The Drosophila *spn-D* Gene Encodes a RAD51C-Like Protein That Is Required Exclusively During Meiosis

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ABSTRACT

In Drosophila, mutations in double-strand DNA break (DSB) repair enzymes, such as *spn-B*, activate a meiotic checkpoint leading to dorsal-ventral patterning defects in the egg and an abnormal appearance of the oocyte nucleus. Mutations in *spn-D* cause an array of ovarian phenotypes similar to *spn-B*. We have cloned the *spn-D* locus and found that it encodes a protein of 271 amino acids that shows significant homology to the human RAD51C protein. In mammals the *spn-B* and *spn-D* homologs, XRCC3 and RAD51C, play a role in genomic stability in somatic cells. To test for a similar role for *spn-B* and *spn-D* in double-strand DNA repair in mitotic cells, we analyzed the sensitivity of single and double mutants to DSBs induced by exposure to X rays and MMS. We found that neither singly mutant nor doubly mutant animals were significantly sensitized to MMS or X rays. These results suggest that *spn-B* and *spn-D* act in meiotic recombination but not in repair of DSBs in somatic cells. As there is no apparent ortholog of the meiosis-specific DMC1 gene in the Drosophila genome, and given their meiosis-specific requirement, we suggest that *spn-B* and *spn-D* may have a function comparable to DMC1.

MEIOTIC recombination and DNA repair are intimately linked in all organisms. Both processes use a common set of enzymes that catalyze the reaction known as recombinational repair of double-strand DNA breaks. This process restores the normal DNA sequence without nucleotide loss by DNA synthesis, using as template the information provided by homologous DNA sequences, usually present on a homologous chromosome (Kanaar *et al.* 1998; Pâques and Haber 1999; Jasin, 2000). The key players that perform this reaction are highly conserved, in particular, the Rad51 recombinase and its accessory proteins. In eukaryotic cells the Rad51 protein is a functional homolog of the bacterial RecA protein (Baumann and West 1998). *Saccharomyces cerevisiae* cells contain, in addition to Rad51 itself, three Rad 51 paralogs: Rad55, Rad57, and the meiosis-specific Dmc1 (Sung 1997). Seven members of the Rad51 protein family have been identified in humans: RAD51 (Shinohara *et al.* 1993), DMC1 (Habu *et al.* 1996), XRCC2 (Cartwright *et al.* 1998b; Liu *et al.* 1998), XRCC3 (Tebbs *et al.* 1995; Liu *et al.* 1998; Pierce *et al.* 1999), RAD51B (Albala *et al.* 1997; Cartwright *et al.* 1998a), RAD51C (Dosanjh *et al.* 1998), and RAD51D (Cartwright *et al.* 1998a; Kawabata and Saeki 1998; Pittman *et al.* 1998).

In Drosophila, mutations in one of these genes, *spindle-B* (*spn-B*), which encodes a protein that is most similar to XRCC3, were first isolated on the basis of their effects on oogenesis, where curiously, a dorsal-ventral patterning defect of the egg is the most obvious phenotype of homozygous mutant females. In addition to these patterning defects, mutations in *spn-B* also cause defects in the appearance of the oocyte nucleus (González-Reyes *et al.* 1997; Ghabrial *et al.* 1998). *spn-B* mutations do affect recombination, but this effect is hard to detect, because the patterning defects prevent most of the eggs from developing into viable progeny (Ghabrial *et al.* 1998). *spn-B* is a member of the spindle class genes, which also include *spn-A*, *spn-C*, *spn-D*, *spn-E*, and *spn-F* on the third chromosome (Tearle and Nüsslein-Volhard 1987) as well as *okra*, *zucchini*, and *squash* on the second chromosome (Schüpbach and Wieschaus 1991). Similar to *spn-B*, *okra* encodes a component of the recombinational DSB repair machinery, the *Drosophila* Rad54 ortholog (Ghabrial *et al.* 1998). On the other hand, *spn-E* encodes an RNA-dependent ATPase (Gillespie and Berg 1995) with no direct connection to DNA repair. While the molecular nature of other spindle class genes is still unknown, earlier studies suggested that *spn-C/mus301* and *spn-D* might also encode proteins that act in recombinational repair of double-strand DNA breaks (Boyd *et al.* 1981; Ghabrial *et al.* 1998; Ghabrial and Schüpbach 1999).

Interestingly, the dorsal-ventral patterning defects in

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spn-B mutants are dependent upon the activation of a meiotic checkpoint (Ghabrial and Schüpbach 1999). As has been elegantly demonstrated in a variety of organisms, notably in yeast and humans, unrepaired DNA breaks can activate a set of checkpoint proteins, which halt progression through the cell cycle until the DNA is repaired. A meiotic checkpoint similar to that described in yeast exists in Drosophila and is activated in *spn-B* mutant egg chambers due to the compromised repair of DSBs induced during meiotic recombination. Components of the meiotic checkpoint in Drosophila include the proteins Mei-41 and Dmchk2, which are homologous to ATR/Mec1 and Rad53, respectively (Ghabrial and Schüpbach 1999; Abdu *et al.* 2002). Checkpoint activation results in a significantly lower level of Gurken protein—a TGFα-like signaling molecule whose levels and localization within the oocyte play a central role in dorsal-ventral patterning (reviewed in Nilson and Schüpbach 1999).

In this study, we show that the dorsal-ventral defects of *spn-D* mutants are suppressed by mutations in *mei-W68* and *mei-41*, suggesting that, as seen for *spn-B* and *okr*, mutations in *spn-D* activate the meiotic checkpoint. Cloning the *spindle-D* (*spn-D*) gene revealed that this locus encodes a Rad51C-like protein. In mammals, the Rad51 paralogs XRCC3 and Rad51C have a role in somatic cells, where they contribute to genomic stability and are involved in genetic recombination processes (reviewed in Godthelp *et al.* 2002). The Drosophila XRCC3 homolog, Spn-B, does not show any somatic defects, raising the possibility that Spn-B is entirely dedicated to meiosis. Alternatively, it is possible that Spn-B is acting in the soma, but might be redundant with other Rad51-like proteins. The human XRCC3 physically interacts with both Rad51 and Rad51C (Schild *et al.* 2000; Masson *et al.* 2001), suggesting that if there is redundancy for *spn-B*, it might be with *spn-D*. We found that *spn-B* and *spn-D* single mutants as well as the double mutants have little or no effect on the repair of double-strand DNA breaks in somatic cells and that the essential role of the two genes is in the process of meiotic recombination.

MATERIALS AND METHODS

Drosophila strains: *spn-D* was meiotically mapped to 91 cM on 3R and is uncovered by *Df(3R)T1-P* (Ghabrial *et al.* 1998). Four EMS-induced alleles of *spn-D*, *spn-D*^{349}, *spn-D*^{150}, (Tearle and Nüsslein-Volhard 1987), *spn-D*^{CX} (Ghabrial *et al.* 1998), and *spn-D*^{225} (González-Reyes *et al.* 1997), were identified previously. Despite repeated outcrossing, no homozygous viable chromosome of *spn-D*^{CX} was recovered. Also, no nucleotide changes were identified in sequencing the coding region of *spn-D*^{CX}. In previous genetics tests *spn-D*^{150} has been shown to behave as an amorphic allele (González-Reyes *et al.* 1997). The *spn-B*^{BU} allele and the rescue construct have been described previously (Ghabrial *et al.* 1998). Mutant stocks of *mei-41* and *mei-W68* were obtained from the Bloomington Stock Center. Green fluorescent protein (GFP)-spnD was expressed in the ovary using a *nanos*-Gal4-VP16 expression system (Van Doren *et al.* 1998).

Molecular cloning: Blast searches of genomic DNA from the region of 3R corresponding to the predicted location of *spn-D* revealed the presence of DNA sequences with homology to Rad51. At the time, no corresponding predicted genes or expressed sequence tags (ESTs) were available, so 3′ and 5′ rapid amplification of cDNA ends (RACE; CLONTECH, Palo Alto, CA) experiments were performed. Briefly, ovarian mRNA was purified from Ore-R females using Dynabeads (Dynal Biotech, Great Neck, NY) as previously described (Tomancak *et al.* 1998). For 3′ RACE the gene-specific primer TCCTTGGGTCACCTGGTTGAAC was used. For 5′ RACE the gene-specific primer GTTCAACCAGGTGACCCAAG was used. The putative *spn-D* sequence was confirmed by sequencing a cDNA amplified from a cDNA ovarian library (Stroumbakis *et al.* 1994).

Rescue construct: A 2.2-kb *Xba*I/*Sal*I fragment and a 7-kb *Sal*I/*Xba*I fragment from phage 10.1 (Fleming *et al.* 1990) were separately subcloned in PBS. The 7-kb *Sal*I/*Xba*I fragment was then digested with *Sal*I/*Pst*I, and a 2.8-kb fragment was purified. A triple ligation using the following fragments (the 2.2-kb *Xba*I/*Sal*I, the 2.8-kb *Sal*I/*Pst*I, and pCasper cut with *Xba*I and *Pst*I) was performed to obtain pCs-*spn-D*. To make the UASp-GFP::*Spn-D* fusion the entire GFP-coding sequence was amplified by PCR, using modified primers to create an *Asp718* restriction site at the 5′ end and a *Sac*I site at the 3′ end. The 1.3-kb *spn-D* genomic region was amplified by PCR using modified primers to create *Sac*I (5′) and *Xba*I (3′) restriction sites. A triple ligation using the following fragments, GFP *Asp718/Sac*I, genomic spn-D *Sac*I/*Xba*I, and pUASp cut with *Asp718/Xba*I, was performed to obtain UASp-GFP::*spn-D*. *P*-element-mediated germ-line transformation of both constructs was carried out according to standard procedures.

Sequencing of mutant alleles: Genomic DNA was prepared from flies of the genotype *spn-D**/*spn-D** according to standard procedures (Sambrook *et al.* 1989). The coding region was sequenced and the sequences were compared with the wild-type genomic sequence of the parental chromosome using the MacVector (Kodak/IBI) program.

Northern blot: Whole RNA was extracted from wild-type (Oregon-R) ovaries using Trizol reagent (GIBCO BRL, Gaithersburg, MD). The RNA was separated on a denaturing gel, blotted onto nitrocellulose membrane, and probed with radiolabeled DNA fragments using standard molecular biology techniques. The probe used was an EST (RE19845), which covers the entire *spn-D* open reading frame.

Antibody staining of ovaries: Immunolocalization of Grk was performed as described previously (Queenan *et al.* 1999).

Tests of DNA repair in mitosis: To test the requirement in mitotic double-strand break repair, *spn-B*^{BU}, *spn-D*^{150} single and double mutants were exposed during larval development to the mutagen methyl methanesulfonate (MMS; Sigma, St. Louis) or to ionizing radiation. Sensitivity to MMS was measured as described previously (Ghabrial *et al.* 1998). To determine the X-ray sensitivity, crosses of the appropriate genotypes were made in duplicate, one to be treated with X rays and the other to serve as control. The crosses were transferred daily and 2 days after the transfer, the larvae were irradiated with an Astrophysics Research Corporation X-ray machine operated at 145 kV and 5 mA for 90 sec, which corresponds to a dose of ~1200 rad. After eclosion, the percentage of the mutant flies was determined, and the sensitivity to X rays was expressed as the fraction of the expected percentage of the mutant flies in the treated vial as compared to the progeny of untreated control vials.

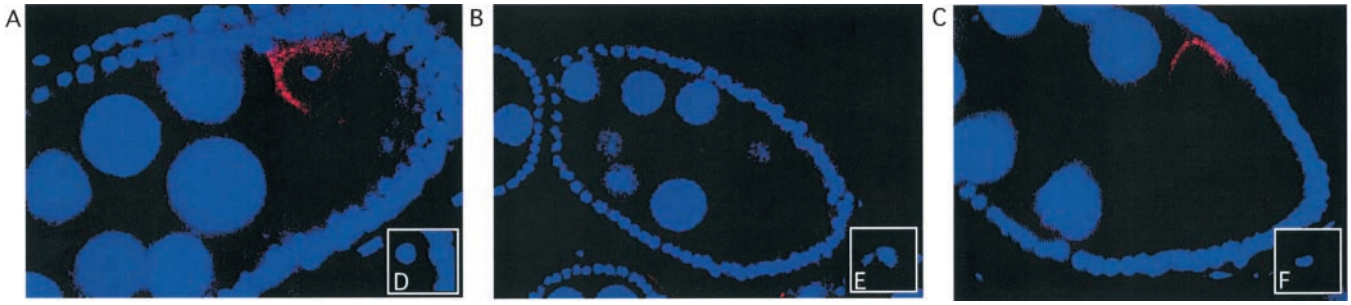

FIGURE 1.—Grk accumulation and karyosome morphology in wild-type and mutant Drosophila egg chambers. (A–F) DNA is blue and Grk protein is red. (A) Wild-type egg chamber (Ore-R); Grk protein is localized at the dorsal anterior cortex of the oocyte. (B) *spn-D*^{349}/*spn-D*^{150} mutant egg chamber; Grk protein is reduced. (C) *meiW-68*; *spn-D*^{349}/*spn-D*^{150} double-mutant egg chamber; Grk protein is localized as in wild type. (D) Karyosome from wild-type egg chamber. (E) Karyosome from *spn-D*^{349}/*spn-D*^{150} mutant egg chamber. (F) Karyosome from *meiW-68*; *spn-D*^{349}/*spn-D*^{150} double-mutant egg chamber.

RESULTS

***spn-D* mutations are suppressed by eliminating activation of the meiotic checkpoint:** The *spn-B* patterning defects can be suppressed by blocking the formation of double-strand DNA breaks (DSBs) during meiosis using mutations in *mei-W68* or by eliminating the checkpoint by using mutations in the checkpoint gene, *mei-41* (GHABRIAL AND SCHÜPBACH 1999). To study whether the ovarian phenotype in *spn-D* is also due to unrepaired double-strand DNA breaks, flies double mutant for *mei-W68* and *spn-D* were generated (Figure 1 and Table 1). In double-mutant flies we observed suppression of dorsal-ventral patterning defects (Table 1) and a corresponding dramatic increase in the accumulation of Grk protein (Figure 1C), as compared to *spn-D* single mutants (Figure 1B). We also observed a significant suppression of oocyte nuclear defects (Figure 1, D–F). In wild-type egg chambers the DNA has a spherical shape and is condensed in a structure called the karyosome (Figure 1D). In contrast, in *spn-D*^{150} mutant egg chambers, the DNA is found in variety of conformations, including wild-type shape (33%), oblong shape (31%), or fragmented (36%; Figures 1E and 4A). In egg chambers from the *spn-D* and *mei-W68* double-mutant flies, the appearance of the DNA within the oocyte is fully restored to wild type (Figure 1F).

To confirm that the patterning defects in *spn-D* mutants are caused by activation of the same meiotic checkpoint activated in *spn-B* mutants, flies double mutant for *mei-41* and *spn-D* were generated. We found that *mei-41* suppressed up to 96% of the eggshell defects found in *spn-D* mutants (Table 1). However, we observed that even residual Mei-41 protein activity still affected the oocyte nuclear morphology. Only a minor suppression of the oocyte nuclear defect was found in the flies double mutant for both *mei-41*^{D1} (presumably hypomorphic allele) and *spn-D*, whereas in the flies double mutant for both *mei-41*^{D3} (a putative null allele, SIBON *et al.* 1999) and *spn-D*, most of the egg chambers had wild-type-like oocyte nuclear morphology (χ^2 significance of $P = 0.0005$; Table 2).

The spindle-D gene encodes a Rad51C-like protein: Homology searches of the region of chromosome 3 corresponding to the location of the *spn-D* locus re-

TABLE 1

Eggshell phenotypes of *spn-D* alone and in combination with *mei-W68* and *mei-41*

Maternal genotype	Wild-type-like eggshell (%)	Mutant eggshell (%)	*n*
spn-D^{349}	43	36 (21[a])	980
spn-D^{150}	22	78	589
spn-D^{225}	84	16	478
mei-W68/*CyO*; *spn-D*^{150}/*spn-D*^{150}	30	70	1003
mei-W68; *spn-D*^{150}/*spn-D*^{150}	98	2	1173
mei-W68/*CyO*; *spn-D*^{349}/*spn-D*^{150}	39	61	1113
mei-W68; *spn-D*^{349}/*spn-D*^{150}	100	0	1287
mei-41/*Clb*; *spn-D*^{150}/*spn-D*^{150}	51	49	2168
mei-41; *spn-D*^{150}/*spn-D*^{150}	96	4	1261
mei-41/*Clb*; *spn-D*^{349}/*spn-D*^{150}	64	36	1726
mei41; *spn-D*^{349}/*spn-D*^{150}	95	5	1451

[a] Dorsalized eggs with dorsal appendage material around the lateral and ventral side of the egg.

TABLE 2

Karyosome phenotypes of double-mutant combinations of *spn-D* with *mei-41*

Maternal genotype	Wild-type-like oocyte nucleus (%)	Mutant oocyte nucleus (%)	*n*
mei-41^{D1}/Clb; spn-D^{349}/spn-D^{150}	47	53	54
mei-41^{D1}; spn-D^{349}/spn-D^{150}	60	40	59
mei-41^{D3}/Clb; spn-D^{349}/spn-D^{150}	51	49	48
mei41^{D3}; spn-D^{349}/spn-D^{150}	86	14	63

vealed the existence of genomic DNA with homology to *Rad51*-like genes. This *spn-D* candidate gene was cloned (see MATERIALS AND METHODS) and the gene structure was determined from comparison of cDNA sequences and the corresponding genomic sequence (Figure 2A). A search of the Berkeley *Drosophila* Genome Project (BDGP) database reveals three recently identified ESTs (GenBank nos. AY71162, BI578285, and BI580876) corresponding to the *spn-D* transcript that confirms the gene structure of *spn-D*. *spn-D* encodes a protein of 271 amino acids with an apparent molecular weight of 31 kD. Northern blots of wild-type (Oregon-R) ovarian mRNA were hybridized with a probe corresponding to the *spn-D* transcript and revealed a single band of ~1.5 kb (data not shown). Motif searches revealed that the protein belongs to the RecA family and contains the consensus P-loop motif, which is an ATP/GTP-binding site (Figure 2B). Blast searches of DNA and protein databases identified the human RAD51C protein as the most closely related to Spn-D (23% identity and 38% similarity). Phylogenetic analysis using the P-loop domain of RecA-related proteins from human and Drosophila showed that the Drosophila genome includes five *Rad51*-like genes, all of which are putative homologs of mammalian genes, but none of which appear to correspond to *Rad51B* or *DMC1* (Figure 3). It is important to note that *spn-D* represents an additional RecA/Rad51-like protein not included in the list of four such genes identified in the analysis of release 1 of the Drosophila genome (ADAMS *et al.* 2000).

The coding regions of three mutant *spn-D* alleles were sequenced and were found to contain unique single-nucleotide changes. *Spn-D^{225}* and *spn-D^{150}* have nonsense mutations that should truncate the protein after the 40th and 229th residues, respectively (Figure 2B). The *spn-D^{349}* mutation is in the splice acceptor site of the third exon. To demonstrate that these mutations in the *Rad51C*-like gene are responsible for the defects observed in the *spn-D* mutants, two rescue constructs—one containing 9.2 kb of genomic DNA and another containing a fusion GFP::SpnD protein under the control of the Gal4/UAS system—were introduced into flies

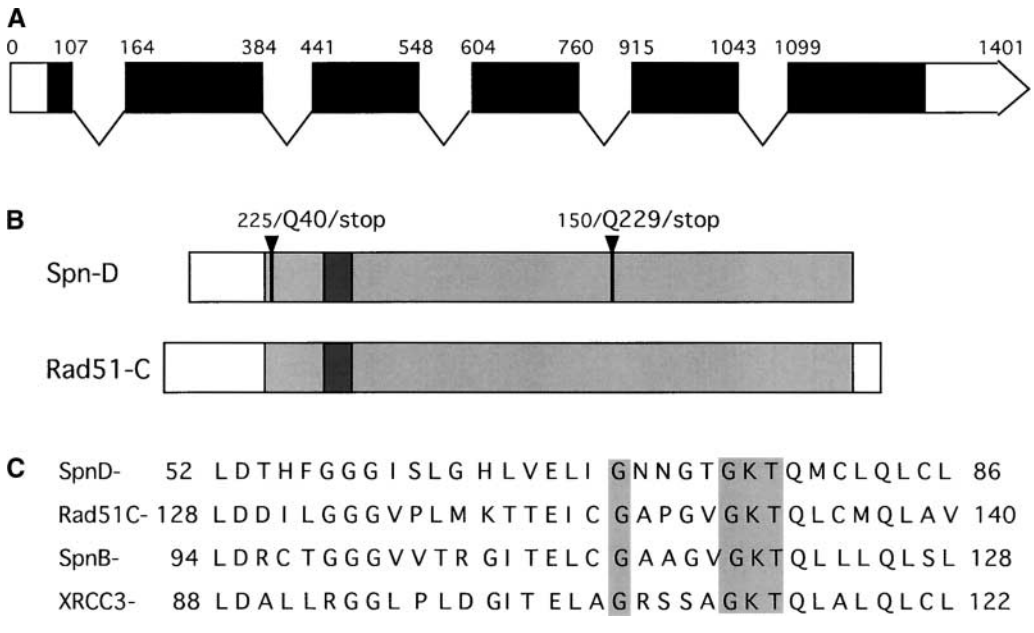

FIGURE 2.—*spn-D* encodes a Rad51C-like protein. (A) *spn-D* gene structure. Untranslated regions are indicated as open boxes, introns are indicated as lines, and coding regions are indicated as solid boxes. Numbers represent the nucleotide length of the gene. (B) Protein structure of Spn-D as compared with RAD51C. Light shading, region of homology between the two proteins; dark shading, P-loop motif, which is an ATP/GTP-binding site. The position of each mutant allele is shown; the allele number is followed by the wild-type amino acid, the amino acid number, and the stop codon. (C) An alignment of the A-type nucleotide-binding domains of Spn-D, human RAD51C, Spn-B, and human XRCC3. Dark shading marks the P-loop motif.

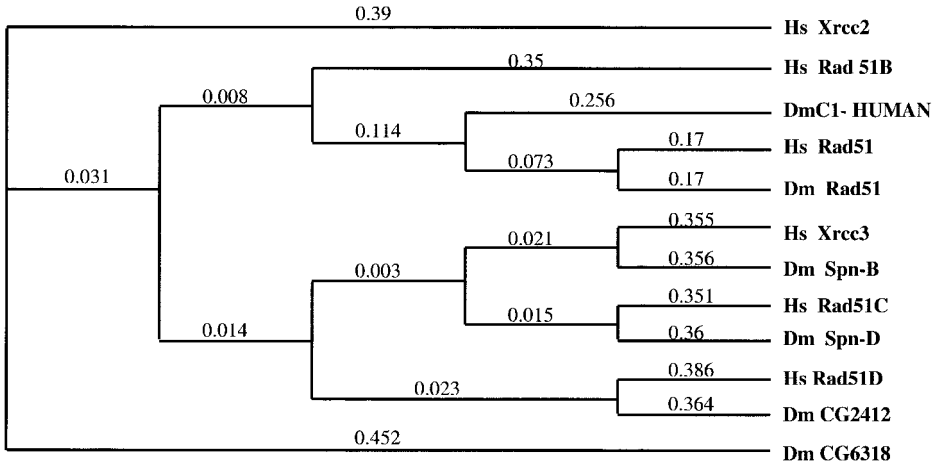

FIGURE 3.—Phylogenetic analysis of human and Drosophila Rec-A related proteins. Hs, human; Dm, Drosophila. The alignment of the sequences and the generation of the tree were performed using the MacVector program. Numbers represent the uncorrected distance, which estimates the proportional differences between sequences.

by *P*-element-mediated transformation. Both transgenes fully rescued all the mutant phenotypes, including the dorsal-ventral patterning defect and the karyosome abnormalities (Figure 4), verifying that *spn-D* indeed corresponds to the Rad51C homolog of Drosophila.

Somatic requirements for *spn-D* and *spn-B* in double-strand DNA breaks: To determine whether the *spn-D* gene is involved in somatic double-strand DNA repair processes, we subjected *spn-D*^{225}, *spn-D*^{150}, *spn-D*^{349}, and *spn-D*^{150}/*spn-D*^{349} larvae to X rays or to 0.08% of MMS and compared the survival rates of wild-type and mutant individuals (Figure 5 and data not shown). Since all of the *spn-D* alleles tested were insensitive to double-strand DNA breaks, we decided to use the *spn-D*^{150} allele—a putative genetic null (GONZÁLEZ-REYES *et al.* 1997) shown to cause the most severe ovarian defects—in this and following experiments. Given the apparent enhancement of certain ovarian defects in double-mutant animals (GONZÁLEZ-REYES *et al.* 1997), we also tested whether the *spn-B*, *spn-D* double mutant would show a somatic DNA repair defect that is not apparent for either single mutation (Figure 5 and GHABRIAL *et al.* 1998). We found that the double mutant larvae, whether treated with 0.08% MMS or exposed to X rays, were viable similarly to their heterozygous control siblings (Figure 5).

Given the molecular relatedness of the genes and their similar requirement in the ovary, we also tested whether an increase in the dose of *spn-B* can compensate for mutations in *spn-D*. Introduction of extra copies of *spn-B* into *spn-D* mutant flies did not rescue the eggshell and the karyosome defects of *spn-D* mutations (data not shown). While a single copy of this transgene can rescue the *spn-B* mutant phenotype, even two copies of this transgene—in addition to the two endogenous copies of *spn-B*—are unable to substitute for *spn-D*. Thus, despite the similarities of their mutant phenotypes and the molecular similarities between the two proteins, *spn-B* and *spn-D* have at least some distinct nonoverlapping functions.

DISCUSSION

The roles of Spn-D and Spn-B: In eukaryotes, repair of DSBs can occur by at least two pathways, nonhomologous end joining (NHEJ) and homologous recombination (HR). HR contributes to the generation of genetic

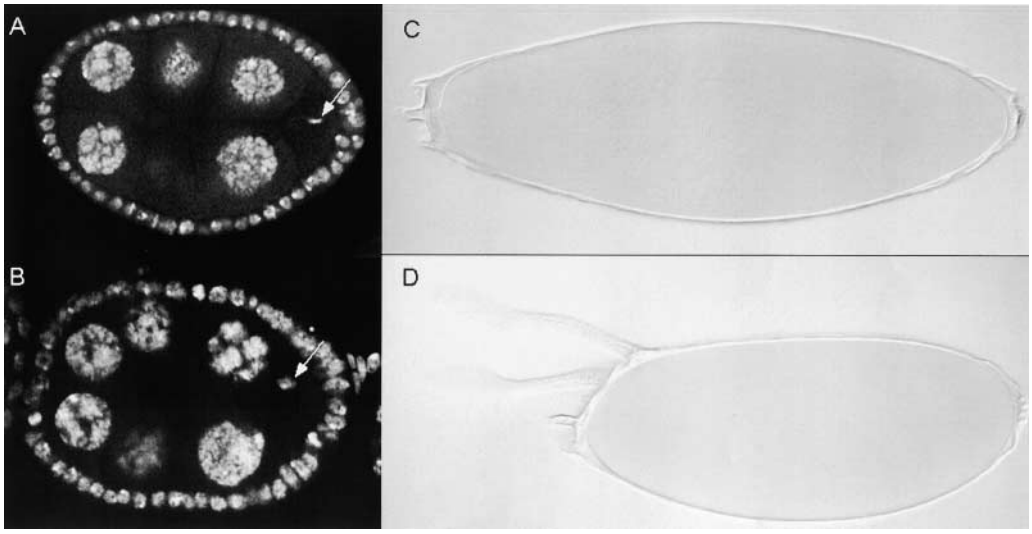

FIGURE 4.—Rescue of mutant karyosome and eggshell by the UASp-*SpnD* construct. The *nanos*-Gal4 line was used to drive expression of the GFP::SpnD protein in the germ line. (A and B) DNA stained with Hoechst is labeled in white. The arrows point at the karyosome. (A) Mutant karyosome from *spn-D*^{349} egg chamber. (B) Wild-type-like karyosome in a *nanos*-Gal4/UASp-GFP::*SpnD*; *spn-D*^{349} egg chamber. (C) Strongly ventralized eggshell from a *spn-D*^{349} mutant. (D) Wild-type-like eggshell from a *nanos*-Gal4/UASp-GFP::*SpnD*; *spn-D*^{349} female.

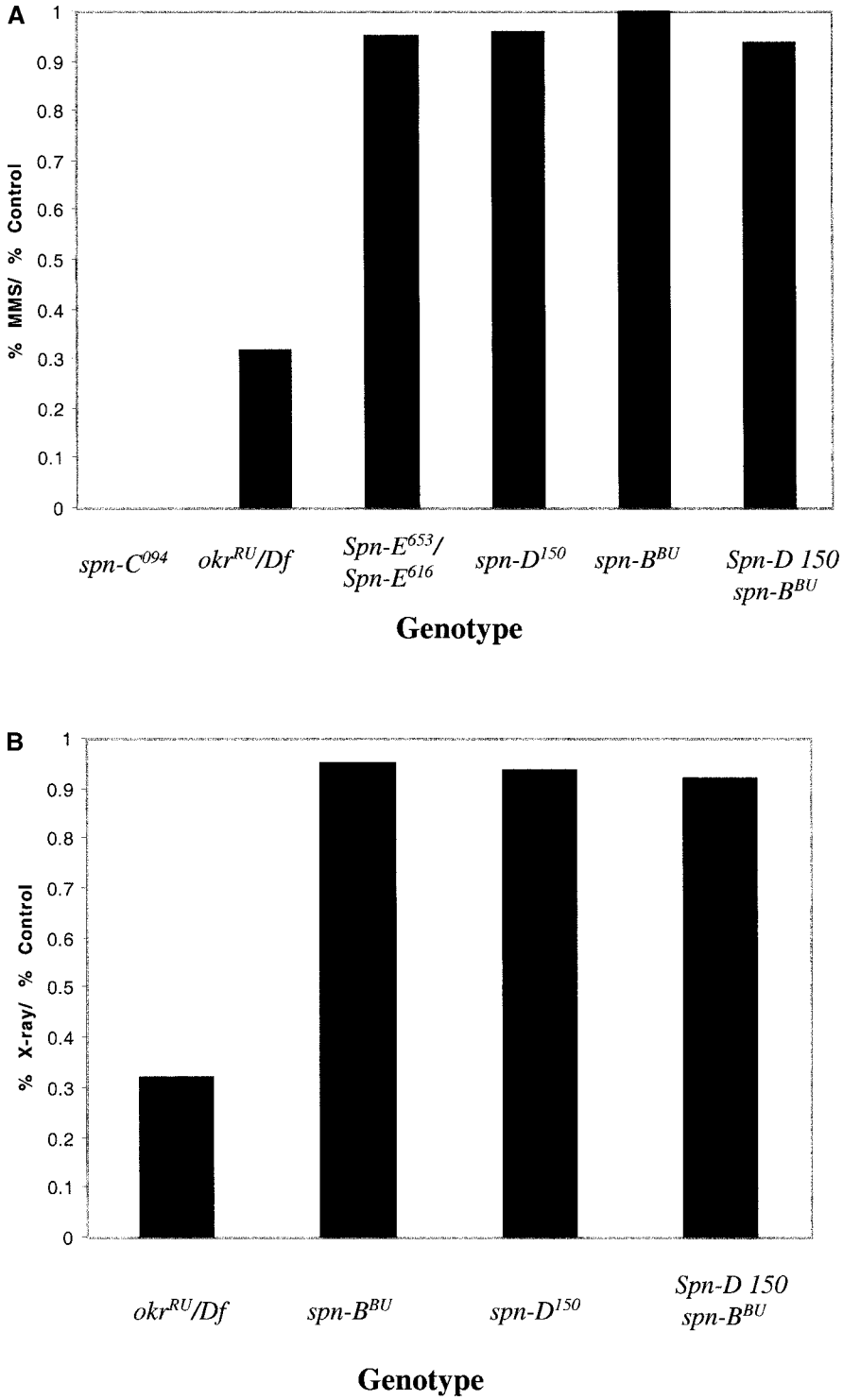

FIGURE 5.—MMS (A) and X-ray (B) sensitivity of *spn-B*, *spn-D* mutants. Each graph shows the ratio of percentage expected (treated) to percentage expected (control) for the different genotypes.

diversity and the faithful germ-line transmission of genetic information during meiosis (PÂQUES and HABER 1999) and is also utilized in somatic tissues to repair damaged DNA. The extent to which one DSB repair pathway is used in the soma, relative to the other pathway, has been found to vary widely between species (VAN DEN BOSCH *et al.* 2002).

The Rad51 protein, which is a functional homolog of the bacterial RecA protein, is central to the recombination process. All eukaryotes for which the entire genome sequence is known contain members of the RecA-like protein family. In the *Drosophila melanogaster* and *Caenorhabditis elegans* genome, orthologs of two proteins, which are specific to recombination during meiosis, Dmc1 and Mnd1, have not been found (DERNBURG *et al.* 1998; GERTON and DERISI 2002). *DMC1* encodes a meiosis-specific protein that has been shown to bias template choice to interhomolog events, rather than

to intersister events, during meiotic recombination (Bishop *et al.* 1992). It was suggested that Mnd1p operates downstream of Dmc1p binding to DNA and that Mnd1p may be a cofactor for strand invasion (Gerton and DeRisi 2002). The absence of *DMC1* and *MND1* homologs in both Drosophila and *C. elegans* raises the issue of whether flies and worms have evolved recombination mechanisms that differ from those of yeast, mouse, and human. In Drosophila and *C. elegans* the synaptonemal complex (SC) formation precedes recombination, in contrast to yeast. It has been suggested that the SC of Drosophila and *C. elegans* may impose structural constraints, thus directing recombinations toward interhomolog events (Sekelsky *et al.* 2000). An alternative mechanism that would ensure interhomolog events is the functional substitution of other Rad-51-like proteins for Dmc1. In mitotic cells, the human RAD51 protein has been considered to be the only protein to catalyze homologous pairing during the recombinational repair process (Gupta *et al.* 1999; Mazin *et al.* 2000). However, it has been shown that the human XRCC3 interacts with RAD51C and that this stable complex can catalyze homologous-pairing activity *in vitro* (Kurumizaka *et al.* 2001). Together with previous work (Ghabrial *et al.* 1998), this study shows that mutations in *spn-B,* the Drosophila XRCC3 homolog, and *spn-D*, the Drosophila Rad51C, cause defects in meiotic recombination, but do not seem to affect the repair of DSBs in somatic cells. The ability of the XRCC3-RAD51C complex to mediate strand invasion together with the meiotic-specific requirement of *spn-B* and *spn-D* suggests that *spn-B* and *spn-D* together may perform a meiosis-specific role equivalent to DMC1.

The meiotic checkpoint does not globally affect egg chamber development: Mutations in *spn-D* and in other DNA repair components result in the activation of a meiotic DNA damage checkpoint that affects the accumulation of Gurken protein in the oocyte cytoplasm as well as the compaction of DNA in the oocyte nucleus. These effects may be mediated by the protein Vasa (Ghabrial and Schüpbach 1999). Activation of the meiotic checkpoint appears to affect the accumulation/translation of more than one protein in oogenesis: in addition to Grk, the levels of Fs(1)K10 protein are known to be reduced (Ghabrial *et al.* 1998), and since *grk* and *fs(1)K10* mutations do not exhibit karyosome defects, one might expect that an additional target of the checkpoint and Vasa regulation could be protein(s) involved in oocyte nuclear DNA compaction. It is even conceivable that such a protein(s) may be the more conserved target of a meiotic checkpoint.

It is curious that in Drosophila, activation of the checkpoint by mutations such as *spn-D* does not halt oogenesis entirely. It would seem more efficient if unrepaired DNA breaks would block the development of the entire egg chamber and not affect only a subset of proteins such as Gurken and Fs(1)K10. It has been demonstrated that in early egg chambers of spindle-class mutants development is delayed: the synaptonemal complexes, which normally resolve in region 2b of the germarium, persist into region 3 of the germarium and can still be found in stage 2 egg chambers (Huynh and St. Johnston 2000). Despite these delays in meiotic progression, the mutant egg chambers continue to develop, they reach a normal size, and are laid. This may reflect the substantial contributions of the nurse cells to oocyte development and the fact that the nurse cell cycle is independent of the cell cycle of the oocyte nucleus. Even though the nurse cells are connected to the oocyte via ring canals, a very effective system of cell cycle insulation must exist between these cells, given that the nurse cells undergo many rounds of endoreplication while the oocyte nucleus is progressing through prophase of meiosis I.

We suggest that during normal meiosis, the initial checkpoint-induced developmental delay is sufficient to allow full repair of all DNA breaks; however, under conditions in which damaged DNA cannot be fully repaired (e.g., *spn* class mutants), oogenesis eventually proceeds and is driven mainly by nurse cell development. In such cases, although development of the egg chamber proceeds, the downregulation of Gurken, FS(1)K10, and perhaps other oocyte-specific proteins is never relieved and the resulting eggs are morphologically abnormal. The need to independently regulate nurse and oocyte cell cycles may account for why the activation of the meiotic checkpoint does not have a more profound effect on the growth and development of the affected egg chamber. Alternatively, it is also possible that in Drosophila a salvage process is activated, similar to what has been described for yeast: in certain strains containing *zip1*, *zip2*, and *dmc1* mutants, some cells complete sporulation after a delay in meiotic prophase progression (Bailis *et al.* 2000).

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