Roles for *Caenorhabditis elegans rad-51* in Meiosis and in Resistance to Ionizing Radiation During Development

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ABSTRACT

We have investigated the role of *Caenorhabditis elegans* RAD-51 during meiotic prophase and embryogenesis, making use of the silencing effect of RNA interference (RNAi). *rad-51* RNAi leads to severe defects in chromosome morphology in diakinesis oocytes. We have explored the effect of *rad-51* RNAi in mutants lacking fundamental components of the recombination machinery. If double-strand breaks are prevented by *spo-11* mutation, *rad-51* RNAi does not affect chromosome appearance. This is consistent with a role for *rad-51* downstream of the initiation of recombination. In the absence of MRE-11, as in the absence of SPO-11, *rad-51* depletion has no effect on the chromosomes, which appear intact, thus indicating a role for MRE-11 in DSB induction. Intriguingly, *rad-51* silencing in oocytes that lack MSH-5 leads to chromosome fragmentation, a novel trait that is distinct from that seen in *msh-5* mutants and in *rad-51* RNAi oocytes, suggesting new potential roles for the *msh-5* gene. Silencing of the *rad-51* gene also causes a reduction in fecundity, which is suppressed by mutation in the DNA damage checkpoint gene *rad-5*. But not in the cell death effector gene *ced-3*. Finally, *rad-51* depletion is also seen to affect the *soma*, resulting in hypersensitivity to ionizing radiation in late embryogenesis.

The *RAD51* gene, homologous to the *Escherichia coli* RecA gene, was originally isolated in *Saccharomyces cerevisiae* (Aroussekra et al. 1992; Basile et al. 1992; Shinohara et al. 1992). Mutations in this gene confer on yeast cells an enhanced sensitivity to genotoxic agents, a reduction in mitotic recombination, and an impaired meiosis. Homologs and paralogs of the *RAD51* gene have been found in all eukaryotes investigated so far. Among these, *DMC1* encodes for a protein highly similar in sequence and function to Rad51, but is specifically required only in meiotic recombination (Bishop et al. 1992).

Much of our understanding of the enzymology and genetic control of meiotic recombination comes from studies in *S. cerevisiae* (reviewed in Paques and Haber 1999). Meiotic recombination includes two coupled processes: the formation and processing of double-strand breaks (DSBs). The *SPO11* gene product, which is responsible for initiating recombination, induces enzymatic DNA cleavage resulting in DSBs (Caö et al. 1990; Bergerat et al. 1997; Keeney et al. 1997). *Mre11* is required for the induction and processing of DSBs (Johzuka and Ogawa 1995; Ogawa et al. 1995; Usui et al. 1998). Rad51 catalyzes the strand-invasion and strand-exchange reaction between homologous DNA molecules (Sung 1994). A number of genes are involved in the control of meiotic crossing over, including *MSH4* and *MSH5*, which are required for reciprocal exchange between, but not within, homologs (Ross-Macdonald and Rieder 1994; Hollingsworth et al. 1995).

Orthologs of *SPO11*, *MRE11*, *MSH4*, *MSH5*, and *RAD51* have been identified in *Caenorhabditis elegans* (Dernburg et al. 1998; Rinaldo et al. 1998; Takanami et al. 1998; Zalevsky et al. 1999; Kelty et al. 2000; Chin and Villeneuve 2001). Surprisingly, the meiosis-specific *DMC1* gene present in fungi, plants, and mammals is absent in *C. elegans* as well as in *Drosophila melanogaster*. Mutants in *spo-11, mre-11*, *msi-4*, and *msh-5* are all defective in chiasma formation. It has been shown that *spo-11* is required for the induction of meiotic recombination and that artificially induced breaks bypass the requirement for *spo-11* (Dernburg et al. 1998). Conversely, γ-irradiation of *mre-11* null mutants does not lead to induction of chiasmata: after treatment, lethality is increased and the chromosomes in nuclei of oocytes at the diakinesis stage appear abnormal. *mre-11* in *C. elegans* is therefore required for repair of radiation-induced DSBs during meiotic prophase (Chin and Villeneuve 2001). A further role of *MRE-11* in DSB induction is hypothesized on the basis of the intact appearance of univalents in unirradiated *mre-11* mutant oocytes, but this role has not yet been clearly proven. Treatment of *msh-5* hermaphrodites with γ-rays does not induce chiasma formation, and therefore radiation-induced breaks do not bypass the requirement for *msh-5* in chiasma formation. In contrast to what is observed in *mre-11* mutants, after the γ-irradiation of *msh-5* mutants,
the chromosomes at diakinesis appear morphologically intact and lethality is not significantly increased; therefore, DNA repair is not affected by the \textit{msh-5} mutation (\textit{Kelly et al.} 2000).

Although the recombination machinery is highly conserved among eukaryotes, metazoa have developed a germ cell line distinct from the somatic cell line, requiring a different level of gene regulation compared to unicellular eukaryotes. In \textit{C. elegans} physiological and damage-induced apoptosis has been described in the female germ line of hermaphrodites (Gumienny \textit{et al.} 1999; Gartner \textit{et al.} 2000). Unlike somatic cells, germ cells respond to genotoxic stress with programmed cell death and by inducing a transient cell cycle arrest, although these two responses are spatially separate. Mitotic germ cells respond with cell cycle arrest, whereas pachytene-stage meiotic germ cells undergo apoptosis. Interestingly, silencing of the \textit{rad-51} gene mediated by RNA interference (RNAi) also induces cell cycle apoptosis in the same checkpoint-mediated fashion as ionizing radiation (IR). In both cases, cell death uses the same basic execution machinery (such as the \textit{ced-3}, \textit{ced-4}, and \textit{ced-9} genes) and requires other genes such as \textit{mrt-2} (Ahmed and Hodgkin 2000), \textit{rad-5}, and \textit{him-7} that are likely to be involved in a meiotic DNA damage checkpoint. \textit{spo-11} mutation partially suppresses the increase in apoptosis caused by \textit{rad-51} RNAi, suggesting that oocyte precursor apoptosis may be triggered by the accumulation of recombination intermediates (Gartner \textit{et al.} 2000).

RNA interference of the \textit{rad-51} gene in \textit{C. elegans} leads to a number of visible phenotypes, such as (i) high levels of embryonic lethality (Takanami \textit{et al.} 1998), (ii) increase in the frequency of males (Gartner \textit{et al.} 2000), (iii) reduced fertility (Takanami \textit{et al.} 1998), and (iv) hypersensitivity to \gamma- radiation in the germ line (Takanami \textit{et al.} 2000) and \textit{in soma} (this article).

In this article, the effects of \textit{rad-51} RNAi in several genetic backgrounds are analyzed in detail to dissect the pathways in which \textit{rad-51} is involved in \textit{C. elegans} in the germ line and to establish the role of this gene in the somatic cells of this nematode.

\section*{MATERIALS AND METHODS}

\textbf{Strains and maintenance:} All the strains were maintained and cultured according to Sulston and Hodgkin (1988). Unless otherwise specified, all the experiments were performed at 18\textdegree C.

The following \textit{C. elegans} strains used in this work were kindly provided by the \textit{Caenorhabditis} Genetics Center:

\textbf{N2:} wild-type strain (Bristol variety; Brenner 1974)
\textbf{NL917:} \textit{mut-7(pk204)III} (Ketting \textit{et al.} 1999)
\textbf{AV106:} \textit{spo-11(ok779)IV/\textit{nt1[unc-32(n754) let-3]}(IV;V)} (Dernburg \textit{et al.} 1998)
\textbf{MT1522:} \textit{ced-3(n771)IV} (Ellis and Horvitz 1986; Xue \textit{et al.} 1996)
\textbf{CB1392:} \textit{nuc-1(e1392)X} (Hevelone and Hartman 1988)

\textbf{CB1256:} \textit{him-3(e1256)IV} (Hodgkin \textit{et al.} 1979; Zetka \textit{et al.} 1999)

The strain NL936: \textit{mut-7(pk204)III, unc-32} was a gift from Ronald Plasterk. The strain SP506: \textit{rad-5(mn159)III} (Hartman and Herman 1982) was a gift from Anton Gartner. The strain AV112: \textit{mre-11(ok179)/IV/\textit{nt1[unc-32(n754) let-3]}(IV;V)} (Chin and Villeneuve 2001) and the strain \textit{mshe-3(m23)IV/\textit{nt1[unc-32(n754) let-3]}(IV;V)} (Kelly \textit{et al.} 2000) were gifts from Anne Villeneuve.

\textbf{RNA interference:} Young adults were injected with double-strand RNA (dsRNA) [corresponding to nucleotides (nt) 513–945 of the \textit{rad-51} cDNA, GenBank accession no. AP01201] at a concentration of 500 \mu g/ml in proximity of the gonad (Firke \textit{et al.} 1998). Injected worms (\textit{P}0) were individually cloned, left to lay eggs overnight and then transferred every 12 hr onto fresh seeded plates, and the progeny were screened until only unfertilized oocytes were laid. Eggs laid during the first night after injection were discarded because they were unlikely to be affected. \textit{F}1 hermaphrodites were individually cloned, transferred every 24 hr onto fresh seeded plates, and the progeny were screened. To rule out any mechanical or chemical stress due to the RNA injection procedure as a possible cause of the phenotype observed in \textit{P}0, the same sample of RNA was also injected into \textit{mut-7} worms, which are known to be RNA-interference resistant (Ketting \textit{et al.} 1999). The brood size, the percentage of unhatched eggs, and the percentage of male progeny produced by the \textit{mut-7} \textit{P}0 were comparable to those of untreated \textit{mut-7} hermaphrodites (Tables 1 and 2).

Injection of \textit{rad-5} hermaphrodites (which are temperature sensitive) was performed taking further precautions: worms were placed on a cool (4\textdegree) clean plate and injected one at a time. After performing the injection, a cold recovery buffer was immediately added.

For each strain used, between 10 and 40 hermaphrodites were injected and an equal number of matching age hermaphrodites were individually cloned to provide a control group and their progeny were screened as described.

In analyzing the brood size, the total number of (hatching and unhatching) eggs laid for each given strain was considered. The mean values and standard deviations of control and \textit{rad-51} RNAi worms are reported in Table 3.

\textbf{Genetic cross:} Eighteen hours after dsRNA injection, five \textit{mut-7 unc-32} \textit{P}0 hermaphrodites were crossed with three or four wild-type (N2) males, the parents were passed onto fresh seeded plates every 12 hr and the \textit{F}1 and \textit{F}2 progenies were screened and cloned as described above. Five uninjected \textit{mut-7 unc-32} \textit{P}0, hermaphrodites were similarly crossed with wild-type males, and the \textit{F}1 and \textit{F}2 progenies were screened in the same way. We also injected \textit{mut-7 unc-32} \textit{P}0 hermaphrodites with a control dsRNA corresponding to an open reading frame (ORF) in which RNAi leads to embryonic lethality due to zygotic effect before the ventral enclosure stage (Maeda \textit{et al.} 2001; C. Rinaldo, unpublished results). While the self-fertilized \textit{F}1 progeny of such hermaphrodites show no embryonic lethality, when the injected \textit{mut-7 unc-32} \textit{P}0 hermaphrodites are crossed with the wild-type males, the heterozygous \textit{F}1 progeny turn out to be 100\% unviable.

\textbf{Imaging of meiotic chromosomes at diakinesis:} Adult hermaphrodites 48 hr after dsRNA injection (\textit{P}0) and matching age syngenic control worms were microdissected in cold PBS (phosphate-buffered saline), 0.25 m\textit{l} levamisole. Worms were sliced with 25-gauge needles near the head and tail, and the gonads were released. Samples were then fixed with cold acetone; incubated for 25 min in PBS, 1 \mu g/ml 4',6-diamidino-2-phenylindole (DAPI); washed in PBS; mounted on a dried 2\% agarose pad; and observed under a Zeiss Axiosvert 10 fluo-
TABLE 1

Effects of rad-51 RNAi on embryo viability and brood size

<table>
<thead>
<tr>
<th>rad-51 RNAi</th>
<th>N2</th>
<th>Injected mut-7</th>
<th>mut-7</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P0</td>
<td>F1</td>
<td>Adults</td>
</tr>
<tr>
<td>N</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eggs laid</td>
<td>6</td>
<td>35</td>
<td>4</td>
</tr>
<tr>
<td>Average no. of eggs</td>
<td>163</td>
<td>138</td>
<td>231</td>
</tr>
<tr>
<td>Dead embryos</td>
<td>391</td>
<td>4409</td>
<td>2</td>
</tr>
<tr>
<td>Dead embryos frequency</td>
<td>0.40</td>
<td>0.91</td>
<td>0.002</td>
</tr>
</tbody>
</table>

Results obtained when RNAi is performed in the wild-type strain N2 were compared with those obtained with an identical procedure in the RNAi-resistant mutant mut-7 (see materials and methods for details). N, the number of individuals whose progeny were scored.

RESULTS

Embryonic lethality mediated by RNAi of rad-51 is due to maternal effect: The embryonic lethality arising in the late offspring of the rad-51 dsRNA-injected hermaphrodites (P0, Table 1, Figure 1) and reinforced in the surviving F1 progeny (F2) is consistent with a maternal effect. However, a pronounced maternal effect might obscure a milder zygotic effect. To discriminate between maternal and zygotic effects, rad-51 RNAi was performed in the double mutant mut-7 unc-32 and an appropriate genetic cross was set up (Figure 2). The mut-7 is a recessive mutation that confers resistance to RNAi (Grishok et al. 2000); unc-32 is a high-penetrance behavioral marker linked to mut-7. Homozygous mut-7 unc-32 hermaphrodites injected with rad-51 dsRNA were crossed with wild-type males. Parental mut-7 unc-32 hermaphrodites are not affected by RNAi, but F1 heterozygotes derived from such a cross will, however, inherit interfering agents and be sensitive to rad-51 RNAi. Embryonic lethality is expected to arise only if expression of the rad-51 gene is required during F1 embryonic development. A low level of embryonic lethality (4%) is observed throughout the F1 offspring, comparable to that observed in the untreated control mut-7 unc-32 hermaphrodites (Figure 2).

In the following generation, F1 heterozygous hermaphrodites (in which RNAi is active) are allowed to self-fertilize and the progeny are screened. About one-quarter of the offspring (F2) will be homozygous for mut-7 and insensitive to any residual effect of dsRNA injection. Embryonic lethality in this subpopulation can reflect only a parental defect. A 93% embryonic lethality is observed in the F2. The ratio between Unc and non-Unc worms among the survivors is the same as in the control experiment, indicating that the fate of the zy-
mut-7 unc-32 homozygous hermaphrodites were injected with rad-51 dsRNA and crossed with wild-type males. mut-7 unc-32 homozygous hermaphrodite parents are not affected by interference (RAD-51+/H11001). F1 heterozygotes derived from the cross are sensitive to rad-51 RNAi (RAD-51−). In the control cross mut-7 unc-32 homozygous hermaphrodites were not injected with dsRNA and therefore mut-7 unc-32/H11001/H11001 F1 heterozygotes express RAD-51 (RAD-51+). The level of F1 embryonic lethals in the experiment and in the control (4 and 4.5% respectively) is comparable. mut-7 unc-32/+ F1 heterozygous hermaphrodites carrying interfering agents (RAD-51−) were allowed to self-fertilize and the progeny were screened. We expect one-quarter of the offspring (F2) to be homozygous for mut-7 unc-32 (RAD-51−). We observe 93% embryonic lethality in the F2 population. One-quarter of the surviving F2 progeny show an Unc phenotype.

Embryonic lethality is a common feature among meiotic mutants (Dernburg et al. 1998; Zalevsky et al. 1999; Žetka et al. 1999; Kelly et al. 2000; Chin and Villeneuve 2001) and mutations in these genes also result in an increase in the percentage of male progeny. In C. elegans, a high incidence of X0 males (known as between rad-51 and three well-characterized meiotic genes: spo-11, involved in DSB induction (Dernburg et al. 1998); mre-11, involved in DSB processing (Chin and Villeneuve 2001); and msh-5, promoting crossovers (Kelly et al. 2000). Mutants in all three meiotic genes display diakinesis oocytes containing 12 individual DAPI-stained bodies (corresponding to six bivalents) are detectable in oocytes of wild-type worms (Figure 3A, left), highly unshaped and poorly condensed chromosomes, often grouped in bunches, are observed in all the oocytes of rad-51 RNAi worms (Takanami et al. 1998, 2000; and Figure 3A, right). To dissect the pathways in which rad-51 is involved in C. elegans meiosis, we explored the epistatic relationships between rad-51 and three well-characterized meiotic genes: spo-11, involved in DSB induction (Dernburg et al. 1998); mre-11, involved in DSB processing (Chin and Villeneuve 2001); and msh-5, promoting crossovers (Kelly et al. 2000). Mutants in all three meiotic genes display diakinesis oocytes containing 12 individual DAPI-stained bodies corresponding to achiasmate univalent chromosomes (Dernburg et al. 1998; Chin and Villeneuve 2001; Kelly et al. 2000; and Figure 3, B–D, left).

When rad-51 interference is performed in a spo-11 background, we observe 12 DAPI-stained bodies corresponding to properly condensed univalents (Figure 3B, right) such as those displayed by the spo-11 oocytes in the presence of a functional rad-51 (Dernburg et al. 1998; Figure 3B, left). In the above experiment we do not observe any defective chromosome structures like...
TABLE 2

Effects of rad-51 RNAi on incidence of males

<table>
<thead>
<tr>
<th>rad-51 RNAi</th>
<th>N2: control</th>
<th>Injected mut-7</th>
<th>mut-7: control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F₁</td>
<td>F₂</td>
<td>F₁</td>
</tr>
<tr>
<td></td>
<td>590</td>
<td>416</td>
<td>1886</td>
</tr>
<tr>
<td>Males</td>
<td>3</td>
<td>27</td>
<td>1</td>
</tr>
<tr>
<td>Males frequency</td>
<td>0.005</td>
<td>0.065</td>
<td>0.0005</td>
</tr>
</tbody>
</table>

Results obtained when RNAi is performed in the wild-type strain N2 were compared with those obtained with an identical procedure in the RNAi-resistant mutant mut-7 (see MATERIALS AND METHODS for details). N, the number of viable progeny scored.

We observed that rad-5 is the only genetic background (besides mut-7) in which the rad-51 RNAi does not have a significant effect on brood size. Reduced fecundity is the only rad-51 RNAi-induced phenotype suppressed by the rad-5 mutation: rad-51 RNAi in the rad-5 mutant strain leads to abnormal chromosome appearance in diakinesis oocytes (Figure 3E, right) and embryonic lethality.

A significant, although modest, reduction in brood size is caused by rad-51 interference in a ced-3 background (ced-3 being a cell death effector gene; Xue et al. 1996), suggesting a very partial involvement, if any, of apoptosis in reducing fertility in rad-51-interfered worms. rad-51 RNAi has a modest effect on the brood size also in a spo-11 genetic background.

We also analyzed the effects of rad-51 RNAi on fertility in two control strains respectively mutated in the genes nuc-1 (involved in apoptosis well downstream from ced-3 when commitment to death is irreversible; Wu et al. 2000) and him-3 (acting in meiotic recombination downstream of rad-51; Zetka et al. 1999; C. Rinaldo, unpublished results). In both cases rad-51 RNAi effectively reduces fertility.

rad-51-interfered worms are hypersensitive to IR in soma: We have investigated the effect of γ-radiation on somatic cells during late embryonic development of rad-51-interfered worms.

Wild-type worms subjected to 20 Gy of γ-rays as embryos and rad-51-depleted worms that have not been subjected to γ-rays exhibit a body morphology that is indistinguishable from wild-type untreated worms. Most F₁ rad-51 RNAi adults that have been irradiated as embryos display gross abnormalities in the gonads and in the vulva (88% of all the adults). These defects appear only sporadically in the wild-type population treated with 120 Gy of γ-rays (<1% of all the treated worms). The gonad defects (observed in 75% of the γ-treated RAD-51-depleted worm population) might in part be a consequence of defects in germ line growth or survival, although anomalies in arm migration and gonad elongation are likely to be due to somatic defects. That somatic tissues depleted of RAD-51 are hypersensitive to γ-radiation is demonstrated by the vulva defects:

those observed in RAD-51-depleted oocytes in a wild-type background (Figure 3A, right). This observation shows that, in C. elegans, rad-51 acts downstream of spo-11 during meiotic prophase and is consistent with the results on germline apoptosis in rad-51 RNAi/spo-11 background described by Gartner et al. 2000.

rad-51 RNAi, in a mre-11(ok179) background, leads to the appearance of DAPI-stained bodies corresponding to properly condensed univalents in diakinesis nuclei (Figure 3C, right). This observation provides evidence that mre-11, known to be required for the repair of DSBs during meiosis (Chin and Villeneuve 2001), is also necessary in C. elegans in the initial steps of recombination for DSB induction.

The MSH-5 protein is required for both normal and radiation-induced meiotic crossing over, but is not required for DNA repair during meiosis (Kelly et al. 2000). rad-51 RNAi in a msh-5(me23) background results in the appearance of chromatin that distinctly differs both from that seen in msh-5 mutants (where 12 intact-appearing univalents are observed) and from the poorly condensed appearance of the chromatin in rad-51 RNAi oocytes. While we did not detect any DNA condensation defects, we did detect, in addition to a partial aggregation of bodies, very small DAPI-stained spots, pointing to the occurrence of DNA fragmentation (Figure 3D, right). Chromosome fragmentation is not observed in rad-51 RNAi oocytes in a wild-type background.

Brood-size reduction by rad-51 RNAi requires rad-5: In an attempt to understand which pathways lead to the reduced fertility of the rad-51 RNAI hermaphrodites (Takanami et al. 1998; Table 1), we compared brood-size variations induced by rad-51 RNAI in genetic backgrounds affecting meiosis, DNA damage checkpoints, and apoptosis (Table 3).

Gartner et al. (2000) have demonstrated that in a rad-51-interfered background there is a net increase in checkpoint-dependent cell death of oocyte precursors. In the ced-3 mutant germ cells, both physiological and DNA damage-induced apoptosis are inhibited. In the meiotic checkpoint mutant rad-5, physiological germ cell death occurs, but DNA damage-induced apoptosis is absent.
19% of a RAD-51-depleted population, which had been treated with γ-radiation, shows protruding vulva, bursting at the vulva (Figure 4B), and egg-laying defects (Figure 4C).

**DISCUSSION**

Embryonic lethality resulting from *rad-51* RNAi is due to maternal effect: Embryonic lethality can be explained if most embryos are aneuploid, since proper chromosome disjunction has not taken place during meiosis I in the affected parent, and if they also carry chromosome aberrations. An increase in X chromosome nondisjunction is confirmed by the observed Him phenotype. However, the incidence of males observed in the progeny of RAD-51-depleted F1 (6.5%, see Table 2) is lower than that observed in RNAi or null mutations of other meiotic genes such as him-3 (20%, Zetka et al. 1999) and spo-11 (50%, Dernburg et al. 1998). One possible explanation is that, since males are hemizygous for the X-linked genes, aberrant X chromosomes may be subjected to strong negative selection. Although meiotic defects are sufficient to account for the embryonic lethality observed as a result of RAD-51 depletion, we do not rule out a likely effect in mitosis: it is conceivable that RNAi in the hermaphrodite parent might prevent transmission of the RAD-51 protein, which fertilized eggs may normally inherit and which might be required for DNA repair during the first cell divisions. However, it should be borne in mind that the occurrence of DSBs in *C. elegans* may be less deleterious during mitotic cell division in soma (where transmission of broken chromosomes could be allowed by the holocentric organization) than in meiosis (where chromosomes adopt a monocentric organization). Studies on null mutants of the *rad-51* gene will be necessary to rule out any requirement for *rad-51* during early embryogenesis.

The *rad-51* gene is involved in DNA damage response in the soma: Eukaryotic RAD51 genes are involved in the cellular response to genotoxic agents and in particular in the DSB repair pathway in somatic cells. In *S. cerevisiae*, rad51 mutants are viable although hypersensitive to genotoxic agents (Game and Mortimer 1974; Shinohara et al. 1992). In the mouse, null mutations in *RAD51* are lethal during early embryogenesis and this arrest in development can be explained as the result of a failure in the repair of DNA damage (Lim and Hasty 1996; Tsuzuki 1996). We show that *C. elegans* *rad-51* RNAi, followed by γ-ray treatment during late embryogenesis, causes several developmental defects mostly affecting those postembryonic cell lineages lead-

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**Figure 3.**—DAPI staining of oocyte nuclei in the proximal portion of the gonad corresponding to the diakinesis stage. Chromosome morphology in wild type (N2) (A), *spo-11(ok79)* (B), *mre-11(ok179)* (C), *msh-5(me23)* (D), and *rad-5(mn159)* (E) is shown on the left. Chromosome morphology in the corresponding strains 48 hr after injection with *rad-51* dsRNA is shown on the right. Some univalents in B and C are out of focus as they are located on different focal planes (see asterisks). Arrows indicate DAPI-stained spots with a diameter smaller than the average univalent (chromosome fragments). Bar, 5 μm.
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Figure 4.—Effect of \( \gamma \)-radiation on development of F\(_1\) rad-51 RNAi worms. (A) rad-51 RNAi adult hermaphrodite that has not been treated with \( \gamma \)-radiation (vulva in particular). (B) rad-51 RNAi adult hermaphrodite that has been treated with 20 Gy of \( \gamma \)-rays during embryogenesis (bursting at the vulva). (C) rad-51 RNAi adult hermaphrodite that has been treated with 20 Gy of \( \gamma \)-rays during embryogenesis (vulva showing defects in egg laying). Bar, 10 \( \mu \text{m} \).

New roles of mre-11, rad-51, and msh-5 in C. elegans: Chromosomes at the diakinesis stage, when RAD-51 is depleted, appear poorly condensed and associated in bundles. However, in this context if DSBs are inhibited by the absence of SPO-11, the chromosomes appear intact and properly condensed. Therefore, we can conclude that the abnormal morphology of chromosomes in rad-51 RNAi oocytes is a consequence of a defect in repair of meiotic DSBs. This is consistent with RAD-51

Table 3

<table>
<thead>
<tr>
<th>Brood size of untreated RAD-51(+) and corresponding RAD-51-depleted worms</th>
<th>wt</th>
<th>wt&lt;sup&gt;*&lt;/sup&gt;</th>
<th>nuc-1&lt;sup&gt;*&lt;/sup&gt;</th>
<th>him-3&lt;sup&gt;*&lt;/sup&gt;</th>
<th>spo-11&lt;sup&gt;*&lt;/sup&gt;</th>
<th>rad-5&lt;sup&gt;<em>&lt;/sup&gt; ced-3&lt;sup&gt;</em>&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average no. of eggs</td>
<td>224 ± 138</td>
<td>87 ± 86</td>
<td>13 ± 20</td>
<td>212 ± 127</td>
<td>115 ± 25</td>
<td>203 ± 10</td>
</tr>
<tr>
<td>Average no. of eggs</td>
<td>26 ± 87</td>
<td>13 ± 86</td>
<td>5 ± 15</td>
<td>212 ± 127</td>
<td>115 ± 25</td>
<td>203 ± 10</td>
</tr>
<tr>
<td>Average no. of eggs</td>
<td>13 ± 86</td>
<td>5 ± 15</td>
<td>212 ± 127</td>
<td>115 ± 25</td>
<td>203 ± 10</td>
<td>10</td>
</tr>
</tbody>
</table>
| Analysis using z-statistics shows that in all the strains indicated with the asterisks untreated and rad-51 interfered populations are significantly different (\( P < 0.001 \)).

N<sub>F1</sub>, the number of F<sub>1</sub> individuals whose progeny were scored.

<sup>a</sup>The data relative to the RNAi-resistant mut-7 worms treated with the same procedure used to induce RNAi in the other strains. Only in the rad-5 genetic background and in the RNAi-resistant control strain mut-7 are untreated and rad-51 interfered populations not significantly different.
playing a role downstream of DSB induction. Interestingly, in mre-11 mutant oocytes IR induces the appearance of large chromosome aggregates (Chin and Ville-Neuve 2001) partially reminiscent of the cytological phenotype of rad-51 RNAi untreated oocytes. It is conceivable that MRE-11 and RAD-51 act in contiguous steps of recombination (such as the 5’ to 3’ resection of DSBs and strand invasion), which are critical for both crossover and noncrossover, and that the chromosome aggregates may be the result of abortive attempts to repair DSBs using alternative pathways.

We have shown here that depletion of RAD-51 in an mre-11 background (exactly as in a spo-11 background) leads to the appearance of properly condensed univalents at diakinesis, indicating that DSBs are not formed in the mre-11 mutant. We therefore demonstrate that MRE-11 is required for initiation of meiotic recombination in C. elegans and thus the dual function of MRE-11, i.e., meiosis-specific induction of programmed DSBs and DNA repair, is conserved in evolution from fungi to metazoa.

Although DNA repair is not affected by the msh-5 mutation (Kelly et al. 2000), rad-51 RNAi in a msh-5 mutant (Figure 3D) leads to chromosome fragmentation in oocytes. The RAD-51 depletion is therefore the cause of such fragmentation. We suggest that when a functional RAD-51 protein is available in the msh-5 mutants, DSBs are resolved as noncrossovers, for which MSH-5 is not required, resulting in undamaged, properly condensed univalents. In the absence of RAD-51 in a msh-5 background, DSBs cannot be resolved either as crossovers or as noncrossovers and therefore chromosomes are fragmented.

The diffused appearance of chromosomes resulting from RAD-51 depletion is somehow dependent on a functional MSH-5; in fact it is not observed when rad-51 RNAi is performed in the msh-5 mutant. MSH-5 may be involved in the stabilization of crossover intermediates (but not of noncrossover intermediates) and, in the absence of RAD-51, MSH-5 may improperly recognize some alternative substrate and indirectly contribute to the abnormal chromosome morphology characteristic of rad-51 RNAi oocytes. Our findings may also suggest a physiological role for MSH-5 protein in regulating chromatin organization during meiotic recombination. For instance, it might locally release a constraint promoting an “open” state of the chromatin that would normally regress once the exchange has been completed.

rad-51 cross-talk with the meiotic checkpoint gene rad-5 and fertility determinants: In self-fertilizing C. elegans hermaphrodites, spermatogenesis is completed after the L4/adult molt. Subsequent differentiation of germ cells gives rise exclusively to oocytes, which are produced in great excess. The number of spermatocytes normally acts as the limiting factor in determining the brood size. Oocyte precursor apoptosis could account for the reduction in fertility induced by rad-51 RNAi if it resulted in the spermatocytes outnumbering the residual oocytes. Alternatively, apoptosis would have to affect hermaphrodite spermatogenesis as well as oogenesis. rad-51 RNAi affects the brood size of the P₀ as well as that of the F₁ (Table 1). In spite of the dramatic increase in oocyte precursor cell death described by Gartner et al. (2000), we observed that, at the end of fertile life, the gonads of the P₀ rad-51 RNAi hermaphrodites still contain a large number of developing oocytes, the spermathecae are devoid of sperms, and unfertilized oocytes are laid (data not shown). Therefore, although spermatogenesis in these worms should not be affected by RNAi because the dsRNA is injected after spermato-genesis has been completed, oocytes still outnumber the spermatocytes. Furthermore, rad-51 RNAi reduces fertility in a cel-3 background. Therefore apoptosis does not seem to be the cause of the reduced fecundity observed. However, since rad-5 brood size is not affected by rad-51 RNAi, we propose that, in response to rad-51 RNAi, RAD-5 is able to activate not only apoptosis but also alternative pathways contributing to brood-size contraction. Surprisingly, we observed that rad-51 interference has an effect on brood size in the spo-11 genetic background also. This observation leads us to envisage rad-51 performing other functions during gametogene-sis: for example, besides acting downstream of spo-11 during homologous recombination, it may also play a role in the premeiotic S phase.

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