significantly in late pachytene compared to \textit{syp-3(me42); rec-8(RNAi)} mutants, while they are no longer present at that stage in any of the other aforementioned backgrounds. Altogether, these results indicate that NHEJ can also contribute to meiotic DSB repair when homologous chromosomes are unavailable.

DISCUSSION

Relationship between chromosome organization and mode of DSB repair: Although the stable association between homologs is impaired in both \textit{syp-3} mutants, these mutants differ in their kinetics of disappearance of RAD-51 foci, which may reflect differences in the timing and/or mode of repair. In the case of \textit{syp-3(ok758)}, and similar to \textit{syp-1} and \textit{syp-2} null mutants, high levels of both RAD-51 foci and apoptosis are observed in late prophase, correlating with a persistent clustered organization of chromosomes. In contrast, in \textit{syp-3(me42)}, a timely disappearance of RAD-51 foci and near wild-type levels of apoptosis correlates with chromosomes dispersing upon exit from transition zone. Moreover, in \textit{syp-3(me42); rec-2} double mutants where chromosomes remain in an extended polarized nuclear organization, elevated levels of RAD-51 are observed in late prophase [once again similar to \textit{syp-2} and \textit{syp-3(ok758)} null mutants], supporting the idea that DSB repair correlates...
with chromosome dispersal and loading of SC components. Together, these data raise the possibility that chromosome configuration (i.e., clustered vs. dispersed) may affect the capacity for and/or timing of DSB repair. Under this scenario, the clustered organization would inhibit repair, whereas the dispersed organization would permit progression of repair.

Whereas the idea that progression of DSB repair is influenced by the clustered vs. dispersed state of the chromosomes accounts nicely for some aspects of our observations, it does not, however, readily explain all features of progression of DSB repair in the syp-3 mutants. During wild-type meiosis, a barrier to sister chromatid repair is proposed to operate so that repair occurs between nonsister chromatids from homologous chromosomes (Hollingsworth et al. 1995; Schwacha and Kleckner 1997; Wan et al. 2004; Webber et al. 2004; Niu et al. 2005). However, in the syp-3 mutants, given that DSBs are formed but homologs are not stably paired, interhomolog repair is also impaired. In syp-3(ok758) mutants, repair may occur in a delayed fashion when chromosomes finally redisperse in late pachytene and the relationship between sister chromatids is proposed to become less stringent (ColatlacoVo et al. 2003). In syp-3(me42) mutants, the timing of disappearance of RAD-51 foci suggests that the barrier preventing sister chromatid recombination may be lifted prematurely. It is possible that in addition to promoting the dispersal of chromosomes, the inappropriate loading of SC central region proteins in the syp-3(me42) mutant also affects the relationship between sister chromatids in a manner that permits intersister recombination during early-mid prophase when interhomolog recombination is favored during wild-type meiosis. In the accompanying article (Smolikov et al. 2007), we demonstrated that some of the SYP-1 protein appears localized between sister chromatids in the syp-3(me42) mutant; this localization may allow it to function in promoting intersister recombination events.

The syp-3(me42) mutant has also enabled us to investigate the contribution of NHEJ to DSB repair in the germline. Although NHEJ is not a primary mode of repair in the germline (Martin et al. 2005; Clejan et al. 2006), NHEJ has recently been observed to participate in meiotic DSB repair in brc-2 mutants where homologous recombination is impaired due to the misregulation of RAD-51 (Martin et al. 2005). In this work, we demonstrate that NHEJ can participate in germline DSB repair when both homolog-mediated and sister chromatid-mediated recombination are impaired due to synopsis defects and defective sister chromatid cohesion. This suggests that NHEJ may be simply outcompeted by interhomolog or intersister-mediated homologous recombination. Alternatively, NHEJ may be under regulatory constraints that are released concomitant with changes in chromosome configuration and structural constraints.

**DSB repair does not drive early changes in chromosome configuration during C. elegans meiosis:** Our studies are consistent with a model by which changes in chromosome configuration affect DSB repair progression. However, we also addressed whether the reverse also applied, which is whether DSB repair drives early changes in chromosome configuration during meiosis. While this is the case for budding yeast and Sordaria (Trelles-Sticken et al. 1999; Storlazzi et al. 2003), it does not seem to be the case in C. elegans (MacQueen et al. 2002; ColatlacoVo et al. 2003; this article). First, the formation of DSBs per se is not required for chromosomes to acquire a polarized nuclear organization upon entrance into meiosis, for SC assembly between

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**Figure 4.—Uncoupling the exit from a clustered organization from meiotic DSB formation.** DAPI-stained mid-pachytene nuclei (projections are halfway through 3D data stacks). syp-3(me42); spo-11 and syp-3(ok758); spo-11 are indistinguishable from the corresponding syp-3 single mutants. Bars, 2 μm. Histograms depict the quantification of RAD-51 foci in germlines of the indicated genotypes (as described in Figure 1). The average number of nuclei scored per zone (n) for spo-11, syp-3(me42); spo-11, syp-3(ok758); spo-11 were: zone 1 (n = 138), zone 2 (n = 158), zone 3 (n = 145), zone 4 (n = 138), zone 5 (n = 127), zone 6 (n = 102), and zone 7 (n = 90).
homologs or for redispersal of chromosomes upon progression into pachytene, as determined through the analysis of spo-11 mutants (Dernburg et al. 1998). Second, in syp-1, syp-2, and syp-3(ok758) null mutants, chromosomes persist in a polarized nuclear organization that is not altered when meiotic DSB formation is abrogated in the absence of SPO-11 (Figure 4; MacQueen et al. 2002; Colaiácovo et al. 2005). Third, in the case of syp-3(me42), the premature chromosome redispersal characteristic of this mutant is also not altered in a syp-3(me42); spo-11 mutant (Figure 4). Fourth, while the levels of RAD-51 are increased in syp-3(me42); rec-8(RNAi) double mutants compared to syp-3(me42), the altered progression of DSB repair does not lead to a change in chromosome organization within the nuclei and chromosomes still redisperse prematurely as in syp-3(me42). Therefore, initiation and/or progression of DSB repair are not directly driving early changes in chromosome configuration during meiosis. This is, however, distinct from a later wave of chromosome organization that is observed to occur between the early and late pachytene stages and that has been proposed to be under recombination-dependent regulation in C. elegans (Carlton et al. 2006).

In conclusion, SYP-3 is a novel SC component that plays a central role in the regulated formation of the SC central region between homologous chromosomes. Comparisons between different syp-3 mutants are consistent with a model in which changes in chromosome configuration may modulate the mode and timing of DSB repair employed during prophase progression. Therefore, our findings support the view that a complex system of checks and balances acts to coordinate these processes to achieve a successful outcome of meiosis.

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LITERATURE CITED


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